The Mechanism of the Alkylation Step in Ergosterol Biosynthesis

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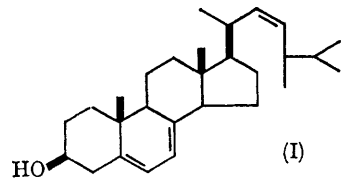
THIS Communication describes the chemical synthesis of [24-^{3}H]lanosterol and [26,27-^{14}C]-lanosterol and establishes the mechanism of the alkylation step in ergosterol (I) biosynthesis.

Previous workers have shown that the extra methyl group of the ergosterol side chain is derived from methionine, and that when ergosterol is biosynthesised in the presence of [*Me*-$^2H_3$] methionine only two of the deuterium atoms are incorporated into the ergosterol side-chain.[1] We have recently reported that 24-methylenelanosterol[2] is efficiently incorporated by the whole yeast cells into ergosterol.[2] We now report that the *C*-alkylation reaction in ergosterol biosynthesis is accompanied by the migration of a hydrogen atom from C-24. For our mechanistic study we needed [26,27-^{14}C]-lanosterol and [24-^{3}H]lanosterol and these were synthesised by the methods described below.

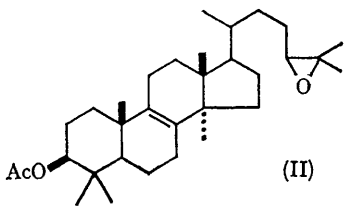
24,25-Epoxylanosteryl acetate[2,3](II), m.p. 181—182°, on treatment with aqueous perchloric acid–methanol gave the methoxy-alcohol (III), m.p. 134°. Photolysis of compound (III) in the presence of lead tetra-acetate resulted in cleavage of the C-24–C-25 bond and furnished the aldehyde (IV), m.p. 144—146°. Wittig reaction with triphenyl-[2-^{14}C]isopropylidenphosphorane followed by lithium aluminium hydride reduction then gave [26,27-^{14}C]lanosterol (V), m.p. 125—127°.

The aldehyde (IV) was reduced with tritiated sodium borohydride and the resulting alcohol oxidised without purification to furnish the tritiated aldehyde (VII). This was converted into [24-^{3}H]-lanosterol (VI) by the method described above.

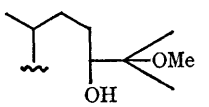
Doubly-labelled lanosterol (V + VI, $^3H/^{14}C$ = 3·2) was converted into ergosterol (I, $^3H/^{14}C$ = 3·2) by the whole cells of *Saccharomyces cerevisiae* in 14·7% yield. The derived ergosterol retained all the tritium of its precursor, lanosterol. Biosynthesised ergosterol was ozonised and 2,3-dimethylbutyraldehyde isolated as the dimedone derivative. The derivative had the same $^3H/^{14}C$ ratio, equal to 3·3, as for ergosterol. When 2,3-dimethylbutyraldehyde was equilibrated under basic conditions prior to the formation of the dimedone derivative, no tritium was lost ($^3H/^{14}C$ = 3·3). In a control experiment it was demonstrated that under the equilibration conditions the α-hydrogen of 2,3-dimethylbutyraldehyde was freely exchanged with the medium. An aqueous solution of 2,3-dimethylbutyraldehyde obtained from the ozonolysis of non-radioactive ergosterol was left at room temperature for 24 hr. in the presence of aqueous sodium hydroxide which contained tritiated water. The medium had 3·98 × 10^4 counts/minute/mg. atom of hydrogen. After neutralisation, the aldehyde was isolated as the dimedone derivative and this contained 3·87 × 10^4 counts/minute/mmole. This represents 0·98 mg. atom of tritium incorporated per mmole of the aldehyde. In a control experiment it was shown that, under the conditions of formation of the dimedone derivative, no random incorporation of label in the derivative occurred. 2,3-Dimethylbutyraldehyde ($^3H/^{14}C$ = 3·3), obtained from the biosynthetic ergosterol, was oxidised to furnish the corresponding acid ($^3H/^{14}C$ = 3·2), thus demonstrating that the tritium was not located at C-23 of ergosterol.



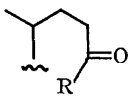
(I)



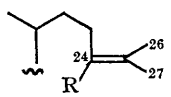
(II)



(III)

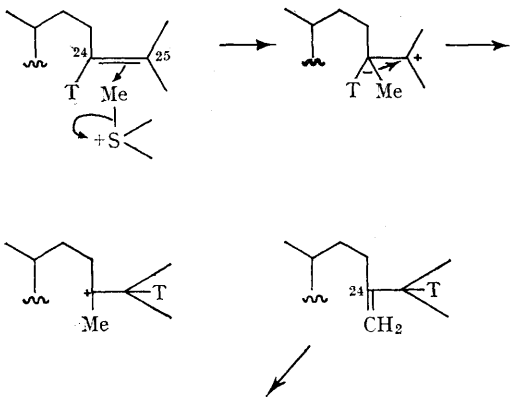


(IV) R = H
(VII) R = ^{3}H



(V) R = H; C-26, 27 = ^{14}C
(VI) R = ^{3}H

These experiments conclusively establish that in the biological conversion of lanosterol into ergosterol, although the 24-hydrogen atom of the former is retained in ergosterol, it is not located at C-24 or C-23. Mechanistic considerations suggest that the 25-hydrogen of ergosterol should be the one that was originally present at C-24 in lanosterol.



Fucosterol, Ergosterol, 24-Methylene-steroids

These results and the fact that 24-methylene-lanosterol is efficiently incorporated into ergosterol[2] establish the following sequence of events for the *C*-alkylation step in ergosterol biosynthesis.

In our view a scheme of the type indicated above is involved in the biosynthesis of ergosterol, tuberculostearic acid,[4] 24-methylene-steroids, polyporenic acids, fucosterol,[5] and related compounds.

The intermediacy of cyclopropane derivatives in the biosynthesis of methyl and methylene groups has previously been considered;[6,7] however, our results make such proposals unlikely, at least in the case of ergosterol.

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[3] We are most grateful to Dr. J. F. McGhie for giving us the experimental details for the large-scale preparation of lanosteryl acetate.
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