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The Synthesis of Labelled 24-Methylenelanosterol and its Conversion into Ergosterol

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Continuing studies on the mechanism of biosynthesis in the steroid field (Wilton, Akhtar & Munday, 1966), we now report observations pertinent to the biosynthesis of ergosterol. Recent work from several Laboratories has shown (Alexander, Gold & Schwenk, 1957; Parks, 1958; Badar, Guglielmetti & Arigoni, 1964; Castle, Blondin & Nes, 1963) that biological systems elaborate the normal steroid side chain of the type (I) into branched-chain structures of the types (II) and (III) through the participation of *S*-adenosyl-methionine (see Scheme 1).

Lederer (1964) reported that ergosterol (IV) when biosynthesized in the presence of [*Me*-D₃]-methionine, incorporated only two of the three deuterium atoms. This observation has given rise to considerable speculation, and a number of compounds of the type shown in Scheme 2 have been postulated as precursors of ergosterol (Lederer, 1964). The intermediary role, however, of these compounds (a, b, c and d; Scheme 2) in ergosterol biosynthesis has hitherto not been subjected to experimental verification.

We now report the first synthesis of 24-methylene-

Scheme 1.

Scheme 2.

[23,25- $^3\text{H}_3$]-lanosterol (XII) and its conversion into ergosterol (IV) by the whole cells of *Saccharomyces cerevisiae* (LK₂G₁₂) (see Scheme 3).

Lanosteryl acetate (V) (Lewis & McGhie, 1956), on treatment with *m*-chloroperbenzoic acid, gave the *monoepoxide* (VI, partial structure), m.p. 181–182°, which with HBr–acetic acid then gave compound (VII). The bromohydrin (VII) was oxidized without purification, to furnish the *bromo ketone* (VIII), m.p. 135°. On treatment with zinc–acetic acid, compound (VIII) gave the *acetoxo ketone* (IX), m.p. 128°, which was equilibrated with NaOH in the presence of tritiated water (0.08 ml.; 450 mc). This treatment resulted in the hydrolysis of the 3-acetate and the introduction of tritium at positions 23 and 25. The derived alcohol (X) was acetylated with acetic anhydride and pyridine to furnish the tritiated acetate (XI) (1.1×10^7 counts/100sec./mg.). The final stage of our synthesis was accomplished by treating the ketone (XI) with Wittig's triphenylphosphinemethylene reagent to yield 24-methylene[23,25- $^3\text{H}_3$]lanosterol (XII), m.p. 154° (9×10^6 counts/100sec./mg.). All new compounds (VI, VIII, IX and XII) gave satisfactory elemental analysis and had the expected nuclear-magnetic-resonance and infrared spectra.

The stage was now set for investigating the incorporation of 24-methylenelanosterol (XII) into ergosterol (IV). Previous workers (Schwenk & Alexander, 1957; Corwin, Schroeder & McCullough, 1956) have failed to achieve the incorporation of precursors other than acetate and mevalonate into ergosterol by whole yeast cells. Incorporation of squalene and lanosterol into ergosterol by using a cell-free homogenate has been reported before (Schwenk & Alexander, 1957); however, the extremely low yields obtained by previous workers made this system inadequate for the present work. We have now developed a method that allows the incorporation of radioactive lanosterol (Akhtar, Hunt & Parvey, 1966) into ergosterol by the whole cells of *Saccharomyces cerevisiae* in a dramatically improved yield. This method was used for the incorporation of 24-methylenelanosterol into ergosterol and is described below.

24-Methylenelanosterol (XII) (1 mg.; 9×10^6 counts/100sec.) was emulsified and added to a sterilized medium (8000 ml.) (Klein, Eaton & Murphy, 1954). After addition of an inoculum (1–3 mg.) the incubation was carried out at 30° under an atmosphere of N_2 for 48 hr. The N_2 supply was then replaced by O_2 and the incubation

Scheme 3.

Scheme 4.

was continued for a further 24 hr. The cells were harvested by centrifugation at 3600 rev./min. in an MSE 18 centrifuge, refluxed with ethanolic 5% (w/v) KOH (50 ml.) for 1 hr. and the non-saponifiable material was extracted with ether. The ether extract, which contained 28% (2.5×10^6 counts/100 sec.) of the original radioactivity, was fractionated by preparative thin-layer chromatography into two bands, A and B. The less polar band A contained 11.6% (1.05×10^6 counts/100 sec.) of the original radioactivity and was not characterized. The more polar band B contained 17% (1.5×10^6 counts/100 sec.) of the original radioactivity and was extracted with ether, diluted with ergosterol (1 g.) and crystallized to constant radioactivity (563 counts/100 sec./mg.; total activity $563 \times 1000 = 5.6 \times 10^5$ counts/100 sec.). This represents 6.2% incorporation of 24-methylenelanosterol into ergosterol (7 μ g. of 24-methylenelanosterol converted/g. dry wt. of yeast cells). Confirmatory evidence for the incorporation of 24-methylenelanosterol (XII) into ergosterol (IV) was provided by ozonolysis to yield 2,3-dimethylbutyraldehyde, which was isolated as the dimer derivative. This derivative retained quantitatively the radioactivity of the original ergosterol.

In conclusion we suggest the pathway shown in Scheme 4 for the biosynthesis of ergosterol side chain.

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Amino Acid Sequence in the Active Site of Phosphoglucomutase from Rabbit Muscle

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Phosphoglucomutase (α -D-glucose 1,6-diphosphate) is an enzyme widely distributed in Nature that catalyses the interconversion of glucose 1-phosphate and glucose 6-phosphate. The presence of phosphate in this enzyme was demonstrated by Jagannathan & Luck (1949), who also showed that it may readily be exchanged with phosphate in the

substrates and thus that it is probably involved in the active centre of the enzyme. Later this phosphate group was shown to be covalently bound to a serine residue (Anderson & Jollès, 1957). Milstein & Sanger (1961), using ^{32}P -labelled enzyme, showed that a unique sequence around the serine phosphate residue could be isolated from phospho-