The Stereochemistry of Hydrogen Elimination at C-6, C-22, and C-23 during Ergosterol Biosynthesis by *Aspergillus fumigatus* Fres.

BY T. BIMPSON, L. J. GOAD,* and T. W. GOODWIN

(*Department of Biochemistry, The University of Liverpool, P.O. Box* 147, *Liverpool,* L69 3BX)

SEVERAL reports have appeared concerning the stereochemistry of hydrogen elimination during double bond formation in various sterols.[1] We have described[1c,d] the stereochemistry of hydrogen elimination from C-22 and C-23 during introduction of the 22-*trans*-double bond into poriferasterol by the phytoflagellate, *Ochromonas malhamensis*. We used mevalonic acid (MVA) labelled stereospecifically with tritium at either C-2 or C-5. We now describe the results of similar studies on the biosynthesis of ergosterol by *Aspergillus fumigatus* Fres., and show an interesting and unexpected difference between this fungus and the alga, *O. malhamensis*, in the stereochemistry of hydrogen eliminations during the elaboration of the 22-double bond.

Three cultures of *A. fumigatus* were grown[2] in the presence of (3*R*)-[2-^{14}C-(5*R*)-5-$^{3}H_1$] MVA (I; 4 μc of ^{14}C); (3*R*)-[2-$_{14}$C-(2*R*)-2-$^{3}H_1$] MVA (II; 2 μc of ^{14}C), and (3*R*)-[2-^{14}C-(2*S*)-2-$^{3}H_1$] MVA (III; 2 μc of ^{14}C), respectively. After 6—7 days the cultures were harvested and the non-saponifiable lipids extracted.

Chromatography of the non-saponifiable lipid from the (3*R*)-[2-^{14}C-(5*R*)-5-$^{3}H_1$]MVA incubation gave squalene (^{3}H : ^{14}C = 11·31) and ergosterol (V), which was further purified by $AgNO_3$–silica gel t.l.c. and then crystallised to constant specific activity after the addition of carrier ergosterol (^{3}H : ^{14}C = 13·56; ^{3}H : ^{14}C atomic ratio = 5·99 : 5). A portion of the labelled ergosterol was converted by chromic acid oxidation into 5α-hydroxyergosta-7,22-diene-3,6-dione[3] (m.p. 229—237°; ^{3}H : ^{14}C = 11·55; ^{3}H : ^{14}C atomic ratio 5·10 : 5). The drop in the ^{3}H : ^{14}C ratio upon introduction of the 6-keto-group proves that tritium was present at C-6 in the ergosterol and is in agreement with previous reports that the 6α-hydrogen atom of a precursor sterol (*e.g.*, IV) is lost during 5-double bond formation.[1b] Ozonolysis of a second portion of the labelled ergosterol gave 2,3-dimethylbutyraldehyde, isolated as the dimedone derivative (m.p. 149°; ^{3}H : ^{14}C = 13·69; ^{3}H : ^{14}C atomic ratio = 1·21 : 1). The presence of a tritium atom in the 1,2-dimethylbutyraldehyde demonstrated that the 5-*pro*-*R*-hydrogen atom of MVA, which becomes the 23-*pro*-*R*-hydrogen of the sterol is retained in the terminal portion of the ergosterol side chain, presumably at C-23.[4] It therefore follows that the 23-*pro*-*S*-hydrogen atom must be eliminated[4] from an ergosterol precursor to produce the Δ^{22}-bond.

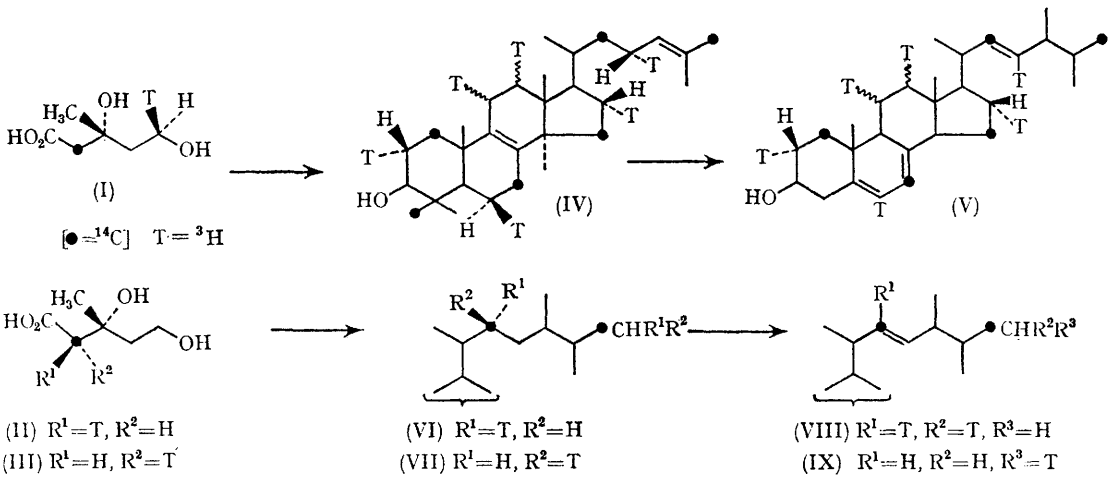
The stereochemistry of hydrogen elimination at C-22 was investigated by degradation of the ergosterol biosynthesised in the presence of 3*R*-[2-^{14}C-(2*R*)-2-$^{3}H_1$] MVA (II) and 3*R*-[2-^{14}C-(2*S*)-2-$^{3}H_1$] MVA (III), respectively. The ergosterol samples were first purified by $AgNO_3$–silica gel t.l.c., crystallised after addition of carrier ergosterol, and then degraded according to the following sequence.[5] Oppenauer oxidation gave ergosta-4,7,22-trien-3-one (m.p. 131—133°) which was isomerised with dry HCl to give ergosta-4,6,22-trien-3-one (m.p. 105—106°). Reduction of the latter produced ergosta-4,22-dien-3-one (m.p. 128—130°) which was ozonised to give 3-oxobisnor-4-cholen-22-al (m.p. 153—155°). Finally, oxidation with chromic acid gave 3-oxobisnor-4-cholenic acid (m.p. 263—265°, decomp.). The ^{3}H : ^{14}C ratios are given in the Table. In the degradation of the ergosterol biosynthesised from 3*R*-[2-^{14}C-(2*R*)-2-$^{3}H_1$] MVA, oxidation of the aldehyde to the acid resulted in a large drop in the ^{3}H : ^{14}C ratio, demonstrating the presence of a tritium atom at C-22 of the ergosterol. By contrast, there was a relatively small decrease† in the ^{3}H : ^{14}C ratio upon oxidation of the 3-oxobisnor-4-cholen-22-al derived from the

TABLE

	(3*R*)-[2-^{14}C-(2*R*)-2-$^{3}H_1$]MVA d.p.m. of ^{3}H : d.p.m. of ^{14}C	(3*R*)-[2-^{14}C-(2*S*)-2-$^{3}H_1$]MVA d.p.m. of ^{3}H : d.p.m. of ^{14}C
MVA	8·58	8·59
Ergosterol	4·06	3·20
Ergosta-4,7,22-trien-3-one	3·83	3·02
Ergosta-4,6,22-trien-3-one	3·77	3·15
Ergosta-4,22-dien-3-one	3·95	3·08
3-Oxobisnor-4-cholen-22-al	3·82	2·75
3-Oxobisnor-4-cholenic acid	2·55	2·46

† In the present work with (3*R*)-[2-^{14}C-(2*R*)-2-$^{3}H_1$]MVA and (3*R*)-[2-^{14}C-(2*S*)-2-$^{3}H_1$]MVA, loss of tritium accompanied by some randomisation of the stereospecific label was observed. A similar result has previously been noted in *O. malhamensis* incubation (ref. 1c) and ascribed to the reversibility of the isopentenyl pyrophosphate–dimethylallyl pyrophosphate enzymic isomerisation.

ergosterol labelled with 3*R*-[2-^{14}C-(2*S*)-2-$^{3}H_1$] MVA. These results, which are complementary, provide evidence that the 22-*pro*-*S*-hydrogen atom, which is derived from the 2-*pro*-*S*-hydrogen atom of MVA, is lost from a sterol precuror *malhamensis* was used, in which the 22-*pro*-*R*- and 23-*pro*-*R*-hydrogen atoms are lost during poriferasterol biosynthesis. It is interesting to speculate whether these differing stereochemical eliminations are (a) determined by the nature of the



[partial structures (VI) and (VII)] during formation of the C-22—C-23 double bond of ergosterol [(VIII) and (IX)].

The present demonstration that the 22-*pro*-S- and 23-*pro*-*S*-hydrogen atoms are eliminated during 22-double bond formation in ergosterol by *A. fumigatus* Fres. is in direct contrast to our previous observations,[1c,d] when the alga *O.* C-24 alkyl group (b) general to all fungi and algae, respectively, or (c) vary from one genus (or even species) to another.

We thank Dr F. W. Hemming for a culture of *A. fumigatus* Fres., and the S.R.C. for financial support.

(*Received, January 6th*, 1969; *Com.* 011.)

[1] (a) A. M. Paliokas and G. J. Schroepfer, *J. Biol. Chem.*, 1968, **243**, 453; (b) M. Akhtar and M. A. Parvez, *Biochem. J.*, 1968, **108**, 527; M. Akhtar and S. Marsh, *ibid.*, 1967, **102**, 462; (c) A. R. H. Smith, L. J. Goad, and T. W. Goodwin, *Chem. Comm.*, 1968, 926; (d) 1259; (e) E. Caspi, J. B. Greig, P. J. Ramm, and K. R. Varma, *Tetrahedron Letters*, 1968, 3829; (f) L. Canonica, A. Fiecchi, M. Galli Kienle, A. Scala, G. Galli, E. Grossi Paoletti, and R. Paoletti, *Steroids*, 1968, **12**, 445; (g) G. F. Gibbons, L. J. Goad, and T. W. Goodwin, *Chem. Comm.*, 1968, 1212.

[2] K. J. Stone and F. W. Hemming, *Biochem. J.*, 1967, **104**, 43.

[3] A. Burawoy, *J. Chem. Soc.*, 1937, 409.

[4] M. Akhtar, M. A. Parvez, and P. F. Hunt, *Biochem. J.*, 1968, **106**, 623.

[5] D. A. Shepherd, R. A. Donia, J. A. Campbell, B. A. Johnson, R. P. Holysz, G. Slomp, J. E. Safford, R. L. Pederson, and A. C. Ott, *J. Amer. Chem. Soc.*, 1955, **77**, 1212; M. E. Herr and F. W. Heyl., *ibid.*, 1952, **74**, 3627; F. Johnson, G. T. Newbold, and F. S. Spring, *J. Chem. Soc.*, 1954, 1302.