Tetrahedron Letters No.3, pp. 181-185, 1969. Pergamon Press. Printed in Great Britain.

STEREOCHEMICAL DIFFERENCES IN THE BIOSYNTHESIS OF C_{27}-Δ^7-STEROIDAL INTERMEDIATES[1]

E. Caspi and P. J. Ramm

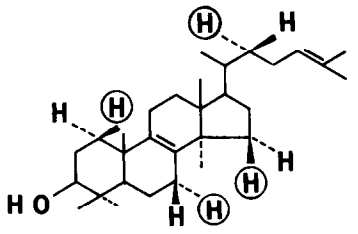
Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts

(Received in USA 1 November 1968; received in UK for publication 10 December 1968)

In a previous communication[2], we have proved that cholesterol biosynthesized from 2R-2T-2-^{14}C-mevalonic acid (T = ^{3}H) in a rat liver preparation retained five tritium atoms. We further showed that the tritium atom at C-1 has the expected β-configuration while that at C-7 had undergone an inversion from the α- to the β-configuration during the transformation from lanosterol to cholesterol. It was then apparent that this transformation proceeds *via* the elimination of a 7β-hydrogen in the formation of the Δ^7-intermediate. Ultimately the Δ^7-double bond of the later 5,7-diene intermediate is reduced by the addition of 7α- and 8β-hydrogen atoms. Later work by Gibbons, Goad and Goodwin[3] has confirmed our results.

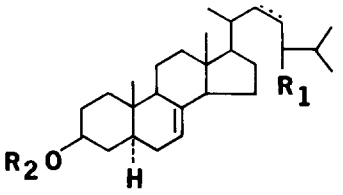
Since the biosynthesis of sterols in yeast also involves Δ^7-intermediates, we undertook to evaluate the mechanism of formation of this double bond in this organism. In view of the poor incorporation of mevalonic acid into yeast sterols, *in vivo*, we have carried out our investigations using yeast homogenates. It has been reported previously that in such systems, mevalonic acid is converted to 3β-hydroxy-ergost-5,7,22,24(28)-tetraene rather than ergosterol.[4]

Yeast cells[5] (10 g.) were suspended in 0.1M phosphate buffer, pH 7.0 (20 ml), and homogenized for 2 minutes in a Bronwill MSK apparatus with glass beads (0.5 mm. diameter, 14 g.) under adiabatic cooling conditions with carbon dioxide. 2R-2T-2-^{14}C-mevalonic acid (1.5 μmole; T/^{14}C ratio 8.7; 2.2 x 10^7 d.p.m. ^{14}C) was incubated with the total homogenate[6] for 12 hours at 26°. After conventional workup, the recovered non-saponifiable residue (7.3 x 10^6 d.p.m. ^{14}C) was fractionated chromatographically (tlc), and the fractions extensively purified to yield squalene (I) (T/^{14}C ratio 8.4, atomic ratio (a.r.) 6:6), lanosterol (II) (T/^{14}C ratio 8.4, a.r. 6:6) and a mixture of sterols with R_f values very close to that of ergosterol. On co-crystallisation of a portion of the mixed sterols with ergosterol, the radioactivity became separated from the crystalline material as expected[4]. One aliquot of the sterols was then hydrogenated over nickel sponge in dioxan (a), and another portion was first acetylated and then hydrogenated over nickel sponge in ethyl acetate (b). We have confirmed that ergosterol



II*

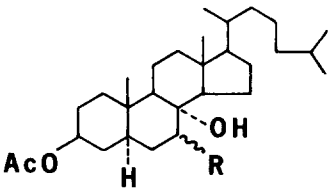
*Pertinent protons originating from C-2 of MVA are indicated; those from pro-2R are encircled.



III $R_1=CH_3$; $R_2=H$; Δ^{22}-

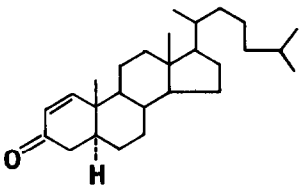
IV $R_1=CH_3$; $R_2=Ac$;

V $R_1=H$; $R_2=Ac$;



VI R=OH(α)

VII R=O



X

TABLE

Experiment	2R-2T-2^{14}C-MVA		2S-2T-2^{14}C-MVA	
Product	T/^{14}C	Atomic Ratio	T/^{14}C	Atomic Ratio
Squalene (I)	8.4	6:6	7.4	6:6
Lanosterol (II)	8.4	6:6	7.4	6:6
3β-Acetoxy-5α(H)-cholest-7-ene (V)	6.4	3.81:5	5.8	3.92:5
3β-Acetoxy-7α,8α-dihydroxy-5α(H)-cholestane (VI)	6.5	3.87:5	5.6	3.78:5
3β-Acetoxy-6α-hydroxy-5α(H)-cholestan-7-one (VII)	6.5	3.87:5	4.4	2.97:5
3β-Hydroxy-5α(H)-cholestane (VIII)	6.4	3.81:5	5.7	3.85:5
5α(H)-cholestan-3-one (IX)	6.5	3.87:5	5.8	3.92:5
5α(H)-cholest-1-en-3-one (X)	6.5	3.87:5	4.6	3.11:5

when hydrogenated under conditions (a) gives 3β-hydroxy-5α(H)-ergost-7,22-diene (III) and the acetate under conditions (b) gives 3β-acetoxy-5α(H)-ergost-7-ene (IV). Co-crystallisation of the products from hydrogenation (a) and (b) with authentic specimens of III and IV, respectively, again led to the separation of the radioactivity from the solids in each case. Since 3β-hydroxy-ergost-5,7,22,24(28)-tetraene is converted to ergosterol by hydrogenation over sponge nickel[4], it was apparent that in our experiment no radioactivity was associated with either of these sterols. However, when the product from hydrogenation (b) was co-crystallized with an authentic sample of 3β-acetoxy-5α(H)-cholest-7-ene (V), 18% of the radioactivity remained associated with the crystalline material. Clearly the biosynthesized product had retained a C-27 cholesteryl structure. The crystallized (V) showed a T/^{14}C ratio of 6.4 (a.r. 3.81:5), thereby indicating that only four tritium atoms are present in contrast to cholesterol derived from rat livers where five tritium labels are retained.

Our objective now was to locate the carbon atom from which tritium was missing, and we first focused on C-7. The diluted biosynthetic (V) was treated with osmium tetroxide in pyridine giving 3β-acetoxy-7α,8α-dihydroxy-5α(H)-cholestane[7] (VI) (T/^{14}C 6.5; a.r. 3.87:5). Oxidation of VI (chromium trioxide-pyridine)to 3β-acetoxy-8α-hydroxy-5α(H)-cholestan-7-one (VII) proceeded without loss of tritium (T/^{14}C 6.5; a.r. 3.87:5) revealing the absence of isotopic hydrogen at C-7 of (V). This observation is of considerable interest since it indicates that the 7α-hydrogen of lanosterol was removed in the formation of the 7-double bond. It may be recalled that in the biosynthesis of cholesterol in rat livers it is the 7β-hydrogen from lanosterol that is lost.

We now undertook to determine the configuration of the tritium[2] at C-1, and for this purpose the 3β-hydroxy-5α(H)-cholestane (VIII) was prepared[8]. The purified (VIII) (T/^{14}C ratio 6.4; a.r. 3.81:5) was oxidized with 8N chromic acid to 5α(H)-cholestan-3-one (IX) (T/^{14}C ratio 6.5; a.r. 3.87:5) which was dehydrogenated with dichlorodicyanobenzoquinone (DDQ) giving 5α(H)-cholest-1-en-3-one (X) (T/^{14}C ratio 6.5; a.r. 3.87:5), without loss of tritium. Since DDQ dehydrogenation of steroidal 5α-3-ketones proceeds with loss of the 1α and 2β axial hydrogens[9], then the tritium at C-1 must have the expected 1β-configuration.

In another experiment 2S-2T-2-^{14}C-mevalonic acid (0.8 μmole;T/^{14}C ratio 8.0; 1.1 x 10^7 d.p.m. ^{14}C) was incubated with a yeast homogenate. The recovered non-saponifiable residue (4.3 x 10^6 d.p.m. ^{14}C) gave, after purification as described above, squalene (I) (T/^{14}C ratio 7.4; a.r. 6:6), lanosterol (II) (T/^{14}C ratio 7.4; a.r. 6:6), and the sterol mixture. After

hydrogenation under conditions (b) and dilution with authentic material, 3β-acetoxy-5α(H)-cholest-7-ene (V) ($T/^{14}C$ ratio 5.8; a.r. 3.92:5) was obtained. Here again the biosynthesized cholesteryl derivative retained only four tritium atoms. Transformation of (V) via the 7,8-diol (VI) ($T/^{14}C$ ratio 5.6; 3.78:5) to the ketol (VII) proceeded with the loss of one tritium atom as evidenced by the $T/^{14}C$ ratio 4.4 corresponding to an atomic ratio of 2.97:5. This proves the presence of a tritium atom at C-7 in the biosynthesized C_{27} product derived from 2S-2T-$2^{14}C$-mevalonic acid and confirms the loss of the 7α-hydrogen in the transformation from lanosterol.

The approach to the stereochemistry of the tritium atom at C-1 was the same as used in the previous case. The 3β-hydroxy-5α(H)-cholestane (VIII) ($T/^{14}C$ ratio 5.7; a.r. 3.85:5) was oxidized to the ketone (IX) ($T/^{14}C$ ratio 5.8; a.r. 3.92:5) which was treated with DDQ to give 5α(H)-cholest-1-en-3-one (X) ($T/^{14}C$ ratio 4.6; a.r. 3.11:5). Clearly, in this case, a tritium is lost during the dehydrogenation step and this tritium must have the expected 1α-configuration[9].

In summary, it may be concluded that the biosynthesis of the C_{27}-cholesteryl analogues in yeast homogenates differs from that of cholesterol in rat liver homogenates in respect to the stereochemistry of formation of the Δ^7-olefin. In the transformation of lanosterol to C_{27} sterols it is the 7β-hydrogen that is eliminated in the rat liver preparation, while the 7α-hydrogen is lost in the yeast homogenate. It is of interest that the biosynthesis of poriferasterol in Ochromonas malhamensis proceeds in a manner analogous to that of cholesterol in rat livers[10].

REFERENCES

1. This work was supported by Grant P-500H from the American Cancer Society and Grant K3-16614 from the National Cancer Institute.
2. E. Caspi, J. B. Greig, P. J. Ramm and K. R. Varma, Tetrahedron Letters, 3829 (1968).
3. G. F. Gibbons, L. J. Goad and T. W. Goodwin, Chem. Comm., 1212 (1968).
4. H. Katsuki and K. Bloch, J. Biol. Chem. 242, 222 (1967).
5. Saccharomyces cerevisiae (culture 156 - a gift from Professors Carl and Gertrude Lindegren, Southern Illinois University, Carbondale, Illinois) was grown for 20 hours at 26° in medium (pH 5.0) containing, per liter, $MgSO_4$ $7H_2O$ (1 g.) KH_2PO_4 (2 g.), Bacto peptone (Difco; 6 g.), yeast extract (Difco; 4 g.), and glucose (40 g.). The harvested cells were washed twice with 0.1M phosphate buffer (pH 7.0).
6. The following cofactors were added: G.S.H. (150 mg.), A.T.P. (100 mg.), D.P.N. (50 mg.), T.P.N. (50 mg.) and glucose-6-phosphate (25 mg.).
7. L. F. Fieser and M. Fieser, Steroids, p. 241. Reinhold Publishing Co., New York (1959).
8. Under condition specified by Cornforth et al., Biochem. J. 65, 94 (1957), we have obtained in addition to the expected 3β-hydroxy-5α(H)-cholest-8(14)-ene the saturated 5α(H)-cholestanol (VIII).
9. A. B. Turner and H. J. Ringold, J. Chem. Soc.(C), 1720 (1968).
10. A. R. H. Smith, L. J. Goad and T. W. Goodwin, Chem. Comm., 926 (1968).