Biosynthesis of Terpenes and Steroids. Part IV.† Specific Hydride Shifts in the Biosynthesis of Lanosterol and β-Amyrin

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The hydride shifts accompanying 2,3-epoxysqualene cyclisation to lanosterol in yeast and to β-amyrin in peas have been checked using 2,3-epoxy[11,14-$^3H_2$]squalene. The results support the Ruzicka–Eschenmoser hypothesis and not a plausible alternative which was considered. The synthesis of 2,3-epoxy[11,14-$^3H_2$]squalene by two routes is described.

THE intermediacy of 2,3-epoxysqualene in polycyclic triterpenoid biosynthesis has been established in mammalian tissue,[1-3] in higher plants,[4-7] a mould [8] and in yeast.[9] The rearrangements postulated in the Ruzicka

† Part III, D. H. R. Barton, G. Mellows, and D. A. Widdowson, *J. Chem. Soc.* (*C*), 1971, 110.

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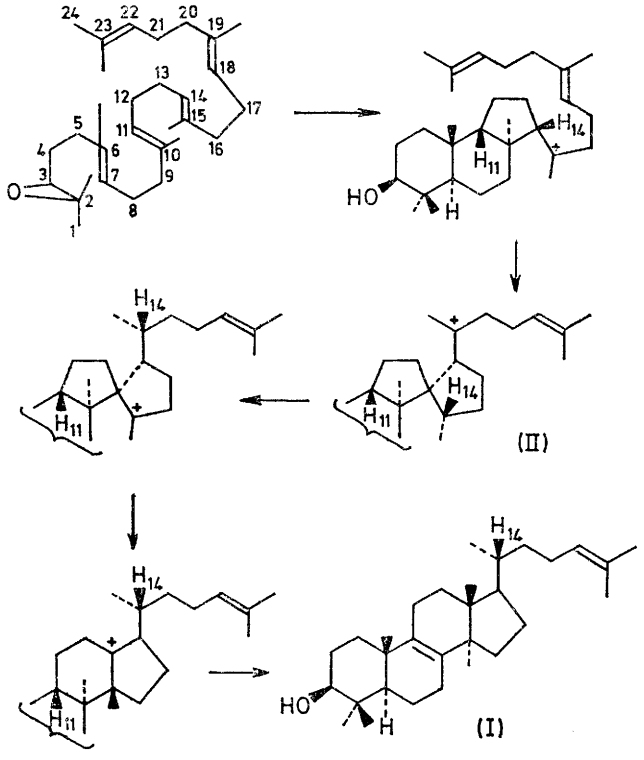
theory [10,11] of lanosterol (I) biosynthesis from squalene, have also received experimental support. In particular, two consecutive 1,2-methyl shifts have been shown to occur.[12-14] However, at the outset of this work the details of the hydrogen migrations had not been determined.

An alternative mode of cyclisation to that of Ruzicka, which involves a 1,3-hydrogen migration has been suggested [15] on the basis of enzyme studies. The ultimate positions of the migrating hydrogens were the same as in the Ruzicka proposal.

A similar mechanism involving a spiro-carbonium ion intermediate (II) could conceivably operate in which a chemically more facile [16,17] 1,5-hydrogen shift occurs



SCHEME 1

(Scheme 1). Such migrations are known to take place in biological systems.[17,18] Thus, the hydrogen at C(14) of squalene would become attached to C(20) of lanosterol. Lanosterol derived from 2,3-epoxy[11,14-$^3H_2$]squalene would then be labelled only at C(20), since the hydrogen at C(11) of squalene is lost in the cyclisation process.[19]

Our first approach to the synthesis of the labelled 2,3-epoxysqualene involved exchange of the bis-triphenylphosphonium salt of 1,4-dibromobutane, followed by reaction [20-22] of the corresponding bis-phosphorane with geranylacetone.[23-25] The radioactivity in the unchanged ketone recovered from the reaction indicated that considerable scrambling of the label had occurred, presumably by exchange of the enolisable hydrogens. Such scrambling has been observed before in Wittig reactions.[26,27] An alternative isotopically more specific, sequence involving the preparation of [2-$^3H_1$]farnesyl bromide and coupling with nickel carbonyl [28] was therefore developed. Nitroethane was labelled at C-1 with tritium using base-catalysed exchange with tritiated water.[29] Nef degradation [29] of nitro[1-$^3H_2$]ethane afforded [1-$^3H_1$]acetaldehyde which was converted into dibromo[1-$^3H_1$]ethane with phosphorus trichloride dibromide [30] in light petroleum. Dehydrobromination with one equivalent of the sodium salt of butyldigol gave [1-$^3H_1$]vinyl bromide. This was immediately converted into [1-$^3H_1$]vinylmagnesium bromide in tetrahydrofuran which was treated with *trans*-geranylacetone to give [2-$^3H_1$]nerolidol.

Formaldehyde dimedone, obtained by ozonolysis of the labelled nerolidol, contained only 0·9% of the total activity. The radioactive nerolidol therefore had 0·9% of the label at C-1 and, by difference, 99·1% of the label at C-2.

[2-$^3H_1$]Farnesyl bromide, prepared from the nerolidol by treatment with phosphorus tribromide in ether, was allowed to react with nickel carbonyl in dimethylformamide [28] to give a mixture of coupled products in 45% yield. The ^1H n.m.r. spectrum of inactive material indicated that as well as primary/primary coupling, some tertiary/primary coupling had occurred. *trans*-[11,14-$^3H_2$]Squalene was separated from the mixture as the thiourea clathrate by dilution with approximately 25% by weight of inactive *trans*-squalene. The specific activity of the diluted all-*trans*-[11,14-$^3H_2$]squalene indicated that the all-*trans*-isomer constituted approximately 25% of the hydrocarbons formed by the coupling

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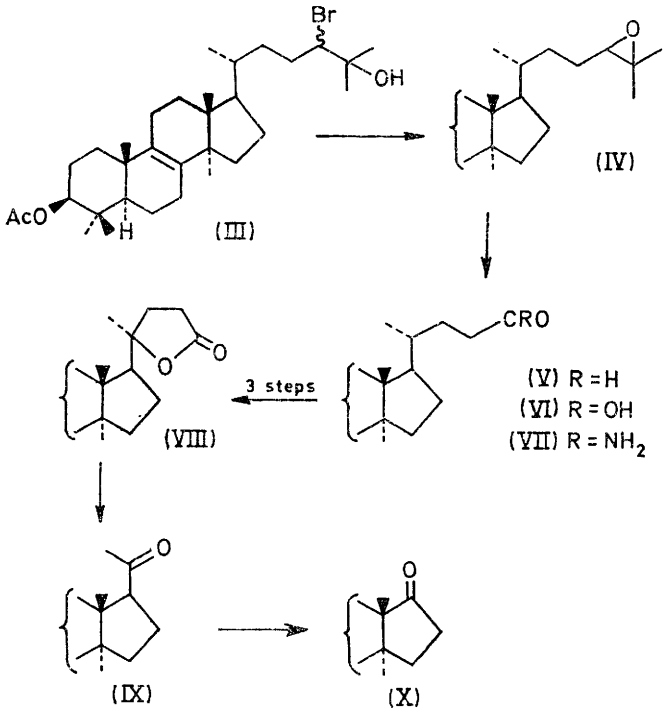
reaction. Well established procedures[3] led to *trans*-2,3-epoxy[11,14-$^3H_2$]squalene.

Incubation of this precursor with a cell-free system of *Saccharomyces cerevisiae*,[9] dilution with lanosterol, and benzoylation of the non-saponifiable extract gave radioactive lanosterol benzoate. The incorporation into lanosterol, not allowing for the probable utilisation of one enantiomer of the epoxide, was 8·5%.

Reduction of the benzoate with lithium aluminium hydride, followed by acetylation, gave lanosteryl acetate which was degraded as illustrated in Scheme 2.



SCHEME 2

Treatment of lanosteryl acetate with *N*-bromosuccinimide in aqueous tetrahydrofuran at 0° afforded the bromohydrin (III) which was immediately cyclised to a mixture of epoxides (IV) with potassium carbonate in acetone–methanol. Oxidative cleavage with periodic acid[31] gave the aldehyde (V) which was oxidised by an adaptation of the procedure described by Brown.[32] The acid chloride, obtained by treatment of the acid (VI) with either thionyl chloride or oxalyl chloride in benzene, was converted into the amide (VII).

Photolysis of (VII) with iodine and lead tetra-acetate[33] afforded the desired lactone (VIII) as a mixture of C(20) epimers, together with the hexanor-ketone (IX). The latter is probably formed by further oxidation of the γ-imino-lactone[33] intermediate. Also formed in the reaction were minor amounts of the $\Delta^{7,9(11)}$-analogues of both the major products. The hexanor-ketone was degraded directly to the 17-ketone (X) by autoxidation with oxygen and potassium t-butoxide in t-butyl alcohol,[34] and cleavage[35] of the intermediate hydroperoxide.

The specific activities of the degradation products are given in Table 1. The γ-lactone, in which the hydrogen originally attached to C(20) of lanosterol, had been removed, retained 99% and the 17-ketone (X) only 5%, of the original activity. The hexanor-ketone isolated from the amide photolysis after alkaline hydrolysis had suffered almost complete loss of activity (8% of starting amide). The remaining label present at C(17) was removed by conversion into (X). The results show that 94% of the original activity was present at C(17) in accordance with a biosynthetic path involving two 1,2-hydrogen shifts as originally proposed[10,11] or consecutive 1,2- and 1,3-shifts as proposed by van Tamelen.[15] The path outlined in Scheme 1 is eliminated. This result is in agreement with that recently reported by Richards and his co-workers[36] during the preparation of the present paper. They showed that, in a rat liver homogenate, label from C(14) of squalene labelled with tritium at C(9), C(11), C(14), and C(16) and in the methyl groups attached to C(10) and C(15) was transferred only to C(17) of lanosterol.

Goodwin and his co-workers[37,38] have demonstrated that β-amyrin (XI) biosynthesised from [2-^{14}C,(4*R*),-4-$^3H_1$]mevalonic acid is labelled at the positions predicted by the Ruzicka hypothesis.[11] An alternative mechanism also consistent with their results is outlined in Scheme 3. Incorporation of 2,3-epoxy[11,14-$^3H_2$]-squalene would distinguish between these. The former mechanism predicts that the label would appear at C(18), and Scheme 3 at C(19), α or β depending on whether the terminal stages are a 1,3-shift or two 1,2-shifts respectively. In both schemes label will also appear at C-9.

A 11,14-*cis–trans* isomer mixture of 2,3-epoxy-[11,14-$^3H_2$]squalenes, prepared[28] from [2-$^3H_1$]farnesyl bromide in which 5·9% of the activity was located at C(1) was incubated with a homogenate from *Pisum sativum*.[39] Dilution with β-amyrin and recovery of the benzoylated triterpenoid fraction gave radioactive β-amyrin benzoate. Treatment with selenium dioxide in acetic acid under reflux for 1 hr. gave β-amyradienyl-II benzoate (XII)[38,40] retaining 47·9% of the original

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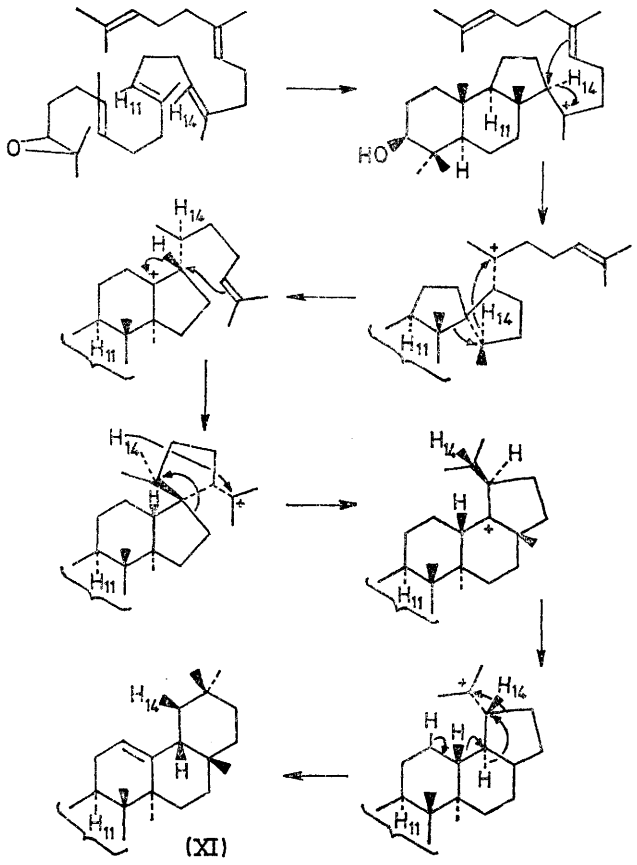


SCHEME 3

activity (Table 2). Further treatment of the dienyl benzoate with selenium dioxide in acetic acid at reflux, for 18 hr.[41] gave the diene-dione (XIII) with an overall

TABLE 1

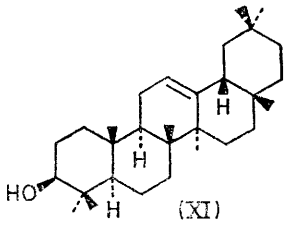
Compound	Specific activity (decomp./min./mmole)	Relative activity (%)
Lanosteryl benzoate	$8{\cdot}9 \times 10^4$	101
Amide (VII)	$8{\cdot}8 \times 10^4$	100
Lactone (VIII)	$8{\cdot}76 \times 10^4$	99
17-Ketone (X)	$4{\cdot}3 \times 10^3$	5

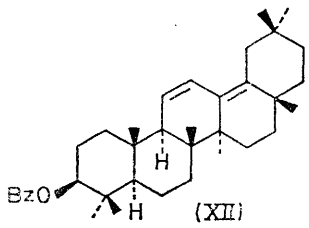
TABLE 2

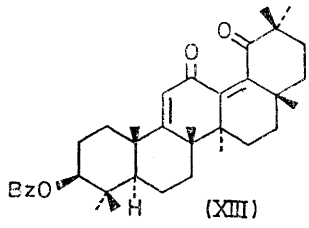
Compound	Observed relative activity	Theoretical relative activity
β-Amyrin benzoate	100	100
Diene (XII)	47·9	50·6
Dienedione (XII)	3·9	1·5

loss of 96·3% of label. These results are entirely in accord with the Ruzicka hypothesis, and eliminate Scheme 3.

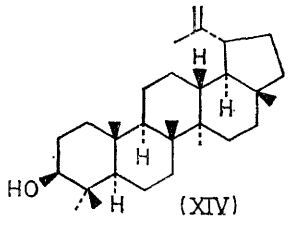
Recently Cornforth [42] has emphasised that X-group mechanisms could play a part in the formation of triterpenes. When applied to β-amyrin biogenesis this would involve the formation of intermediates between 2,3-epoxysqualene and β-amyrin which have hitherto not been demonstrated. The following experiments were carried out to test Cornforth's hypothesis assuming that the X-group could be OH.

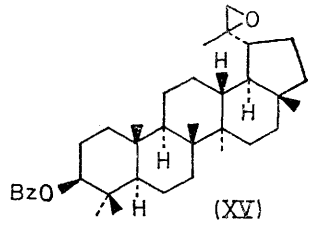


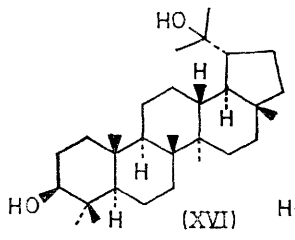


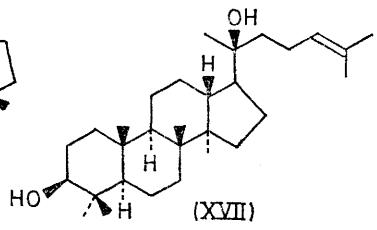


Lupeol (XIV) was oxidised to the C(3) ketone with chromium trioxide and [2-$^3H_2$]lupen-3-one prepared by base-catalysed exchange with tritiated t-butyl alcohol and potassium t-butoxide. Reduction with lithium aluminium hydride gave [2-$^3H_2$]lupeol, which was benzoylated. The [2-$^3H_2$]-mixed epoxides (XV) were prepared with monoperphthalic acid and reduced with









lithium aluminium hydride to give [2-$^3H_2$]lupan-3β,-20-diol (XVI). [2-$^3H_2$]Dammarendiol II (XVII) was similarly prepared by tritiation of the 3-keto-derivative, dipterocarpol.*

When [2-$^3H_2$]lupan-3β,20-diol (XVI) and [2-$^3H_2$]-dammarenediol II (XVII) were incubated with pea homogenate no incorporation into β-amyrin could be detected. A trace of radioactivity could be detected in the β-amyrin from [2-$^3H_2$]lupeol (XIV). These results suggest that if the lupane and dammarane

* Kindly supplied by Professor G. Ourisson.

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nuclei are formed in the conversion from 2,3-epoxysqualene into β-amyrin, they do not exist as hydroxy-quenched intermediates.

EXPERIMENTAL

M.p.s were determined on a Kofler hot stage apparatus. Rotations and, unless otherwise stated, i.r. spectra were measured in chloroform solution. Radioactivity was assayed in a Beckmann or Nuclear Enterprises NE 8310 Scintillation Counter using Nuclear Enterprises type NE 213 liquid scintillator. When unspecified, p.l.c. refers to preparative-layer chromatography on 1 mm. silica G.F. plates.

[1,4-$^3H_4$]*Tetramethylene-1,4-bistriphenylphosphonium Dibromide*.—The inactive salt was tritiated by a method previously described.[27] The recovered material had m.p. 297—299° (lit.,[43] m.p. 290°).

Preparation of [11,14-$^3H_2$]*Squalene from Geranylacetone and* [1,4-$^3H_4$]*Tetramethylene-1,4-bistriphenylphosphonium Dibromide*.—To [1,4-$^3H_4$]tetramethylene-1,4-bistriphenylphosphonium dibromide (1·61 g.) in dry t-butyl alcohol (40 ml.) at 40°, under nitrogen, was added potassium t-butoxide (556 mg.) and the whole was stirred during 7 hr. *trans*-Geranylacetone [23–25] (937 mg.) in t-butyl alcohol (5 ml.) was added and stirring was continued for a further 24 hr. at 40°. The reaction was quenched with water and continuously extracted with ether for 16 hr. The extract was filtered through grade III alumina (60 g.) with light petroleum. The first 100-ml. of filtrate contained all the isomeric squalenes (556 mg., activity $3{\cdot}95 \times 10^8$ decomp./min./mmole). Unchanged *trans*-geranylacetone (118·5 mg.) was removed with benzene–light petroleum (1 : 9). Conversion of the ketone into the semicarbazone derivative and purification by p.l.c. [elution with acetone–chloroform (1 : 4), R_F *ca.* 0·50] gave a specific activity of $6{\cdot}47 \times 10^7$ decomp./min./mmole., m.p. 97—98° (lit.,[44] m.p. 96—97°). This indicates a maximum scrambling of 32·8% in the squalene.

[1-$^3H_2$]*Nitroethane*.—Nitroethane (50 ml.) and tritiated water (12·5 ml.; 2·5 Ci) to which sodium (0·1 mg.) had been added, were stirred at 90° during 18 hr. The mixture was cooled and ether (40 ml.) was added to it; the residual tritiated water was removed. The ether was fractionally distilled to b.p. 40°, and the residual tritiated nitroethane was subjected to further tritiation as above. Dried (Na_2SO_4)[1-$^3H_2$]nitroethane (*ca.* 50 ml.) was recovered by distillation.

[1-$^3H_1$]*Acetaldehyde*.—[1-$^3H_2$]Nitroethane (*ca.* 50 ml.) was dissolved in water (500 ml.) containing sodium hydroxide (32 g.) and added dropwise at 25° to vigorously stirred sulphuric acid [from conc. sulphuric acid (100 ml.) and water (640 ml.)], during ¾ hr. After a further 1 hr. the temperature was raised and the distillate (100 ml.) b.p. >98° was collected in a receiver at 0°. Fractional distillation gave [1-$^3H_1$]acetaldehyde (28 ml.) b.p. <25°.

1,1-*Dibromo*[1-$^3H_1$]*ethane*.—To preformed phosphorus trichloride dibromide from bromine (54·6 ml.) and phosphorus trichloride (67·2 ml.), in light petroleum (b.p. 30—40°) at 0° was added dropwise during ½ hr. [1-$^3H_1$]acetaldehyde (28 ml.) with stirring. After a further 2 hr. at 0°, ice–water (400 ml.) was added dropwise and the mixture was stirred overnight at 25°. The light petroleum layer was separated off and the aqueous phase was extracted with ether. The extracts were combined, washed with water, and dried (Na_2SO_4). The light petroleum and ether were distilled to b.p. 57°. The residue was transferred to a smaller distillation apparatus and fractionated. 1,1-Dibromo[1-$^3H_1$]ethane (30·6 g.) had b.p. 100—104°. Non-radioactive material showed τ 4·12 (1H, q, *J* 7 Hz), 7·50 (3H, d, *J* 7 Hz).

[1-$^3H_1$]*Vinyl Bromide*.—To a stirred solution of sodium (2·01 g.) in butyldigol (87 g.) at 20° was added dropwise 1,1-dibromo[1-$^3H_1$]ethane (16·5 g.) under nitrogen. After 1 hr., the temperature was slowly raised to 80° and the [1-$^3H_1$]vinyl bromide (3 ml.) was distilled through a fractionating column and collected in a trap at −70°.

[2-$^3H_1$]*Nerolidol*.—To magnesium turnings (600 mg.) in dry tetrahydrofuran (5 ml.) was added dropwise to maintain reflux, [1-$^3H_1$]vinyl bromide (3 ml.) under nitrogen. When the reaction was complete the temperature was lowered to 0° and geranylacetone (4·5 g.) in tetrahydrofuran (5 ml.) was added dropwise. After 2 hr. saturated ammonium chloride was added and the stirring was continued for 2 hr. The reaction mixture was poured into water, and extracted with methylene chloride to give an oil (5·0 g.). Elution on grade III alumina (400 g.) with increasing portions of benzene in light petroleum afforded pure [2-$^3H_1$]nerolidol (3·3 g. activity $1{\cdot}8 \times 10^8$ decomp./min./mmole).

Ozonolysis of [2-$^3H_1$]*Nerolidol*.—[2-$^3H_1$]Nerolidol (115·9 mg.), in chloroform (10 ml.) at −40° was ozonised until a persistent blue colour appeared. The solvent was removed *in vacuo* and the ozonide was treated with zinc dust (300 mg.) and acetic acid (0·5 ml.) and water (3 ml.) at 60—70° for 2 hr. Distillation into a saturated aqueous solution of dimedone afforded formaldehyde dimedone derivative which was filtered off and purified by p.l.c. with benzene–ethyl acetate (5 : 1). The band R_F *ca.* 0·65 was crystallised from ethanol to give formaldehyde dimedone as needles of specific activity $2{\cdot}1 \times 10^6$ decomp./min./mmole.

[2-$^3H_1$]*Farnesyl Bromide Isomers*.—To a stirred solution of [2-$^3H_1$]nerolidol (3·2 g.) in ether (6·2 ml.) at 0° was added phosphorus tribromide (4·9 g.) during 15 min. under nitrogen. After 2·5 hr. the reaction mixture was poured onto ice and extracted with ether. The ether extract was washed with 5% sodium hydrogen carbonate solution and water and dried (Na_2SO_4). Removal of solvent gave a mixture of [2-$^3H_1$]farnesyl bromide isomers (4·6 g.). This mixture was used without further purification.

[11,14-$^3H_2$]*Squalene Isomers*.—To [2-$^3H_1$]farnesyl bromide isomers (4·6 g.) in dimethylformamide (15 ml.) at 25° was added nickel carbonyl (3·0 ml.) under nitrogen in a glove bag. After evolution of carbon monoxide had ceased (15 min.) the blood red solution was heated at 50° for 30 min. The green solution was cooled and carbon monoxide was bubbled through it for 2 hr. (The volatile nickel residues were trapped by passing the exit gases through conc. nitric acid). The reaction mixture was poured into water, and extracted with ether to give an oil (3·4 g.). Filtration through grade III alumina (200 g.) with light petroleum (b.p. 40—60°) (250 ml.) gave 10,14-*cis*,*trans*-isomers of [11,14-$^3H_2$]squalene (1·5 g. activity, $4{\cdot}5 \times 10^8$ decomp./min./mmole.).

Separation of All-trans-*Squalene*.—To a solution of thiourea (4·65 g.) in methanol (33·7 ml.) at 50° was added

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a mixture of tritiated squalene isomers (438 mg.) and inactive authentic *trans*-squalene (94 mg.). After being efficiently stirred for 1 hr., the solution was allowed to cool slowly to room temperature, a seed crystal of squalene thiourea clathrate was added, and the stirring was continued overnight. The crystals were washed with light petroleum and the *trans*-squalene (160 mg.) (activity $2\cdot3 \times 10^8$ decomp./min./mmole.) recovered by decomposition of the clathrate with water. This active material, after dilution with inactive *trans*-squalene (45 mg.), was added to the remainder of the tritiated squalene isomers (1·1 g.) and crystallisation of the all-*trans*-squalene clathrate was repeated. The specific activity of the recycled *trans*-[11,14-$^3H_2$]squalene isolated was $3\cdot3 \times 10^8$ decomp./min./mmole.

2,3-*Epoxy*-trans-[11,14-$^3H_2$]*squalene*.—To [11,14-$^3H_2$]-*trans*-squalene (396 mg.) in tetrahydrofuran (26·5 ml.) and water (9·5 ml.) at 4°, under nitrogen, was added *N*-bromosuccinimide (192 mg.) during 10 min. After 1·5 hr. at 0°, the reaction mixture was poured onto ice and extracted with ether. The product was chromatographed on a silica gel column. (25 g., SiO_2 containing 15% water). Light petroleum eluted unchanged squalene. Benzene–light petroleum (7 : 3) eluted the 3-bromo-2-hydroxy[11,14-$^3H_2$]-squalene (130 mg.). The labelled bromohydrin in methanol (3·0 ml.) was stirred with anhydrous potassium carbonate (150 mg.) under nitrogen for 1 hr. at 25°. The reaction mixture was poured into water and extracted with ether. The extract was dried, and the solvent was removed to give 2,3-epoxy-*trans*-[11,14-$^3H_2$]squalene (90 mg., activity $3\cdot3 \times 10^8$ decomp./min./mmole.).

3β-*Acetoxy*-24ξ,25-*epoxylanost*-8-*ene* (IV).—To lanosteryl acetate (6·39 g.) in tetrahydrofuran (105 ml.) and water (25 ml.) at 0°, was added, with stirring, *N*-bromosuccinimide (2·73 g.) during 10 min. The whole was stirred during 2 hr., and then poured into water. Extraction with ether gave crude 3β-acetoxy-24ξ-bromo-25-hydroxylanost-8-ene (III) (7·14 g.). This, in acetone (150 ml.) and methanol (50 ml.), was stirred with powdered anhydrous potassium carbonate (4·5 g.) at 25° for 1½ hr. The reaction mixture was diluted with water and extracted with ether. The extract was washed with water, dried, and solvent was removed. Crystallisation of the product from chloroform–methanol gave 3β-acetoxy-24,25-epoxylanost-8-ene (IV) (5·63 g.) as needles, m.p. 172—190° (lit.,[27] m.p. isomer I 196—198°, isomer II 144—146°); $\nu_{max.}$ 1735s, 1245s, and 1230s cm.$^{-1}$.

3β-*Acetoxy*-25,26,27-*trisnorlanost*-8-*en*-24-*al* (V).—The epoxide (IV) (13·6 g.) was added to a stirred solution of periodic acid (7·70 g.) in dry ether (2 l.) at 25°. After 10 min., water was added and the ether layer was separated, washed with water, and dried. Removal of the solvent gave 3β-acetoxy-25,26,27-trisnorlanost-8-en-24-al (V) (12·4 g.) which crystallised from acetone as fine needles, m.p. 144—147° $[\alpha]_D^{25}$ +58·5° (*c*, 2·4) (lit.,[45] m.p. 144—146° $[\alpha]_D$ +58°); $\nu_{max.}$ (CCl_4) 1720s, 1270s, 2700w, and 1695s cm.$^{-1}$.

3β-*Acetoxy*-25,26,27-*trisnorlanost*-8-*en*-24-*oic acid* (VI).—The aldehyde (V) (12·4 g.) in ether (460 ml.) was vigorously stirred with Browns reagent [32] (154 ml.) diluted with water (308 ml.) during 3·5 hr. at 25°. The ether layer was separated and the aqueous phase was extracted with ether. The extracts were combined, washed with water, dried, and the solvent was removed to give 3β-acetoxy-25,26,27-trisnorlanost-8-en-24-oic acid (VI) (12·8 g.), $\nu_{max.}$ 3600—3500br, 1700s, 1720s, 1240s cm.$^{-1}$. A small portion of the acid was converted into the methyl ester (with excess diazomethane in ether), m.p. 171—173° (plates from chloroform–methanol) $[\alpha]_D^{25}$ +55° (*c*, 1·7) (lit.,[46] m.p. 174—176°, $[\alpha]_D$ +58°).

3β-*Acetoxy*-25,26,27-*trisnorlanost*-8-*en*-24-*carboxamide* (VII).—The acid (VI) (9·0 g.) in dry benzene (400 ml.) and dry dimethylformamide (14·6 g.) was vigorously stirred with thionyl chloride (23·8 g.) at 25° during 2·5 hr. The whole was cooled to 0° and excess of concentrated ammonia solution was quickly added. The precipitate was filtered off and washed with water. Crystallisation from ether afforded 3β-*acetoxy*-25,26,27-*trisnorlanost*-8-*ene*-24-*carboxamide* (VII) (8·8 g.), m.p. 228—230°, $[\alpha]_D^{25}$ +58° (*c*, 1·7); $\nu_{max.}$ 3380m, 3200m, 1650s, 1725s, and 1260s cm.$^{-1}$ (Found: C, 76·1; H, 10·2. $C_{29}H_{47}NO_3$ requires C, 76·1; H, 10·35%).

Photolysis of 3β-*Acetoxy*-25,26,27-*trisnorlanost*-8-*ene*-24-*carboxamide* (VII).—The amide (200 mg.), iodine (330 mg.), and lead tetra-acetate (580 mg.) in chlorobenzene (10 ml.) and chloroform (ethanol-free, 5 ml.) was photolysed for 2 hr. at 15° under dry nitrogen with a high-pressure mercury lamp (125 w). The reaction mixture was filtered and the inorganic residue was washed with chloroform. The extract was washed with 5% aqueous sodium dithionite and water. Removal of the solvent gave a gum (330 mg.) which was heated under reflux with potassium hydroxide (1·5 g.) in ethanol (20 ml.) and water (3 ml.) during 3 hr. The whole was poured into water and was extracted with chloroform to give a neutral fraction (30·3 mg.). Acidification with 2N-sulphuric acid and continuous ether extraction (24 hr.) afforded an acidic fraction. The neutral fraction was reacetylated and purified by t.l.c. by elution with chloroform. The band R_F *ca.* 0·57 afforded 3β-acetoxyhexanorlanost-8-en-20-one (IX) together with some 3β-acetoxyhexanorlanost-7,9(11)-dien-20-one (29·0 mg. total), m.p. 173—176° (needles from benzene–light petroleum), $[\alpha]_D^{23}$ +122° (*c*, 0·4) (lit.,[47] m.p. 166—167° $[\alpha]_D$ +107°); $\nu_{max.}$ (CCl_4) 1740s, 1240s, 1710s, and 1025s cm.$^{-1}$; τ 7·91 (3H, s, 21-CH_3) (Found: C, 77·9; H, 9·8; $C_{26}H_{40}O_3$ requires C, 77·9; H, 10·0%).

The acidic fraction was reacetylated and eluted on p.l.c. with chloroform. The band R_F *ca.* 0·50 afforded 3β-*acetoxy*-25,26,27-*trisnorlanost*-8-*en*-20,24-*olide* (VIII) (80·2 mg.). After three crystallisations from chloroform–methanol a mixture of needles and plates was obtained, m.p. 210—215° (needles), 221—232° (plates), $[\alpha]_D^{24}$ +101° (*c*, 0·6); $\nu_{max.}$ 1750s (γ-lactone), 1725s, 1260s (acetate), 980s cm.$^{-1}$; $\nu_{max.}$ (EtOH) 206·5 nm. (ε 8425); τ 8·60, 8·46 (ratio 2 : 1, 3H, 21-CH_3, mixed epimers) (Found: C, 76·3; H, 9·5. $C_{29}H_{44}O_4$ requires C, 76·3; H, 9·7%).

3β-*Hydroxyoctanorlanost*-8-*en*-17-*one* (X).—3β-Acetoxyhexanorlanost-8-en-20-one (IX) (34·3 mg.), in t-butyl alcohol (5 ml.), in which potassium (250 mg.) had been dissolved was shaken in an atmosphere of oxygen for 1 hr. The flask was flushed with nitrogen, and heated on a steam-bath for 20 min. Half the solvent was removed under vacuum, water was added, and the whole was extracted

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with ether. 3β-Hydroxyoctanorlanost-8-en-17-one contaminated with 25% 3β-hydroxyoctanorlanost-7,9(11)-dien-17-one (23 mg.) was obtained. It had m.p. 172—192° (from ether–light petroleum) [Found: *M* (precision mass. spec.), 330·2559(7). $C_{22}H_{34}O_2$ requires 330·2558(6)].

General Procedure of Feedings to Cell-free Saccharomyces cerevisiae.—To 2,3-epoxy-*trans*[11,14-$^3H_2$]squalene [2·71 mg., 3·3 × 10^8 decomp./min./mmole., containing 0·9% label at C(12), C(13)] and Tween 80 (80—100 mg.) in acetone (1 ml.) was added pH 6·5 phosphate buffer (5 ml.) and the whole vigorously shaken to give an emulsion. This was added to a cell-free preparation of *Saccharomyces cerevisiae* [48] (*ca.* 120 ml.,) and the vessel was washed with pH 6·5 phosphate buffer (2 × 1 ml.). The whole was shaken in a lightly plugged (cotton wool) conical flask in an incubator at 30° during 13 hr. After addition of carrier lanosterol (100·5 mg.) the mixture was heated under reflux (under N_2) with potassium hydroxide (25 g.) in methanol (150 ml.). The reaction mixture was poured into water and exhaustively extracted with ether. Lanosterol was separated as the benzoate (34 mg., activity 8·7 × 10^5 decomp./min./mmole) by p.l.c. on 20% $AgNO_3$–silica gel by elution with benzene–light petroleum (4 : 6).

Degradation of Biosynthetically Labelled Lanosterol.—The isolated lanosterol benzoate was debenzoylated with lithium aluminium hydride and converted into the acetate which was degraded, as described above, to the ketone (1X). The activities of the degraded compounds are given in Table 1. The ketone so obtained contained (by u.v. analysis) 25% of a 7,9(11)-diene analogue. This contaminant does not affect the conclusions derived from the observed radioactivities.

[2-$^3H_2$]*Lupen-3-one and* [2-$^3H_2$]*Dipterocarpol.*—These were prepared by a method described previously.[27]

[2-$^3H_2$]*Dammar-*3β,20β-*dihydroxy-24-ene* (XVII).—Lithium aluminium hydride reduction of [2-$^3H_2$]dipterocarpol in tetrahydrofuran gave the diol (XVII), m.p. 132·5—133·5°, activity 6·3 × 10^9 decomp./min./mmole. Inactive material had m.p. 132·5—133·5° (needles, methanol solvate from chloroform–methanol) $[\alpha]_D^{22}$ +29·5° (lit.,[49] m.p. 131—133°, $[\alpha]_D$ +33°).

[2-$^3H_2$]*Lupeol* (XIV).—Reduction of [2-$^3H_2$]lupeone as above gave [2-$^3H_2$]lupeol (XIV), m.p. 212—213°, activity 4·8 × 10^9 decomp./min./mmole. Inactive lupeol had m.p. 212—213°, $[\alpha]_D^{25}$ +27° (lit.,[50] m.p. 212—216°, $[\alpha]_D$ +28°).

[2-$^3H_2$]*Lupan-*3β-20-*diol* (XVI).—[2-$^3H_2$]*Lupeol* (140 mg.) was treated with benzoyl chloride in pyridine overnight to give [2-$^3H_2$]lupenyl benzoate (150 mg.). This was treated with an excess of monoperphthalic acid in ether (10 ml.) at 23° for 16 hr. The ether solution was washed with 2% sodium hydrogen carbonate solution and water and dried (Na_2SO_4). The product was fractionated by p.l.c. on elution with chloroform–benzene (30 : 70). The band R_F *ca.* 0·4 gave mixed [2-$^3H_2$]lupenyl benzoate epoxides (XV) (141 mg.). The inactive epoxide mixture had m.p. 200—210°, $[\alpha]_D^{26}$ +36·5° $\nu_{max.}$ 1240s cm.$^{-1}$ (Nujol) and was not further characterised. The labelled epoxides (141 mg.) were heated under reflux for 16 hr. with an excess of lithium aluminium hydride in ether. The reaction mixture was decomposed with saturated Rochelle salt solution and extracted into ether. P.l.c. of the product, on elution with methanol–chloroform (4 : 96), gave [2-$^3H_2$]lupan-3β-20-diol (XVI), R_F *ca.* 0·50, (121 mg.), m.p. 238—240° (needles from acetone), activity 1·10 × 10^7 decomp./min./mg. Inactive diol had m.p. 237—240°, $[\alpha]_D^{23}$ +4° (*c* 3·2) (lit.,[50] m.p. 238·5—241°, $[\alpha]_D$ +4°); $\nu_{max.}$ 3580s, 1040s, 1030s cm.$^{-1}$.

Feeding Technique with Pisum sativum *and Work-up Procedure.*—Pea 'supernatant' (25 ml.) containing $MgSO_4$,-$5H_2O$ (3 mg.) [39] was incubated and shaken at 37° during 14 hr., with an aqueous emulsion of 2,3-epoxy[11,14-$^3H_2$]-squalene isomers [70 mg., 1·34 × 10^7 decomp./min., containing 5·9% of label at C(12) and C(13)] and Tween 80. Methanol (60 ml.) containing potassium hydroxide (6 g.) was added and the whole was heated under reflux for 3 hr. with carrier β-amyrin (70 mg.). The non-saponifiable ether extract was benzoylated and the benzoates were eluted on p.l.c. (20% $AgNO_3$–silica gel) with benzene–light petroleum (3 : 7). Recovered β-amyrin benzoate (71 mg.), R_F *ca.* 0·5, was crystallised from chloroform–methanol to constant activity 1·59 × 10^5 decomp./min./mmole, m.p. 237—239°, (lit.,[50] m.p. 230—236°).

β-*Amyradienol*-II *Benzoate* (XII).—β-Amyrin benzoate (XI; 3-$PhCO_2$–) (63 mg., 1·59 × 10^5 decomp./min./mmole). selenium dioxide (90 mg.), water (0·06 ml.), and acetic acid (2·4 ml.) were heated under reflux for 1 hr. Sodium acetate (400 mg.) was added and the whole was heated under reflux for 20 min. The reaction mixture was cooled, poured into water, and extracted with ether. The extract was washed with water, 5% sodium hydrogen carbonate solution and water and was then dried. Removal of the solvent and p.l.c. on 20% $AgNO_3$–silica gel [elution with benzene–light petroleum (3 : 7)], gave β-amyradienol-II benzoate (XII) (56 mg.), R_F *ca.* 0·40, m.p. 251—253° (needles from chloroform–methanol) of activity 7·61 × 10^4 decomp./min./mmole (loss of 52·1% of label, theoretical loss = 49·4%). Inactive diene had m.p. 251·5—253°, $[\alpha]_D$ −33° (lit.,[40] m.p. 249—250°, $[\alpha]_D$ −34°); $\lambda_{max.}$ (EtOH) 251 (ε 32,400), 250 (32,400), and 260 nm. (21,700).

β-*Amyradienedionol Benzoate* (XIII).—β-Amyradienol-II benzoate (XII) (45 mg., 7·61 × 10^4 decomp./min./mmole) and selenium dioxide (67 mg.) were heated under reflux in acetic acid during 18 hr. The whole was poured into water and extracted with ether. The extract was washed with water, 5% sodium hydrogen carbonate solution and water and was then dried. The solvent was removed and the product was chromatographed on p.l.c. by elution with ethyl acetate–benzene (1 : 9). The band R_F *ca.* 0·50 (46 mg.) was removed and crystallisation from aqueous acetic acid gave β-amyradienedionol benzoate (XIII), m.p. 256—258·5° (needles) of activity 5·95 × 10^3 decomp./-min./mmole (loss of 92·2% of label, theoretical loss 96·9%). Inactive material had m.p. 256—258°, $[\alpha]_D^{25}$ −62° (*c*, 1·3), $\nu_{max.}$ (Nujol) 1715s, 1280s, 1695s, and 1650 cm.$^{-1}$; $\lambda_{max.}$ (EtOH) 228 (ε 18,650) and 276·5 nm. (13,250); τ 8·98 (6H, s), 8·92 (3H, s), 8·84 (3H, s), 8·80 (3H, s), 8·65 (6H, s), 8·60 (3H, s), and 4·05 (1H, s) (Found: C, 79·7; H, 8·7. $C_{37}H_{48}O_4$ requires C, 79·8; H, 8·7%).

We thank the S.R.C. for financial support (to G. M.).

[0/1592 *Received, September 16th,* 1970]

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