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THE BIOSYNTHESIS OF [$^{14}C_5$; $^{3}H_4$]-CHOLESTA-5,7,24-TRIEN-3β-OL AND [$^{14}C_5$; $^{3}H_4$]-5α-CHOLESTA-7,24-DIEN-3β-OL FROM (2R)- AND (2S)- [$2^{14}C$; $2^{3}H$]-MEVALONIC ACID BY A YEAST HOMOGENATE

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SUMMARY

Incubation of (2R)- and (2S)-[$2^{14}C$; $2^{3}H$]-MVA with a cell free homogenate of yeast resulted in [$^{14}C_5$; $^{3}H_4$]-cholesta-5,7,24-trien-3β-ol and [$^{14}C_5$; $^{3}H_4$]-5α-cholesta-7,24-dien-3β-ol rather than in C_{28} sterols.

INTRODUCTION

In an earlier publication [1] we described a stereochemical difference between the biosynthesis of cholesterol derivatives in a rat liver preparation and in a yeast homogenate. Described in that paper, among sterols biosynthesized from (2R)-[$2^{14}C$; $2^{3}H$]-mevalonic acid (MVA) and (2S)-[$2^{14}C$; $2^{3}H$]-MVA was a metabolite(s) obtained in significant radioactive yield which on t.l.c. moved closer to ergosterol [1]. At the time, because of lack of reference samples, the metabolite(s) was not characterized, but on hydrogenation of its acetate(s) [2] it gave 5α-cholest-7-en-3β-ol acetate I [1]. The "R"-I and "S"-I both retained four tritium atoms. We have established [3] that tritium atoms are located in the "R"-I at 1β, 15 and 26 and in the "S"-I at 1α, 7 and 26 positions respectively. These results suggested structures II and IV for the metabolite(s). Authentic samples of II and III, and IV and V were prepared [4] and used for the characterization of the product(s). Structure determination was carried out on metabolites derived from (3RS; 2R)-[$2^{14}C$; $2^{3}H$]-MVA.

Incubation of (3RS; 2R)-[$2^{14}C$; $2^{3}H$]-MVA (1·1 × 10^8 d.p.m. of ^{14}C; ^{3}H:^{14}C ratio 10·1) with the yeast

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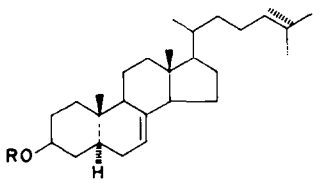
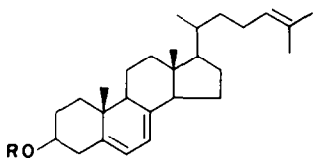
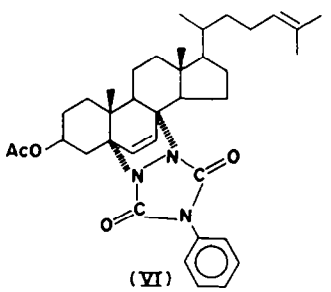


(I) R = Ac; No Δ^{24}
(II) R = H; Δ^{24}
(III) R = Ac; Δ^{24}



(IV) R = H
(V) R = Ac



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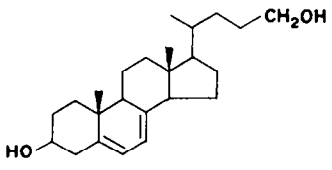
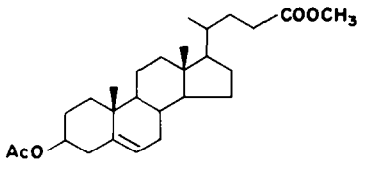


(VII)



(VIII)

Table 1. Specific activities of ^{14}C and ^{3}H:^{14}C ratios of metabolites biosynthesized from (3RS, 2R)-[$2^{14}C$, $2^{3}H$]-MVA by a yeast homogenate and their transformation products*

Compounds	Specific activity ($\times 10^5$ d.p.m. mmol of ^{14}C)	^{3}H:^{14}C ratio Isotopic	Atomic Found	Atomic Calculated
MVA benzhydrylamide		10·1		1:1
Squalene**	—	9·6		6:6
Squalene Hexachloride	3·65	9·4	5·87:6	6:6
Lanosterol	4·52	9·7	6·06:6	6:6
Cholesta-5,7,24-trien-3β-ol acetate (V)				
a from preparative glc**		7·77	4·04:5	4:5
b cocrystallization (V)	2·59	7·75	4·03:5	4:5
c recovered from (VI)	2·40	7·73	4·02:5	4:5
5α-Cholest-7-en-3β-ol acetate (I)	6·00	7·74	4·03:5	4:5
Chole-5,7-diene-3β,24-diol (VII)	0·47	7·31	3·04:4	3:4
5α-Cholesta-7,24-dien-3β-ol acetate (III)	1·22	7·78	4·05:5	4:5
5α-Cholesta-7,24-dien-3β-ol (II)	1·14	7·79	4·05:5	4:5

* Except for the compounds indicated with (**) all products were crystallized at least three times. The results are the average of counts which did not differ by more than 5%. The specific activities were measured at different dilutions. The calculation of atomic ratios is based on squalene.

homogenate gave after work-up an unsaponifiable residue ($3{\cdot}8 \times 10^7$ d.p.m. of ^{14}C) which on fractionation by t.l.c. [1] was resolved into "squalene" ($1{\cdot}29 \times 10^7$ d.p.m. of ^{14}C), "lanosterol" ($1{\cdot}07 \times 10^7$ d.p.m. of ^{14}C) and "ergosterol" ($3{\cdot}8 \times 10^6$ d.p.m. of ^{14}C) zones. The squalene and lanosterol fractions were processed in the conventional manner and counted (table).

The "ergosterol zone" ($3{\cdot}8 \times 10^6$ d.p.m. of ^{14}C) was acetylated and resolved (silica gel–silver nitrate (18%); hexane ethyl-acetate (19:1); developed 3×) into a "dienic" (8% of ^{14}C) and a "trienic" (48% of ^{14}C) fraction. The "trienic" fraction was further purified by sequential argentation t.l.c. in two systems.

An aliquot of the purified triene was diluted with a small amount of V and analyzed by preparative g.l.c. [5]. The emerging peak of V was collected and its radioactivity measured (table). No separation of radioactivity from mass was noted. Another aliquot of the "triene" ($1{\cdot}5 \times 10^4$ d.p.m. of ^{14}C) was diluted with V and crystallized to constant specific activity and constant ^{3}H:^{14}C ratio (table). Hydrogenation of the diluted V gave the expected I (table).

The remainder of the acetylated "trienic" metabolite ($6{\cdot}5 \times 10^4$ d.p.m. of ^{14}C) was diluted with V (150·2 mg) and converted [6] to the phenyl-triazoline adduct VI (117 mg). Treatment of VI (42 mg) with lithium aluminum hydride (LAH) regenerated IV which was purified and counted as V (table). Ozonolysis of VI (59 mg) and reduction of the ozonide with LAH resulted in VII which was recovered and counted. Authentic VII prepared from VIII by bromination (N-bromosuccinimide), dehydrobromination (collidine), and reduction with LAH. The presented evidence conclusively established the structure of the metabolite as IV.

The acetylated "dienic" fraction was purified by multiple argentation t.l.c. and co-crystallized with III (table). Treatment of III with LAH gave II which was crystallized to constant specific activity of ^{14}C and constant ^{3}H:^{14}C ratio (table).

The results establish the structures of the metabolites as II and IV. It is apparent that in the course of the preparation of the homogenate, the C-24 methyl transferase was impaired or destroyed. This was confirmed by the observation that incubation of S-adenosyl-L(methyl-^{14}C)-methionine ($5{\cdot}7 \times 10^7$ d.p.m. of ^{14}C) [7] with the yeast homogenate failed to produce radioactive sterols.

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