Phytochemistry, 1973, Vol 12, pp 1843 to 1864 Pergamon Press Printed in England

REVIEW ARTICLE

STEROLS OF THE FUNGI: DISTRIBUTION AND BIOSYNTHESIS*

JOHN D. WEETE†

Lunar Science Institute, 3303 Nasa Road 1, Houston, TX 77058, U S A

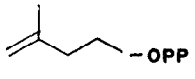
(*Revised Received* 2 *March* 1973 *Accepted* 19 *April* 1973)

Key Word Index—Fungi, sterols, distribution, biosynthesis, review

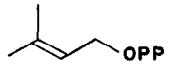
Abstract—The importance of sterols in the growth and reproduction in fungi is becoming increasingly apparent This article concerns the composition and biosynthesis of ergosterol in these organisms Comparison to plant and animal sterol formation are made

INTRODUCTION

BIOLOGICAL polymerization of the five carbon isoprene unit in the form of isopentenyl and dimethylallyl pyrophosphates (Ia, b) leads to a host of compounds such as the mono-(C_{10}), sesqui-(C_{15}), di-(C_{20}), and tri-terpenoids (C_{30}), as well as the carotenoids and rubber which contain greater than thirty carbons. The triterpenes, particularly the sterols, are biologically the most interesting and important of these compounds and are widely distributed throughout the plant and animal kingdoms. In spite of their wide distribution, however, several taxonomic groups are apparently incapable of producing the basic sterol moiety. For example, all insects, the perenosporales fungi, the purple photosynthetic bacterium *Rhodopseudomonas palustris*, the tapeworm *Spirometra mansonoides*, the annelid *Lumbricus terrestris*, the nematode *Turbatrix acete* and others are reportedly not capable of producing sterols [1] Until recently, all procaryotic organisms were also thought to lack the capacity for sterol production, but these compounds have now been identified as products of certain blue-green algae[2,3] and bacteria [4]



(Ia)



(Ib)

Cholesterol is considered the major sterol in animal tissues, C_{29} sterols such as sitosterol are predominant in photosynthetic plants, and ergosterol is the major sterol in most fungi.

* This paper was written at the Lunar Science Institute which is operated by the Universities Space Research Association under Contract No NSR 09-051-001 with the National Aeronautics and Space Administration and by NASA Contract No NAS 9-12622 (J D W) Lunar Science Institute Contribution No 139

† Present address Department of Botany and Microbiology, Auburn University, Auburn, AL 36830, U S A.

[1] CLAYTON, R. B (1971) in *Aspects of Terpenoid Chemistry and Biochemistry* (GOODWIN, T E, ed), Academic Press, New York

[2] DESOUZA, N. J and NES, W. R. (1968) *Science* **162**, 363

[3] REITZ, R C and HAMILTON, J G (1968) *Comp Biochem Physiol* **25**, 401.

[4] SCHUBERT, K, ROSE, G, WACHTEL, H, HÖRHOLD, C. and IKEKAWA, N (1968) *European J Biochem* **5**, 246

Ergosterol was first identified in extracts of ergot in 1889 by Tanret[5] and soon afterward in other fungi by Gerard [5–9] Sterols appear to be principally associated with the membrane elements of the cell, while ergosterol crystals have been observed in some fungal species Sterols are most commonly found as the free sterol (alcohol) but sterol esters may also be readily extracted from fungal tissues Sterol glycosides have also been reported and, recently, an uncharacterized 'water soluble' form of sterols has been reported from yeast

DISTRIBUTION

The sterol content of over 150 fungal species have been reported with many of these being strains of *Saccharomyces cerevisiae* and other yeast species Many of the early investigations were carried out before the development of GLC and MS and, hence, in many instances the total sterol fraction of fungi were reported as containing only ergosterol Other closely related sterols present in low concentrations were often undetected These studies did, however, reveal differences in the sterol levels of many fungal species[10–22] and demonstrated that variations in sterol concentrations could be altered by changing the media composition and culture conditions

In 1930, Bills *et al* [23] reported that 29 yeast species contained from 0 1 to 2 0% ergosterol * Since that time many species representing several taxonomic levels of the fungi have been examined[24,25] and, while it was found that some species contained no detectable sterols, the UT-139 strain of *S cerevisiae* had a total sterol content of 2 90% Generally, yeast appears to have the greatest potential for sterol production Heiduschka and Lindner[26] reported that the ergosterol content of the mycelia of fungal species ranged from 0 29 to 1 17% In a more recent study, McCorkindale and his associates[27] examined the total sterol abundances in the mycelia of 22 phycomycete species and found that their concentrations in representative members of the orders Saprolegniales, Leptomitales, and Mucorales ranged from 0 005 to 0 25% The highest sterol abundances were obtained from the aquatic Saprolegniales while no sterols were detected in the 3 species of the order Peronosporales

* In the above discussion, sterol concentrations are expressed as percentages based on the dry weight of the fungal tissue

[5] TANRET, C (1889) *Compt Rend* **108,** 98
[6] GERARD, E (1892) *Compt Rend* **114,** 1541
[7] GERARD, E (1895) *Compt Rend* **121,** 723
[8] GERARD, E (1898) *Compt Rend* **126,** 909
[9] GERARD, E (1895) *J Pharm Chem* **1,** 601
[10] MACLEAN, I S and HOFFERT, D (1928) *Biochem J* **17,** 720.
[11] PREUSS, L M , PETERSON, W H , STEENBOCK, H and FRED, E B (1931) *J Biol Chem* **90,** 369
[12] PREUSS, L M , GORCIA, H J , GREEN, H C and PETERSON, W H (1932) *Biochem Z* **246,** 401
[13] PREUSS, L M , EICHINGER, E C and PETERSON, W H (1934) *Ztschr Bakt* II **89,** 370
[14] BERNHAUER, K and PATZELT, G (1935) *Biochem Z* **280,** 388
[15] WENCK, P R , PETERSON, W H and GREEN, H C (1935) *Zentr Bakt II* **92,** 324
[16] WENCK, P R , PETERSON, W H and FRED, E B (1935) *Zentr Bakt II* **92,** 330
[17] VANGHELOVICI, M and SERBAN, F (1940) *Acadm Romana Bull Sect Sci Acad Roumaine* **22,** 287
[18] VANGEHELOVICI, M and SERBAN, II, F (1941) *Acad Romana Bull Sec Sci Acad Roumaine* **23,** 436
[19] CAVALLITO, C J (1944) *Science,* **100,** 333
[20] ELLIS, W J (1945) *Australian Coun Sci Ind Res* **18,** 314
[21] ANGELETTI, A and TAPPI, G (1947) *Gazz Chim Ital* **77,** 112
[22] DULANEY, L M , STAPLEY, E O and SIMPF, K (1954) *Appl Microbiol* **2,** 371
[23] BILLS, C E , MASSENGALE, O N and PUCKETT, P S (1930) *J Biol Chem* **87,** 259
[24] APPLETON, G S , KIEBER, R J and PAYNE, W J (1955) *Appl Microbiol* **3,** 249
[25] BLANK, F , SHORLAND, F E and JUST, G (1962) *J Invest Dermat* **39,** 91
[26] HEIDUSCHKA, A and LINDNER, H (1929) *Z Physiol Chem* **181,** 15
[27] MCCORKINDALE, N J , HUTCHINSON, S A , PURSEY, B A , SCOTT, W T and WHEELER, R (1969) *Phytochemistry* **8,** 861

(*Phytophthora infestans*, *Pythium ultimum*, and *P debaryanum*) examined Bean *et al*[28] found similar sterol concentrations in 4 additional aquatic phycomycete species (Table 1)

In spite of the recent advances in GLC the separation of closely related sterol mixtures from biological tissues remains a challenge for the analytical biochemist Sterol extracts from biological materials are often composed of triterpene mixtures containing 4,4-dimethyl, 4α-methyl, and 4-desmethyl sterols The two former triterpene classes of compounds are generally intermediates in the formation of the 4-desmethyl sterols and are often found in lesser concentrations In plant and fungal tissues the 4-desmethyl sterols are 3β-hydroxy derivatives of the parent hydrocarbon cholestane (C_{27}) as well as ergostane (C_{28}) and stigmastane (C_{29}) which differ from the C_{27} hydrocarbon by a C_1 or C_2 substitution at the C-24 position in the side-chain, respectively Derivatives of the C_{28} and C_{29} hydrocarbons are not produced by higher animal systems Individual sterols vary according to the position and degree of unsaturation in the ring and side-chain structures * It cannot be overemphasized that sterol identifications should be regarded as tentative unless combined methods such as GLC (using more than one liquid phase), GC–MS, NMR, IR, and others are used in their separation and identification

The combined works by Bergmann[30,31] and Fieser and Fieser[32] outline the studies prior to 1959 concerning the sterols produced by fungal organisms In his review of plant sterols, Bergmann[30] listed 64 fungal species for which ergosterol was shown to be one of the principal sterols Table 1 includes the trivial and systematic names of sterols which have been reported as fungal products during the past several decades

Phycomycetes

In 1969, McCorkindale and his associates[27] examined 22 phycomycete species using GLC and in some cases MS Since the only fungi examined prior to that time were those having chitinous cell walls typical of higher fungi, these investigators selected representative species of the orders Saprolegniales and Leptomitales (cellulose cell walls) in addition to species of the order Mucorales (chitin cell walls) Of the 6 sterols identified in these fungi, only cholesterol was detected in each of the 3 taxonomic groups, but it was found in only 2 of the Mucorales species Desmosterol, 24-methylcholesterol, and fucosterol were identified for the first time as fungal products in the Saprolegniales and Leptomitales species. In a similar study, Bean *et al* [28] identified cholesterol, 24-ethylcholesterol, 22-dehydrocholesterol, 24-methylcholesterol, and 24-ethyl-22-dehydrocholesterol as products of the aquatic fungi *Allomyces macrogynus*, *Rhizidiomyces apophysatus*, *Rhizophylctis rosea*, and *Hypochytrium catenoides* Only 2 or 3 of these sterols were found in each of these 4 species and no two species were qualitatively or quantitatively similar In the Mucrolaes species,[27,†] only

* Except in special instances, only the 3β-OH sterol derivatives will be considered in this article, see Turner[29] for a comprehensive review of the fungal polyisoprenoid metabolites which contain hydroxyl, carboxyl, and keto groups as well as other structural modifications

† (± *Mucor hiemalis*, ± *Phycomyces blakesleeanus* and *Absidia glauca*, *M dispersus*, *Rhizopus stolonifer*, *Mortierelea rammaniana*, *Thamnidium elegans*, *Zygorhynchus moelleri*, *Syncephalastrium racemosum*, and *Cunninghamella echinulata*)

[28] Bean, G A , Patterson, G W and Motta, J J (1973) *Comp Biochem Physiol* **43B**, 935
[29] Turner, W B (1971) in *Fungal Metabolites*, Academic Press, New York
[30] Bergmann, W (1954) *Ann Rev Plant Physiol* **4**, 383
[31] Bergmann, W (1962) in *Comparative Biochemistry* (Florkin, M and Mason, H S , eds), Vol. 111A, Academic Press, New York
[32] Fieser, L F and Fieser, M (1959) *Steroids*, Reinhold, New York

ergosterol and 22-dihydroergosterol were identified *P blakesleeanus* has also been examined by Goulston and Mercer[33] and Goulston *et al*[34] and, in addition to ergosterol, they identified ergosta-$\Delta^{5,7,24(28)}$-trienol, lanosterol, 24-methylenelanosterol, and possibly 14-demethyl-24-methylene-lanosterol The sterol components of the Mucorales species *R arrhizus* were reported to be ergosterol, ergost-Δ^7-enol (fungisterol), 5-dihydroergosterol, ergosta-$\Delta^{5\ 7,14}$-trienol (tentatively identified) and several minor components which were not identified[35] The sterol constituents of the sporangiospores from this organism were qualitatively the same as those of the mycelia and in similar relative concentrations As mentioned above, no sterols have been detected in species belonging to the *Pythium* and *Phytophthora* genera

Ascomycetes and Fungi Imperfecti

Of the class Ascomycetes, only the yeast sterols have been extensively examined and ergosterol appears to be the principal sterol of these fungi In addition, lanosterol, 4α-methyl-24-methylene-24-dihydrozymosterol, desmosterol, zymosterol, fecosterol, 24(28)-dehydroergosterol, cerevisterol (5,6-dihydroxyergosta-$\Delta^{7\ 22}$-dienol), ergosterol peroxide (5α,8α-epidioxyergosta-$\Delta^{6,22}$-dienol), episterol, 22-dehydroergosterol, 5-dihydroergosterol, and ascosterol have also been identified as yeast products by various investigators[28 36] 5-Dihydroergosterol[36 37] and fungisterol[38] have also been identified from extracts of *Claviceps purpurea*

The Fungi Imperfecti have been examined to a limited extent and considerable variability in their sterol composition has been observed Ergosterol is the most frequently encountered sterol in these fungi, but in some cases it is not the predominant sterol component For example, both ergosterol[39] and 22-dihydroergosterol[40] have been reported by different investigators as the predominant sterol of *Aspergillus flavus* Ergosterol was not detected in several species

Several deuteromycetous fungi such as the dermatophytes *Epidermophyton floccosum*, *Trichophyton interdigitale*, *Microsporum audouini*[25] also produce ergosterol as the major sterol constituent, while others contained lesser quantitites of this sterol relative to another sterol component, brassicasterol (ergosta-$\Delta^{5,22}$-dienol) In some species (*T violaceum*, *T discoides*, and *T megnini*), however, only brassicasterol was detected Other sterols identified as products of deuteromycetous fungi include cerevisterol (*Aspergillus phalloidis*),[41,42] ergosterol peroxide,[43] lanosterol, 24-methylenelophenol and possible its Δ^8 isomer (*A fumigatus*),[34] and 14-dehydroergosterol (*A niger*)[44] Cholesterol was first identified as a fungal product as the only sterol component in extracts of *Penicillium funiculosum*[45]

Ergosterol peroxide has been reported as a fungal product by several investigators, but doubt as to its authenticity as a natural product has arisen by those who claim that it is an

[33] GOULSTON, G and MERCER, E I (1969) *Phytochemistry* **8,** 1945
[34] GOULSTON, G , GOAD, L J and GOODWIN, T W (1967) *Biochem J* **102,** 15C
[35] WEETE, J D , LASETER, J L and LAWLER, G C (1973) *Arch Biochem Biophys* **155,** 411
[36] WIELAND, H and BENEND, W (1943) *Ann Chem* **554,** 1
[37] BARTON, D H R and COX, J D (1948) *J Chem Soc* 1354
[38] TANRET, C (1908) *Compt Rend* **147,** 75
[39] VACHERON, M J and MICHEL, G (1968) *Phytochemistry* **7,** 1645
[40] RAINBO, G W and BEAN, G A (1973) *Phytopathology* (abstr) in press
[41] WIELAND, H and COUTELLE, G (1941) *Ann Chem* **548,** 270
[42] ALT, G H and BARTON, D H R (1954) *J Chem Soc* 1356
[43] WIELAND, P and PRELOG, V (1947) *Helv Chim Acta* **30,** 1028
[44] BARTON, D H R and BRUUN, T (1951) *J Chem Soc* 2728
[45] CHEN, Y S and HASKINS, R H (1962) *Can J Chem* **41,** 1647

oxidation product and artifact of the experimental procedures [43,46–52] Adam *et al* [53] contends that this compound is an experimental artifact because it could only be detected in extracts of *Piploporus betulinus* and *Daedalea quercina* sporophores which had been exposed to daylight for several days and not in fresh extracts Similar results were obtained by Vacheron and Michel[39] who also identified the peroxide as well as cerevisterol which is another sterol thought to be a product of the experimental procedures They suggested that the photo-reaction sequence may proceed as follows

ergosterol → ergosterol peroxide → cerevisterol

It should also be pointed out that ergosterol peroxide has been reported as an intermediate formed in the conversion of lanosterol to ergosterol by yeast and will be discussed in more detail below

Basidiomycetes

Few mushroom fungi have been examined for their sterol composition. A crystalline material was isolated from *D. quercina* and called neosterol,[54,55] but was later identified as a mixed crystal of ergosterol (75·5%) and 5-dihydroergosterol (24·5%) [46,47] These investigators also isolated ergosterol peroxide from the same species Ergosterol has also been identified in lipid extracts from *Clavatia gigantea, Clitocybe illudens*[56] and *Tricholoma rudum*[21] by using non-specific analytical methods Milazzo[57] reported that erogsterol was produced by fourteen basidiomycete fungi which cause white and brown rot Recently, Holtz and Schisler[58] identified ergosterol, fungisterol and 22-dihydroergosterol in extracts of the common edible mushroom *Agaricus campestris* It is interesting to note that no ergosterol was detected in the bracket fungus, *Fomes applanatus* of the Polyporaceae, but (24*S*)-5α-dihydroergosterol and the corresponding ketone (24*S*)-5α-ergosta-$\Delta^{7,22}$-dien-3-one) were identified [59] In a more recent study, Strigina *et al* [60] were also unable to detect ergosterol in this organism, but they identified two sterols, (24*S*)-24-methyl-5α-cholest-7-ene-3β-ol (5α-ergost-Δ^5-enol) and (24*S*)-24-methyl-5α-cholesta-7,16-diene-3β-ol (5α-ergosta-$\Delta^{7,16}$-dienol) This is the first report of a Δ^{16} sterol in a biological system. A minor component was also detected but was not identified

The heterobasidiomycetous fungi have been examined to a more limited extent, that is, the sterol composition of uredospores of only two species of rust fungi have been reported Hougen *et al* [61] identified ergost-Δ^7-enol (fungisterol) as the only sterol component of wheat stem rust (*Puccinia graminis tritici*) spores Other investigators identified ergost-Δ^7-enol or

[46] Breivak, O N , Owades, J L and Light, R F (1954) *J Org Chem* **19,** 1734
[47] Petzoldt, K , Kuhne, M , Blanke, E , Kieslich, K and Kaspar, E (1967) *Ann Chem* **709,** 203
[48] Tanahashi, Y and Takahashi, T (1966) *Bull Chem Soc (Japan)* **39,** 848
[49] Cambie, R C and LeQuesne, P (1966) *J Chem Soc* **C,** 72
[50] Clarke, S M and McKenzie, M (1967) *Nature* **213,** 504
[51] Hamilton, J G and Castrejon, R N (1966) *Fed Am Soc Exp Biol* **25,** 221
[52] Hamilton, J G and Castrejon, R N (1966) *Federation Proc* **25,** 221
[53] Adam, H K , Cambell, I M and McCorkindale, N J (1967) *Nature* **216,** 397
[54] Weiland, H and Asano, M (1929) *Ann* **473,** 300
[55] Weiland, H and Hesse, H (1941) *Ann* **548,** 34
[56] Bentley, R , Lavate, W V and Sweeley, C C (1964) *Comp Biochem Physiol* **11,** 263
[57] Milazzo, F H (1965) *Can J Botany* **43,** 1347
[58] Holtz, R B and Schisler, L C (1972) *Lipids* **7,** 251
[59] Pettit, G R and Knight, J C (1962) *J Org Chem* **27,** 2696
[60] Strigina, L I , Elkin, Y N and Elyakov, G B (1971) *Phytochemistry* **10,** 2361
[61] Hougen, F W , Craig, B M and Ledingham, G A (1958) *Can J Microbiol* **4,** 521

TABLE 1 4,4-DIMETHYL, 4α-METHYL, AND 4-DESMETHYL STEROLS IDENTIFIED IN LIPID EXTRACTS FROM FUNGAL ORGANISMS

Compound No	Sterol (Common name) (systematic name)	Organism†	Converted to *† ergosterol
II	Lanosterol (lanosta-$\Delta^{8\ 24}$-dienol)	Yeast[80 others], *Phycomyces blakesleeanus*[33]	+[88 others]
III	24-Methylene-24-dihydrolanosterol (24-methyl-lanosta-$\Delta^{8,24(28)}$-dienol)	*P blakesleeanus*[33]	+[132]
IV	4α-Methyl-24-methylene-24-dihydrozymosterol (4α-methyl-ergosta-$\Delta^{8\ 24(28)}$-dienol)	*Saccharomyces cerevisiae*[112]	+[112]
V	24-Methylenelophenol (4α-methyl-ergosta-$\Delta^{7\ 24(28)}$-dienol)	*Aspergillus fumigatus*[33]	
VI	Fecosterol (ergosta-$\Delta^{8\ 24(28)}$-dienol)	Yeast[182 183]	
VII	— (ergosta-$\Delta^{5\ 7\ 24(28)}$-trienol)	*P blakesleeanus*[33]	
VIII	14-Dehydroergosterol (ergosta-$\Delta^{5\ 7\ 14\ 22}$-tetraenol)	*A niger*[44]	
IX	24(28)-Dehydroergosterol (ergosta-$\Delta^{5,7\ 22\ 24(28)}$-tetraenol)	Yeast[46 47]	+[184]
X	— (ergosta-$\Delta^{5,7\ 14}$-trienol)	*Rhizopus arrhizus*[35]	
XI	24-Methylenecholesterol (ergosta-$\Delta^{5\ 24(28)}$-dienol)	Aquatic phycomycetes[27]	
XII	24-Methylcholesterol (ergost-Δ^{5}-enol)	*Rhizidiomyces apophysatus*[28] *Hypochytrium catenoides*[28]	
XIII	Fungisterol (ergost-Δ^{7}-enol)	*R arrhizus*,[35] *Ganaderma applanatum* (Fomes applanatus)[60] *Puccinia graminis*[61]	+[152]
XIV	22-Dihydroergosterol (ergosta-$\Delta^{5\ 7}$-dienol)	Yeast,[182 183] Phycomycetes,[27] *Polyporus paragamenus*[185]	
XV	5-Dihydroergosterol (ergosta-$\Delta^{7\ 22}$-dienol)	Yeast,[36 37] *Claviceps purpurea*, *Daedalea quercina*,[47] *Fomes applanatus*,[58] *R arrhizus*,[35] *Polyporus pinicola*[186]	+[172]
XVI	Episterol (ergosta-$\Delta^{7\ 24(28)}$-dienol)	Yeast[33 182]	+[33]
XVII	— (ergosta-$\Delta^{7,16}$-dienol)	*G applanatum*[60]	
XVIII	Brassicasterol (ergosta-$\Delta^{5\ 22}$-dienol)	Certain dermatophytes[25]	
XIX	Ascosterol (ergosta-$\Delta^{8\ 23}$-dienol)	Yeast[182 183]	
XX	Fucosterol (24-ethylidene cholesterol) (stigmast-$\Delta^{5\ 24(28)}$-enol)	*R apophysatus*,[28] *H catenoides*,[28] other aquatic phycomycetes[27]	
XXI	Stigmasterol (stigmasta-$\Delta^{5\ 22}$-dienol)	*Pullularia pullulans*[187], *H catenoides*[28]	
XXII	— (stigmast-Δ^{7}-enol)	*Melampsora lini*[63]	
XXIII	— (stigmasta-$\Delta^{7\ 24(28)}$-dienol)	*M lini*[63]	
XXIV	— (stigmasta-$\Delta^{5,7}$-dienol)	*M lini*[63]	

TABLE 1—*continued*

Compound No	Sterol (Common name) (systematic name)	Organism†	Converted to *† ergosterol
XXV	Cholesterol (cholest-Δ^5-enol)	Certain aquatic phycomycetes[27,28] *Penicillium funiculosum*[45]	
XXVI	Desmosterol (cholesta-$\Delta^{5\ 24}$-dienol)	Yeast, certain aquatic phycomycetes[27]	
XXVII	Zymosterol (cholesta-$\Delta^{8\ 24}$-dienol)	Yeast[182]	
XXVIII	22-Dehydrocholesterol (cholesta-$\Delta^{5\ 22}$-dienol)	*Rhizophlyctis rosea*[28]	
XXIX	Ergosterol (ergosta-$\Delta^{5\ 7\ 22}$-trienol)	All fungi except certain aquatic phycomycetes and rust spores	
XXX	Ergosta-$\Delta^{8(9)\ 22}$-dienol	Yeast[155]	+[155]

* Other potential ergosterol synthesis precursors, which have not been reported as naturally occurring, can be incorporated into the $\Delta^{5,7,22}$ triene sterol by fungal systems and are included in the biosynthesis section of the text

† References are given as superscripts

[182] BARTON, D H R and COX, J D (1949) *J Chem Soc* 214
[183] WIELAND, H , RATH, F and HESSE, H (1941) *Ann Chem* **548,** 34
[184] HAMMAM, A S H (1966) Ph D Thesis, University College of Wales, Aberystwyth
[185] ALT, G H and BARTON, D H R (1952) *Chem Ind (London)* **45,** 1103
[186] HALSALL, T G and SAYER, G C (1959) *J Chem Soc* 2031
[187] MERDINGER, E , KOHN, P and MCCLAIN, R C (1968) *Can J Microbiol* **15,** 1021

stigmasterol, cholesterol, an unknown sterol, and larger amounts of stigmast-Δ^7-enol from uredospores of this same species by GLC retention times [62] Wheat leaf sterols were cholesterol, campesterol, stigmasterol, and sitosterol which are typical of most higher plant systems. Although traces of higher plant sterols may have been present in the spore materials, the lack of similarity in the sterol constituents in these two tissues suggests that sterols isolated from the uredospores are fungal products Further studies involving rust uredospores revealed that the two principal sterols of *Melamspora lini* (flax rust) are stigmast-Δ^7-enol and stigmasta-$\Delta^{7,24(28)}$-dienol, with stigmasta-$\Delta^{5\ 7}$-dienol being found in lesser relative concentrations [63] These studies also revealed that sterols were located intracellularly rather than on the spore surface It is interesting to note that ergosterol has not been identified in the rust fungi Now that the methods are being developed for the growth of axenic cultures of smut and rust fungi on synthetic media, it will be interesting to compare the sterol composition of these tissues with other fungi which are grown readily in culture

Fungisterol has been found in several fungi[64] but it is generally associated with larger amounts of ergosterol [41] There is some confusion in the literature concerning the structure of the sterol designated as fungisterol Saito[65] isolated and identified ergosta-$\Delta^{6,8\ 22}$-trienol from *Penicillium chrysogenum* and named it fungisterol, but Tanret[38] first used this trivial name in reference to ergost-Δ^7-enol isolated from ergot and it is used this way in this article

[62] NOWAK, R , KIM, W K and ROHRINGER, R (1972) *Can J Botany* **50,** 185
[63] JACKSON, L L and FREAR, D S (1968) *Phytochemistry* **7,** 654
[64] HALSALL, T G and SAYER, G C (1959) *J Chem Soc* 2031
[65] SAITO, A (1953) *J Fermentation Technol (Japan)* **31,** 328

BIOSYNTHESIS OF ERGOSTEROL

The formation of sterols by both plant and animal systems occurs through four principal stages (a) conversion of acetate to mevalonate, (b) conversion of mevalonate to squalene, (c) cyclization of squalene, and (d) conversion of the first cyclic intermediate (cycloartenol or lanosterol) to the 4-desmethyl sterol products In recent years, the biosynthesis of phytosterols and cholesterol in animals have been comprehensively reviewed,[66–75] and it appears that the same principal intermediates are involved in the formation of polyisoprenoids by representative species of these groups The decarboxylation of mevalonate, which is formed by way of activated acetate condensation and structural modification, leads to the formation of the basic five-carbon isoprene skeleton in the form of isopentenyl (Ia) and dimethylallyl (Ib) pyrophosphate Subsequent isoprenoid condensations, through the phosphorylated derivatives of geranol and farnesol, gives rise to the C_{30} hydrocarbon squalene which then undergoes cyclization to a tetracyclic 4,4-dimethyl sterol having a 3β-hydroxyl function 4-Desmethyl sterols such as cholesterol are then formed by way of 4α-methyl sterol intermediates These same principal intermediates are also involved in the biosynthesis of ergosterol by fungi The yeast have been the most extensively studied fungi in this area while our knowledge of ergosterol formation by mycelial forms is considerably limited It now appears, however, that an interest in the composition and biosynthesis of fungal sterols is rapidly evolving In the remaining paragraphs of this section, the final stages* of ergosterol formation as it is believed to occur in fungal systems are outlined and discussed while certain aspects of plant and animal sterol biosynthesis are included for comparison

First-cyclic Intermediate

As is the case with cholesterol formation in animals, the first cyclic intermediate in the formation of ergosterol by fungi is lanosterol It is now generally accepted, however, that the 9β,19-cyclopropane derivative of lanosterol, cycloartenol, is the first cyclic intermediate in the synthesis of phytosterols [76–78] Lanosterol has been identified in only a few higher plant species,[73] yet cycloartenol has not been reported as a fungal product and could not be found in yeast [79] Lanosterol was first identified from a fungal source (yeast) by Wieland and Stanley[80] who called it cryptosterol and then later by others [81–83] Since that time,

* Lanosterol → ergosterol, in the subsequent discussions, ergosterol will be considered the final 4-desmethyl product of lanosterol metabolism in fungi although some species do not produce this sterol and it is not predominant in others

[66] BLOCH, K (1952) *Harvey Lectures* **48,** 68
[67] CORNFORTH, J W (1959) *J Lipid Res* **1,** 3
[68] HEFTMANN, E and MOSELTIG, E (1960) in *Biochemistry of Steroids*, Reinhold, New York
[69] CLAYTON, R B (1965) *Quart Rev (London)* **19,** 168
[70] BLOCH, K (1965) *Science* **150,** 19
[71] POPJAK, G and CORNFORTH, J W (1966) *Biochem J* **101,** 553
[72] FRANTZ, I O and SCHROEPFFR, G J (1967) *Ann Rev Biochem* **56,** 691
[73] GOAD, L J (1967) in *Terpenoids in Plants* (PRIDHAM, J B , ed), Academic Press, New York
[74] GOODWIN, T W (1971) *Biochem J* **123,** 293
[75] SCHROEPFER, JR , G J , LUTSKY, B N , MARTIN, J A , HUNTOON, S , FOURCANS, B , LEE, W H and VERMITION, J (1972) *Proc R Soc (London)* **180,** 113
[76] VON ARDENNE, M , OSSKE, G , SCHREIBER, K , STEINFELDFR, K and TUMMLER, R (1965) *Kulturpflanze* **13,** 102
[77] BENVENISTE, P , HIRTH, L , OURISSON, G and SEANCES, C R , (1964) *Acad Agr Fr* **259,** 2284
[78] GOAD, L J and GOODWIN, T W (1966) *Biochem J* **99,** 735
[79] PONSINET, G and OURISSON, G (1965) *Bull Soc Chem Fr* 3682
[80] WIELAND, H and STANLEY, W M (1931) *Ann Chem* **489,** 31
[81] WIELAND, H , PASEDACH, H , and BALLAUF, A (1937) *Ann Chem* **529,** 68
[82] RUZICKA, L , DENSS, R and JEGER, O (1945) *Helv Chim Acta* **28,** 759
[83] RUZICKA, L , DENSS, R and JEGER, O (1946) *Helv Chim Acta* **29,** 204

lanosterol has been identified in several other fungal species [34,81–84] The formation of lanosterol by yeasts has also been demonstrated[85,86] and more recently the squalene-2,3-oxide cyclase responsible for its formation has been isolated from *Phycomyces blakesleeanus* [87] Schwenk and Alexander[88] have also shown that lanosterol is a precursor to 4-desmethyl sterols in yeast by showing that it can be incorporated into ergosterol

The metabolism of lanosterol by animal systems leads principally to the production of cholesterol, while it is converted to ergosterol by most fungal species During this conversion, certain general reactions are common to both animal and fungal systems· (a) loss of the C-4 and C-14 methyl groups, (b) reduction of the C-24 double bond, and (c) a series of nuclear double-bond shifts There are two additional reactions, however, that occur in the formation of ergosterol which do not occur in the animal systems, i e introduction of a methyl group at C-24 and formation of a Δ^{22} double bond in the sterol side-chain With the addition of a reaction that opens the 9β, 19-cyclopropane ring of cycloartenol, the above reactions also occur in green plants during the formation of 4-desmethyl sterols

Demethylation Reactions

Olsen *et al* [89] reported that 3 moles of carbon dioxide were liberated per mole of cholesterol formed from lanosterol The demethylation reaction mechanism was first thought to involve an initial mixed function hydroxylation of the methyl group, stepwise dehydrogenation (oxidized pyridine nucleotide-dependent) of the resulting hydroxymethyl and formyl groups and decarboxylation of the resulting carboxylic acid [89–94] More recently, Miller *et al* [95] have presented evidence for the presence of an aerobic pathway involving the stepwise oxidations of methyl, hydroxymethyl, and formyl groups by reduced pyridine nucleotide-dependent oxidases as the quantitatively important demethylation reaction mechanism occurring in rat liver Moore and Gaylor[96] described the properties of a yeast microsomal preparation which contains sterol demethylase activity and compared them to those of mammalian tissues (liver, skin, and testes) The yeast system closely resembled the mammalian tissues in that stoichiometric amounts of ^{14}C-labeled carbon dioxide were liberated from lanosterol in its conversion to a C_{27} sterol and required glutathione, NAD^+, and a neutral pH The two systems differed, however, in that the yeast system was incapable of demethylating Δ^7-sterols and was stimulated by Mg^{2+} which could be substituted by *S*-adenosylmethionine The latter characteristic was thought to be related to the transmethylation (see below) reaction which does not occur in higher animal systems

The two C-4 methyl groups appear to be sequentially removed [91] Since 4α- rather than the 4β-sterols were more commonly found in nature, it first appeared that the 4β-methyl

[84] Ludwiczak, R S and Wrzeciono, U (1960) *Roczn Chem* **34,** 77
[85] Schwenk, E , Alexander, G J , Fish, C A and Stoudt, T H (1955) *Fed Proc* **14,** 752
[86] Kodicek, E in *CIBA Foundation Symposium on the Biosynthesis of Terpenes and Sterols*, p 173, Churchill, London
[87] Mercer, E I and Johnson, M W (1969) *Phytochemistry* **8,** 2329
[88] Schwenk, E and Alexander, G J (1958) *Archs Biochem Biophys* **76,** 65
[89] Olsen, Jr , J A , Lindberg, M and Bloch, K (1957) *J Biol Chem* **226,** 941
[90] Bloch, K (1965) *Science* **150,** 19
[91] Miller, W L , Kalafer, M E , Gaylor, J L and Delwiche, C V (1967) *Biochemistry* **6,** 2673
[92] Olsen, Jr , J A (1965) *Ergeb Physiol* **56,** 173
[93] Clayton, R B (1965) *Quart Rev Biol* **19,** 168
[94] Gaylor, J L (1964) *J Biol Chem* **239,** 756
[95] Miller, W L , Brady, D R and Gaylor, J L (1971) *J Biol Chem* **246,** 5147
[96] Moore, J T and Gaylor, J L (1968) *Arch Biochem Biophys* **124,** 167

group was the first oxidized Rahman *et al*[97] and Sharpless *et al* [98 99] have recently demonstrated, however, that the 4α-methyl group is removed first and this reaction is followed by a 4β-methyl epimerization The same series of reactions occurred in plant tissues when cycloartanol was converted to 31-norcycloartanol by the fern *Polypodium vulgare* [100]

The participation of a 3-keto steroid in the C-4 demethylation reactions has been known for some time [89 91,101–103] Clayton and his associates[96 98] have presented evidence that the 3-keto group is essential for the epimerization reaction (4β → 4α) which occurs following the loss of the carbon dioxide formed from the original 4α-methyl group This is further supported by Rahimtula and Gaylor[104] who found that 4α-carboxylic acids produced from 4α-methyl sterols by a partially purified rat liver microsomal enzyme were further metabolized to carbon dioxide and the 3-ketosteroid The ketone is subsequently converted to the alcohol by a 3-ketoreductase which requires NADPH This reduction appears to be necessary before oxidation of the second C-4 methyl group can occur

Until recently, the proposed hydroxymethyl and carboxyl intermediates in the demethylation reaction sequence leading to the formation of 4-desmethyl sterols had not been isolated from a biological system The strongest experimental evidence for these compounds being intermediate in the demethylation of lanosterol was that, when chemically synthesized and added to cell-free systems, they could be converted to cholesterol [75] Miller and Gaylor[105,106] have now shown that a rat liver microsomal system is capable of converting 4α-methyl and 4,4-dimethyl sterols to the corresponding 4α-carboxylic and 4β-methyl-4α-carboxylic acids, respectively Hornby and Boyd[107] have identified 4β-methyl-5α-cholesten-4α-carboxylic acid in a similar system The carboxylic acid structures have been confirmed by MS

In animals and fungi, the 14α-methyl appears to be the first removed in the demethylation reaction sequence This is based on the fact that, aside from lanosterol, no 14α-methyl sterols have been isolated and identified from these organisms and that 14-demethyllanosterol could be converted to cholesterol [108,109] It is significant to note, however, that Gustafsson *et al* [110] found 4α,14α-dimethyl-cholest-Δ^8-3β-ol and 4α,14α-dimethyl-cholest-Δ^7-3β-ol as components of human meconium The demethylase enzyme appears to be relatively non-specific since 14α-methyl-cholest-Δ^7-enol can be converted to cholesterol[111] and 4,14α-dimethyl-cholesta-$\Delta^{8,24}$-dienol can be converted to ergosterol by yeast [112]

The presence of 14α-methyl sterols such as cycloeucalenol, pollinasterol, and macdougallin

[97] RAHMAN, R , SHARPLESS, K B , SPENCER, T A and CLAYTON, R B (1970) *J Biol Chem* **245,** 2667
[98] SHARPLESS, K B , SNYDER, T E , SPENCER, T A , MAKESHWARI, K K , GUHN, G and CLAYTON, R B (1968) *J Am Chem Soc* **90,** 6874
[99] SHARPLESS, K B , SNYDER, T E , SPENCER, T A , MAKESHWARI, K K , NELSON, J A and CLAYTON, R B (1969) *J Am Chem Soc* **91,** 3394
[100] GHISALBERTI, E L , DESOUZA, N J , REES, H H , GOAD, L J and GOODWIN, T W (1969) *Chem Commun* 1403
[101] LINDBERG, M , GAUTSHI, F and BLOCH, K (1963) *J Biol Chem* **238,** 1661
[102] BLOCH, K (1959) in *Biosynthesis of Terpenes and Sterols* (WOLSTENHOLME, G E W and O'CONNOR, M , eds), p 4, CIBA Foundation Symposium, Little Brown Company, Boston
[103] SUMDELL, A C and GAYLOR, J L (1968) *J Biol Chem* **243,** 5546
[104] RAHIMTULA, A D and GAYLOR, J L (1972) *J Biol Chem* **247,** 9
[105] MILLER, W L and GAYLOR, J L (1970) *J Biol Chem* **245,** 5369
[106] MILLER, W L and GAYLOR, J L (1970) *J Biol Chem* **245,** 5375
[107] HORNBY, G M and BOYD, G S (1970) *Biochem Biophys Res Commun* **40,** 1452
[108] GAUTSCHI, F and BLOCH, K (1957) *J Am Chem Soc* **79,** 684
[109] GAUTSCHI, F and BLOCH, K (1958) *J Biol Chem* **233,** 1343
[110] GUSTAFSSON, J A and ENEROTH, P (1972) *Proc Royal Soc (London)* **180B,** 179
[111] KNIGHT, J C , KLEIN, P D and SZCZEPANIK, P A (1966) *J Biol Chem* **241,** 1502
[112] BARTON, D H R , HARRISON, D M and WIDDOWSON, D A (1968) *Chem Commun* 17

in several plant species suggests, however, that less preference is given this methyl group as the first site of oxidation in the demethylation sequence by these systems Clayton[69] proposed that demethylation of the 14α-methyl group in plants is hindered by the 9β,19-cyclopropane ring or lack of the $\Delta^{8(9)}$ double bond of cycloartenol It is also interesting to note that the green alga *Chlorella emersonii* accumulates 14α-methyl-5α-ergosta-$\Delta^{8\ 24(28)}$-dienol and 14α-methyl-5α-ergost-Δ^{8}-enol when treated with the hypocholesterolemic agent triparanol [113]

Until recently, little attention has been given to the C-14 methyl removal and, hence, the mechanisms of this demethylation reaction are not well understood It has been assumed that this methyl group is converted to carbon dioxide by the dehydrogenation or oxidation reactions responsible for the C-4 methyl removal However, a recent finding shows that, unlike the C-4 methyl removal, the 32-carbon atom of lanosterol in cholesterol biosynthesis occurs at the aldehyde state of oxidation resulting in the release of formic acid [114] It was also shown that one of the C-15 hydrogen atoms is lost during the lanosterol to cholesterol conversion[115–117] and that it is the α-hydrogen which is lost in the analogous reaction in plants [118] This strongly suggests that a Δ^{14} product may result from the C-14 demethylation

It has been shown that sterols containing the Δ^{14} double bond can be biologically reduced (at the C-15 position) by a *trans* hydrogen of NADPH and a proton from water [119] Further evidence for the participation of Δ^{14} sterols in the formation of cholesterol has been presented through the use of the drug AY-9944 This compound inhibits the Δ^{14} reduction resulting in accumulation of Δ^{14} sterols which are potential intermediates in cholesterol biosynthesis Similar results were obtained by Dickson and Patterson[120] who identified ergosta-$\Delta^{8,14}$-dienol, (24*S*)-stigmasta-$\Delta^{8,14}$-dienol, 4α-methyl-ergosta-$\Delta^{8,14}$-dienol and 4α-methyl-(24*S*)-stigmasta-$\Delta^{8\ 14}$-dienol from the green alga *Chlorella ellipsoidea* grown in media containing 4 ppm of AY-9944 Akhtar *et al* [121] found that yeast also contains the enzyme capable of reducing the Δ^{14} double bond of ergosta-$\Delta^{8,14}$-dienol and converting it to ergosterol

These results coupled with the reports of Δ^{14} sterols in higher plant[122] and fungal tissues[35,44] lends further support to the presence of a pathway containing Δ^{14} sterol derivatives as intermediates in cholesterol and ergosterol formation (see Scheme 2C, below)

Reduction of the Δ^{24} *Double Bond and* C-24 *Alkylation*

There are two possible ways in which the Δ^{24} double bond is reduced in the conversion of lanosterol to 4-desmethyl sterols The Δ^{24} double bond can be reduced by the addition of hydrogen atoms at the C-24 and C-25 positions of the sterol side chain Studies on the biosynthesis of cholesterol by animal systems have shown that the reduction of lanosterol to

[113] Doyle, P J , Patterson, G W , Dutky, S R and Cohen, C F (1971) *Phytochemistry* **10,** 2093
[114] Alexander, K , Akhtar, M , Boar, R B , McGhie, J F and Barton, D H R (1972) *Chem Commun* 383
[115] Canonica, L , Fiecchi, A , Kienle, M G , Scala, A , Galli, G , Paoletti, E G and Paoletti, R (1968) *J Am Chem Soc* **90,** 3597
[116] Akhtar, M , Watkinson, I A , Rahimtula, A D , Wilton, D C and Munday, K A (1968) *Chem Commun* 1406
[117] Akhtar, M , Rahimtula, A D , Watkinson, I A , Wilton, D C and Munday, K A (1969) *European J Biochem* **9,** 107
[118] Gibbons, G F , Goad, L J and Goodwin, T W (1968) *Chem Commun* 1458
[119] Berseus, O (1965) *Acta Chem Scand* **19,** 325
[120] Dickson, L G and Patterson, G W (1972) *Lipids* **7,** 635
[121] Akhtar, M , Brooks, W A and Watkinson, I A (1969) *Biochem J* **115,** 135
[122] Frost, D J and Ward, J P (1970) *Rec Trav Chim* **89,** 1054

24-dihydrolanosterol involves a stereospecific[119,123] addition of hydrogen by a microsomal NADPH dependent enzyme [124,125] The point at which this reduction takes place in the metabolism of lanosterol to cholesterol relative to the demethylation reactions and nuclear double bond shifts is uncertain Since sterols such as cholesterol were only recently reported as fungal products, this reaction has not been studied in the fungi

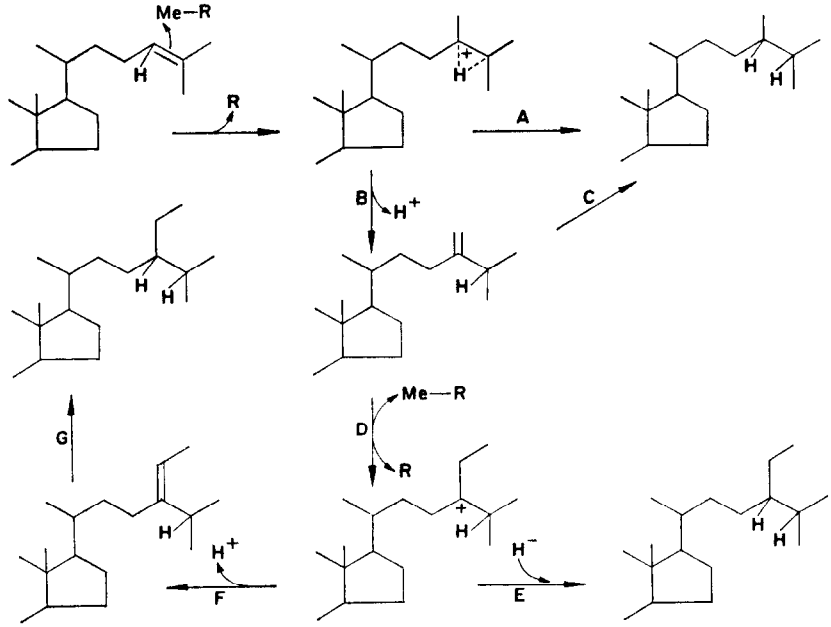
The conversion of lanosterol to C_{28} sterols by fungi involves an alkylation at the C-24 position Using ^{14}C-labeled substrates, it was found that the C-28 alkyl group of ergosterol did not arise from acetate [126] Methionine was found to be an efficient methyl donor[127–130] while *S*-adenosylmethionine was shown to be the most efficient and probable immediate donor[131,132] in yeast A microsomal bound *S*-adenosylmethionine-dependent-Δ^{24}-sterol transmethylase has recently been isolated from yeast [133] This enzyme catalyzes electrophilic transfer of a C_1 group from a positively charged donor (*S*-adenosylmethionine) to an electron-rich site such as the Δ^{24}-double bond of the sterol side-chain [134] An analogous reaction occurs in green plants in the conversion of cycloartenol to 4-desmethyl sterols

Lederer[135,136] has comprehensively reviewed the investigations on the mechanism of alkalation as it occurs in plant and fungal systems (Scheme 1) In the first studies to determine the mechanism of transmethylation in yeast, Alexander and Schwenk[128] found that the tritiated *S*-methyl group of methionine was transferred intact to the C-24 position Since the possibility of a hydrogen-isotope effect existed, Jaurequiberry *et al*[137] re-investigated the alkylation reaction mechanism and found that ergosterol produced by a methionine-less strain of *Neurospora crassa* grown in the presence of deuterated *S*-methylmethionine contained only two of the three original deuterium atoms of CD_3 (Scheme 1B $\rightarrow$ C) Similar results obtained from studies with *Blakeslea trispora*, *Polyporus sulphureus* and a yeast combined with the reports of 24-methylene sterol derivatives reported for several fungi lends further support for this mechanism Furthermore, 24-methylene-lanost-Δ^8-enol can be converted to ergosterol by yeast and *P sulphureus* [138 139]

The formation of ergostane derivatives by algae may not proceed via the same mechanism Tomita and his associates[140] demonstrated that the deuterated methyl of [CD_3] methionine was transferred intact in the formation of ergost-Δ^7-enol by the green alga *Chlorella vulgaris* This suggests that 24-methylene derivatives are not precursors to C_{28} sterols in this organism (Scheme 1A) and possibly other algae

[123] MITROPOULOS, K A and MYANT, N B (1965) *Biochem J* **97,** 26C
[124] AVIGAN, J , GOODMAN, D S and STEINBERG, D (1963) *J Biol Chem* **238,** 1283
[125] GAYLOR, J L (1963) *Arch Biochem Biophys* **101,** 108
[126] HANAHAN, D J and WAKIL, S J (1953) *J Am Chem Soc* **75,** 273
[127] ALEXANDER, G J and SCHWENK, E (1957) *J Am Chem Soc* **79,** 4554
[128] ALEXANDER, G J and SCHWENK, E (1958) *J Biol Chem* **232,** 611
[129] ALEXANDER, G T , GOLD, A M and SCHWENK, E (1957) *J Am Chem Soc* **79,** 2967
[130] ALEXANDER, G T , GOLD, A M and SCHWENK, E (1958) *J Biol Chem* **232,** 599
[131] PARKS, L W (1958) *J Am Chem Soc* **80,** 2023
[132] TURNER, J R and PARKS, L W (1965) *Biochem Biophys Acta* **98,** 394
[133] MOORE, JR , J T and GAYLOR, J L (1969) *J Biol Chem* **244,** 6334
[134] VAN ALLER, R T , CHIKAMATSU, H , DESOUZA, N J , JOHN, J P and NES, W R (1969) *J Biol Chem* **244,** 6645
[135] LEDERER, E (1964) *Biochem J* **93,** 449
[136] LEDERER, E (1969) *Q Rev Chem Soc* **23,** 453
[137] JAUREQUIBERRY, G , LAW, J H , MCCLOSKEY, J and LEDERER, E (1965) *Biochemistry* **4,** 347
[138] AKHTAR, M , PANVEZ, M A and HUNT, P F (1966) *Biochem J* **100,** 38C
[139] BARTON, D H R , HARRISON, D M and MOSS, G P (1966) *Chem Commun* 595
[140] TOMITA, Y , UOMORI, A and MINATO, H (1970) *Phytochemistry* **9,** 555

It has been shown that the 29-carbon atom of 24-ethyl sterols in fungi, algae, and higher plants originates from the *S*-methyl of methionine through a second transmethylation [141–143] The substrate for this reaction is a 24-methylene sterol (Scheme 1D) Castle *et al* [141] have proposed two possible routes by which 24-ethyl sterols may be produced and each of these occur in nature For example, *Ochromonas malhamensis* grown in the presence of [CT_3] methionine produced poriferasterol (24-ethyl (24β)-stigmasta-$\Delta^{5,22}$-dienol) with only four tritium atoms in the 24-ethyl side chain[143] which suggests that the second alkylation reaction proceeds via a 24-ethylidene intermediate[130,145] (Scheme 1F → G) On the other hand, in the formation of stigmast-Δ^{22}-enol by the slime mold *Dictyostelium discordeum*[146] and chondrillasterol (and Δ^7-chondrillastenol) by *C vulgaris*[140] five deuterium atoms are retained in the ethyl side chain when these organisms are grown in the presence of [CD_3]-methionine. These results support the contention that 24-ethylidene sterols are not intermediate in the formation of 24-ethyl sterols in these organisms (Scheme 1E)

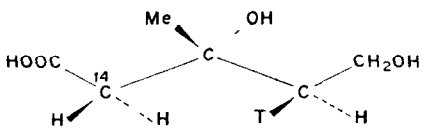


SCHEME 1 ALKYLATION MECHANISMS IN THE FORMATION OF C-24 METHYLENE, METHYL, ETHYLIDENE, AND ETHYL STEROLS AS THEY OCCUR IN PLANT AND FUNGAL ORGANISMS [140,141]

In the initial stages of the alkylation reaction, migration of a hydrogen atom from the C-24 to C-25 position of the sterol side chain was found to occur (Scheme 1) Lanosterol or cycloartenol biologically synthesized from [2-^{14}C, (4*R*)-4-$^{3}H_1$]mevolanate (XXXI) has a tritium atom in the β-position of C-24 and when converted to their respective C-24 alkyl

[141] CASTLE, M , BLONDIN, G and NES, W R (1963) *J Am Chem Soc* **85**, 3306
[142] BADER, S , GUGLIALMETTI, L and ARIGONI, D (1964) *Proc Chem Soc* 16
[143] VILLANUEVA, V R , BARBIER, M and LEDERER, E (1964) *Bull Soc Chim Fr* 1423
[144] SMITH, A R H , GOAD, L J , GOODWIN, T W and LEDERER, E (1967) *Biochem J* **104**, 56C
[145] LENTON, J R , HALL, J SMITH, A R H , GHISALBERTI, E L , REES, H H , GOAD, L J and GOODWIN, T W (1971) *Arch Biochem Biophys* **143**, 664
[146] LENFANT, M , ZISSMANN, E and LEDERER, E (1967) *Tetrahedron Letters* **12**, 1049

isomers, the $^{14}C/^{3}H$ ratio suggests that the original tritium atom in the side chain is retained This has been demonstrated to occur in higher plants,[147] algae,[148 149] and the fungi *A fumigatus*[150] and *Polyporus sulphureus* [146] In a similar study with algae, the $^{14}C/^{3}H$ ratio of fucosterol (stigmasta-$\Delta^{5,24(28)}$-dienol), which could not have a tritium atom in the C-24 position, supports the postulated C-24 to C-25 hydrogen migration during the alkylation reaction [74]



(XXXI)

Formation of Δ^{8}*-4-Desmethyl Sterols*

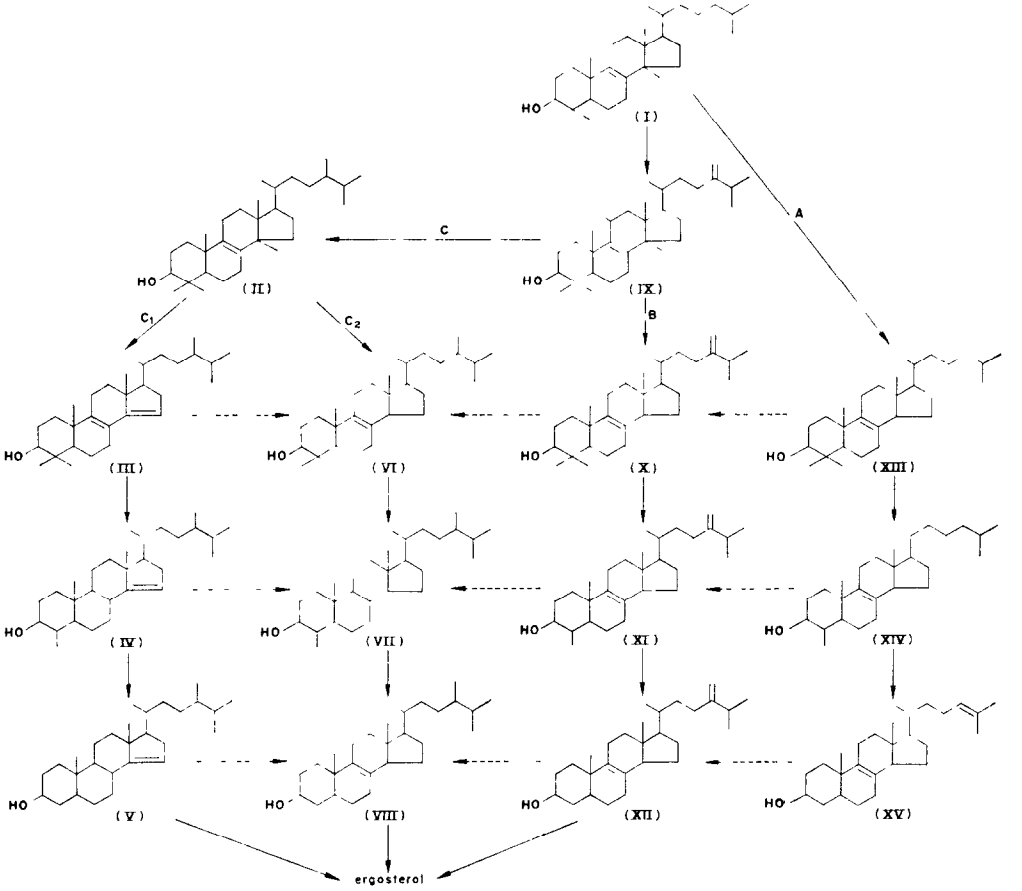
In spite of the several studies which have been carried out on the biosynthesis of ergosterol by yeast, the quantitatively important intermediates after lanosterol remain uncertain Lederer[135] suggested that the first substrate for alkylation is desmosterol Katsuki and Bloch[151] reported experimental evidence that both desmosterol and zymosterol can except methyl groups (at C-24) in whole yeast cells and that no 24-methyl-4,4-dimethyl or 4α-methyl sterols could be detected Moore and Gaylor[133] have isolated a methyltransferase enzyme from a strain of yeast which is capable of converting zymosterol to 24-methylene-dihydrozymosterol These results suggest that pathway A in Scheme 2 may be operative in certain yeast, i e transmethylation occurring at the 4-desmethyl sterol level It should be mentioned, however, that in cell-free yeast preparations desmosterol and zymosterol strongly inhibited the uptake of methionine [151]

Since 24-methylene- and 24-methyl-lanosterol can be converted to ergosterol by *S cerevisiae*,[132,152] it appears that 24-alkyl 4,4-dimethyl sterols can be intermediate in ergosterol formation In addition, 4α-methyl-24-methylene-24-dihydrozymosterol has been isolated from yeast and, along with obtusifoliol (4α,14α-dimethyl-cholesta-$\Delta^{8\ 24}$-dienol) and 4α-methylzymosterol, can be converted to ergosterol by growing yeast cultures [112] These results suggest that the C-24 alkylation reaction can also occur at the 4α-methyl sterol level in the formation of ergosterol by whole yeast cells There is also convincing evidence for the participation of 24-methylated intermediates at the lanosterol level (Scheme 2B and C) in the formation of ergosterol by mycelial fungi 24-Methylene-lanosterol,* 24-methylenelophenol (and possibly its Δ^{8} isomer), and possibly 14-desmethyl-24-methylene-dihydrolanosterol,[34] and others[153] have been identified as fungal products

* Identification of a potential intermediate in a biosynthetic pathway does not sufficiently confirm its intermediary role, see Schroepfer *et al* [75] for suggested criteria for establishing an intermediary role of a sterol in the biosynthesis of cholesterol

[147] RAAB, K H , DESOUZA, N J and NES, W R (1968) *Biochem Biophys Acta* **152,** 742
[148] GOAD, L J and GOODWIN, T W (1965) *Biochem J* **96,** 79P
[149] GOAD, L J and GOODWIN, T W (1969) *European J Biochem* **7,** 502
[150] STONE, K J and HEMMING, F W (1965) *Biochem J* **96,** 14C
[151] KATSUKI, H and BLOCH, K (1967) *J Biol Chem* **242,** 222
[152] AKHTAR, M , PARVEZ, M A and HUNT, P F (1969) *Biochem J* **113,** 727
[153] SHOPPEE, C W (1964) in *Chemistry of the Steroids*, Butterworths, London

(see Table 1) The identification of 24-methylenelophenol (4α-methyl-ergosta-$\Delta^{7,24(28)}$-dienol) suggests that modification of the ring structure ($\Delta^8 \rightarrow \Delta^{5,7}$) in at least some species may occur prior to the completion of demethylation This is consistent with proposed pathways of phytosterol biosynthesis [154]



SCHEME 2 POTENTIAL BIOCHEMICAL PATHWAYS FOR THE CONVERSION OF LANOSTEROL TO Δ^8-4-DESMETHYL STEROLS AS THEY MAY OCCUR IN THE FUNGI

Principal pathways (⟶), interconversions between principal pathways (----→) The systematic names of each sterol component in this figure are listed below and can be identified by the corresponding Roman numeral (I) lanosta-$\Delta^{8,24}$-dienol (lanosterol),*† (II) 24-methyl-lanosta-Δ^8-enol (24-methyl-dihydrolanosterol),*† (III) 4,4-dimethyl-ergosta-$\Delta^{8,14}$-dienol, (IV) 4α-methyl-ergosta-$\Delta^{8,14}$-dienol, (V) ergosta-$\Delta^{8,14}$-dienol,† (VI) 4,4-dimethyl-ergost-Δ^8-enol, (VII) 4α-methyl-ergost-Δ^8-enol, (VIII) ergost-Δ^8-enol, (IX) 24-methylene-lanosta-Δ^8-enol (24-methylene-dihydrolanosterol),*† (X) 4,4-dimethyl-ergosta-$\Delta^{8,24,(28)}$-dienol,* (XI) 4α-methyl-ergosta-$\Delta^{8,24(28)}$-dienol,*† (XII) ergosta-$\Delta^{8,24(28)}$-dienol,* (XIII) 4,4-dimethyl-cholesta-$\Delta^{8,24(25)}$-dienol,* (XIV) 4α-methyl-cholesta-$\Delta^{8,24(25)}$-dienol,*† (XV) cholesta-$\Delta^{8,24(25)}$-dienol *

* Identified as a fungal product

† Can be incorporated into ergosterol by a fungal system

[154] BEVENISTE, P, HEWLINS, M J E and FRITIG, B (1969) *European J Biochem* **9**, 526

In Scheme 2 (C_1 and C_2) pathways of ergosterol synthesis involving 24-methyl sterols having Δ^8 and $\Delta^{8,14}$ points of unsaturation are illustrated * The point in the pathway of ergosterol formation where the Δ^{14} reduction takes place is unknown, but since Δ^{14} sterols appear to be rare in nature it is plausible that the reduction occurs immediately after C-14 methyl removal In this case, Δ^8-sterol formation would proceed via pathway C_2 in Scheme 2 As previously mentioned, certain algae grown in the presence of the AY-9944 inhibitor accumulates Δ^{14} sterols at the 4α-methyl and 4-desmethyl sterol levels [120] The fact that Δ^{14}-4-desmethyl sterols are present suggests that, at least for *C ellipsoidea*, the Δ^{14} reduction occurs at some point subsequent to the demethylation reactions The presence of $\Delta^{5\,7\,14}$ and $\Delta^{5,7\,14\,22}$ sterols in some fungi suggests that additonal structural modifications such as nuclear double bond shifts may also occur prior to Δ^{14} reduction Whether the accumulation of these sterols in certain fungi are due to genetic alterations unique to these species or isolates, or whether Δ^{14} sterols at this level of demethylation and unsaturation are important intermediates in ergosterol biosynthesis is not known Studies using the AY-9944 inhibitor should give a clue concerning their importance and the point of Δ^{14} reduction in ergosterol formation

At this point, there is no reason to believe that the formation of ergosterol by fungi must proceed by way of the major pathways outlined in Scheme 2 Several studies on the formation of ergosterol by yeast and cholesterol by mammalian systems have shown that a number of sterols differing slightly in structure may serve as substrates for the methylation, reduction, and demethylation reactions which occur in these pathways Thus the dotted arrows in Scheme 2 illustrate hypothetical interrelationships between the major pathways which may occur in fungi

Formation of Ergosterol from Δ^8-4-Desmethyl Sterol Precursors

Following the demethylation and alkylation reactions, there appears to be four principal 24-alkyl-Δ^8-4-desmethyl sterol compounds that may serve as potential substrates for the subsequent ring modification which leads to the formation of ergosterol by fungi (a) ergosta-$\Delta^{8,14(28)}$-dienol, (b) ergost-Δ^8-enol, (c) ergosta-$\Delta^{8,14}$-dienol, (d) ergosta-$\Delta^{8\,14,24(28)}$-trienol† (Scheme 3) The $\Delta^{8,24(28)}$ sterol has been identified as a product of the enzyme catalyzed alkylation of zymosterol by a highly purified preparation from yeast [133] Although the $\Delta^{8,14}$ sterol can be converted to ergosterol by yeast,[121] neither it nor the Δ^8 and $\Delta^{8,14,24(28)}$ sterols have been identified as a fungal product

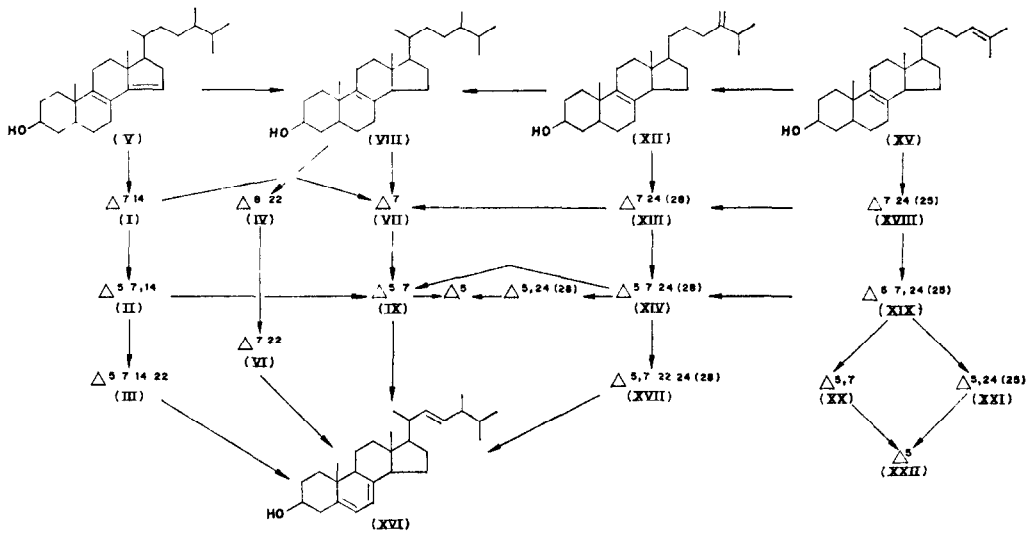
As in the formation of cholesterol, the following reaction sequence also occurs in fungi and leads to the final $\Delta^{5,7}$ ring structure of ergosterol $\Delta^8 \rightarrow \Delta^7 \rightarrow \Delta^{5,7}$ Many of the sterols identified from fungal organisms have points of unsaturation in these positions (see Table 1) and it has been shown that several of these can be converted to ergosterol by yeast and other fungal systems (Scheme 3)

The reaction mechanism of the $\Delta^8 \rightarrow \Delta^7$ conversion in the biosynthesis of cholesterol has been studied using stereo-specifically labeled sterol precursors These studies revealed that the $\Delta^8 \rightarrow \Delta^7$ isomerization involves a loss of the 7β-hydrogen atom while the 7α-hydrogen

* Demethylation of 24-methylene-lanosterol (IX) may also result in a Δ^{14} product and subsequent formation of 24-methylene-$\Delta^{8\,14}$-sterols may be formed in a manner analogous to pathway C_1 in Scheme 2

† As in Scheme 2, the pathway involving 24-methylene-Δ^{14}-sterols is not shown in Scheme 3

was retained.[155–158] Similar results were found in the synthesis of poriferasterol by the alga *O. malhamensis*[159] and in the synthesis of certain sterols by higher plants [153] In contrast, Caspi and Ramm[160] found that the stereochemistry of the Δ^7 formation by a yeast homogenate is the opposite to that of the above systems, i e the 7α-hydrogen atom is lost in the conversion of lanosterol to C_{27} sterols The following three Δ^7 ergostane derivatives have been identified as fungal products and each can be converted to ergosterol by these organisms (Table 1 and Scheme 3): ergosta-$\Delta^{7,24(28)}$-dienol, ergost-Δ^7-enol, and ergosta-$\Delta^{7,22}$-dienol. It is interesting to note that in studies on the formation of ergosterol by *Chlorella* species, the $\Delta^{7,22}$ sterol was not converted to the $\Delta^{5,7,22}$ structure which suggests that a $\Delta^{5,7}$ sterol may be the immediate precursor to ergosterol in that organism [161,162]



SCHEME 3 POTENTIAL PATHWAYS OF ERGOSTEROL BIOSYNTHESIS THROUGH Δ^8-4-DESMETHYL INTERMEDIATES AS THEY MAY OCCUR IN THE FUNGI.

The systematic names of each compound given in this figure is listed below and can be identified by its corresponding Roman numeral or points of unsaturation. (I) ergosta-$\Delta^{7,14}$-dienol; (II) ergosta-$\Delta^{5,7,14}$-trienol,* (III) ergosta-$\Delta^{5,7,14,22}$-tetraenol;* (IV) ergosta-$\Delta^{8,22}$-dienol,*† (V) ergosta-$\Delta^{8,14}$-dienol,† (VI) ergosta-$\Delta^{7,22}$-dienol,*† (VII) ergosta-Δ^7-enol;*† (VIII) ergost-Δ^8-enol: (IX) ergosta-$\Delta^{5,7}$-dienol,*† (X) ergost-Δ^5-enol,* (XI) ergosta-$\Delta^{5,24(28)}$-dienol,* (XII) ergosta-$\Delta^{8,24(28)}$-dienol,*† (XIII) ergosta-$\Delta^{7,24(28)}$-dienol;*† (XIV) ergosta-$\Delta^{5,7,24(28)}$-trienol, (XV) cholesta-$\Delta^{8,24(25)}$-dienol,*† (XVI) ergosta-$\Delta^{5,7,22}$-trienol,* (XVII) ergosta-$\Delta^{5,7,22,24(28)}$-tetraenol,*† (XVIII) cholesta-$\Delta^{7,24(25)}$-dienol, (XIX) cholesta-$\Delta^{5,7,24(25)}$-trienol, (XX) cholesta-$\Delta^{5,7}$-dienol, (XXI) cholesta-$\Delta^{5,24(25)}$-dienol,* (XXII) cholesta-Δ^5-enol*

* Identified as a fungal product

† Can be incorporated into ergosterol by a fungal system

[155] PARKS, L W, BOND, F T, THOMPSON, E. D and STARR, P R (1972) *J Lipid Res* **13**, 311

[156] CANONICA, L, FIECCHI, A, KIENLE, M G., SCALA, A, GALLI, G, PAOLETTI, E G and PAOLETTI, R (1968) *Steroids* **11**, 749

[157] CANONICA, L, FIECCHI, A, KIENLE, M G, SCALA, A, GALLI, G, PAOLETTI, E G. and PAOLETTI, R. (1969) *Steroids* **12**, 445

[158] CASPI, E, GREIG, J. B, RAMM, P J and VARMA, K R (1968) *Tetrahedron Letters* 3829.

[159] GIBBONS, G F, GOAD, L J and GOODWIN, T W (1968) *Chem Commun* 1212.

[160] CASPI, E and RAMM, P. J (1969) *Tetrahedron Letters* **3**, 181

[161] PATTERSON, G W and KARLANDER, E. P (1967) *Plant Physiol* **42**, 1651

[162] PATTERSON, G W and KARLANDER, E P (1968) *Plant Physiol* **43**, suppl S-46

Introduction of the Δ^5 double bond into ring B of the steroid nucleus has been studied in both mammals (rat liver) and fungi (yeast), yet the natural intermediates in either system remains unclear It has been established for the biosynthesis of cholesterol that oxygen[163] and pyridine nucleotide[164] are required in this reaction The double bond formation at the Δ^5 position involves the removal of the α-hydrogen atoms at the C-5 and C-6 positions, i e a *cis* hydrogen elimination [165–168] Two mechanisms for the introduction of this double bond into a Δ^7-intermediate were proposed by Akhtar and his associates [169 170] (a) a hydroxylation-dehydration mechanism in which the first step occurs under aerobic conditions and the second step under anaerobic conditions, and (b) a dehydrogenation mechanism The use of various oxygenated compounds (hydroxyl, keto, and epoxy) in rat liver cell-free preparations for cholesterol synthesis have met with little success in determining the natural intermediate in the $\Delta^7 \rightarrow \Delta^{5,7}$ conversion Akhtar and Parvez[169] used the yeast *S cerevisiae* and the biosynthesis of ergosterol to demonstrate the oxygen-dependent dehydrogenation reaction mechanism These investigators found that whole yeast cells were capable of converting 3α-^{3}H-ergosta-$\Delta^{7,22}$-dienol into ergosterol under aerobic but not anaerobic conditions The stereochemistry of C-5 and C-6 hydrogen removal in yeast is the same as that described for rat liver and was also verified in the fungus *A fumigatus* [171] In addition, they found no conversion of chemically synthesized 3α-^{3}H-ergosta-$\Delta^{7\ 22}$-dien-3β,5α-diol into ergosterol under anaerobic conditions This was analogous to the $\Delta^7 \rightarrow \Delta^{5,7}$ conversion in rat liver preparations The diol was, however, converted to ergosterol in the presence of oxygen Contradictory results are reported in a series of articles by Topham and Gaylor[172–174] that describes a membrane bound 5α-hydroxysterol dehydrase from yeast which catalyzes the anaerobic conversion of ergosta-$\Delta^{7\ 22}$-dien-3β-5α-diol to ergosterol Furthermore, in accordance with Hamilton and Castrejon,[175] they found that 5α,8a-epidioxyergosta-$\Delta^{7,22}$-dienol (ergosterol peroxide) was converted to ergosterol by this enzyme as efficiently as the diol Also, when using 3α-^{3}H-ergosta-$\Delta^{7,22}$-dien-3β,5α-diol as the substrate, the 3α-^{3}H was removed during the conversion to ergosterol Thus, Topham and Gaylor[174] proposed that between a C-5 hydroxylated substrate and the final $\Delta^{5,7\ 22}$ product, the involvement of an intermediate containing a cyclopropane ring between C-3 and C-5 can explain the loss of 3α-^{3}H during the formation of the $\Delta^{5,7}$ double bond system Although several C-5 oxygenated compounds can serve as substrates for the yeast dehydrase enzyme in the formation of ergosterol, the quantitatively important natural intermediate in this reaction remains unknown

The following $\Delta^{5,7}$ sterols have been isolated as fungal products and represent potential ergosterol precursors ergosta-$\Delta^{5,7,24(28)}$-trienol, ergosta-$\Delta^{5,7,22,24(28)}$-tetraenol, ergosta-$\Delta^{5,7}$-dienol, ergosta-$\Delta^{5,7,14,22}$-tetraenol, and ergosta-$\Delta^{5,7,14}$-trienol The first four com-

[163] FRANTZ, JR , I D , DAVISON, A G , DULIT, E and MOBBERLY, M L (1959) *J Biol Chem* **234,** 2290
[164] SCALLEN, T J and SCHUSTER, M W (1968) *Steroids* **12,** 683
[165] GOAD, L J , GIBBONS, G F , LOLGER, L , REES, H H and GOODWIN, T W (1969) *Biochem J* **96,** 79
[166] AKHTAR, M and MARSH, S (1967) *Biochem J* **102,** 462
[167] PALIOKAS, A M and SCHROEPFER, G J (1967) *Biochem Biophys Res Commun* **26,** 736
[168] PALIOKAS, A M and SCHROEPFER, G J (1968) *J Biol Chem* **243,** 453
[169] AKHTAR, M and PARVEZ, M A (1968) *Biochem J* **108,** 527
[170] DEWHURST, S M and AKHTAR, M (1967) *Biochem J* **105,** 1187
[171] BIMPSON, T , GOAD, L J and GOODWIN, T W (1969) *Chem Commun* 297
[172] TOPHAM, R W and GAYLOR, J L (1967) *Biochem Biophys Res Commun* **27,** 644
[173] TOPHAM, R W and GAYLOR, J L (1970) *J Biol Chem* **245,** 2319
[174] TOPHAM, R W and GAYLOR, J L (1972) *Biochem Biophys Res Commun* **47,** 180
[175] HAMILTON, J G and CASTREJON, R N (1966) *Federation Proc* **25,** 221

pounds can be converted to ergosterol by fungi, while the $\Delta^{5,7,14}$ isomer has been only recently identified (tentatively) as a fungal product and it has not been determined if it can be converted to ergosterol

Introduction of the Δ^{22} double bond is another structural modification which occurs in the formation of ergosterol The *trans* Δ^{22} double bond is characteristic of both plant and fungal sterols Stigmasterol is the predominant Δ^{22} sterol in higher plants while its C-24 epimer poriferasterol, chondrillasterol, Δ^{7}-chondrillasterol, and 22-dehydrocholesterol are common in certain algae Cholesta-$\Delta^{5,7,22}$-trienol is found in the protozoan *Tetrahymena pyriformis* [176] Ergosterol is the predominant fungal sterol containing the Δ^{22} double bond, but certain potential ergosterol precursors (see Table 1) as well as Δ^{22}-cholesterol and Δ^{22}-24-ethylcholesterol have been reported in certain aquatic phycomycetes Goodwin[74] has reviewed the few studies concerning the mechanism of Δ^{22}-desaturation. The two carbon atoms involved in this reaction are C-22 and C-23 which originate from the C-2 and C-5 atoms of mevalonate, respectively Using stereospecifically labeled mevalonate, Bimpson *et al* [171] and Bimpson[177] demonstrated that the 22-pro-*S*-hydrogen atoms are eliminated in the formation of the Δ^{22} double bond of ergosterol by *A fumigatus* and *B trispora* In contrast, Smith *et al* [178] found that the stereochemistry of the Δ^{22} desaturase was the opposite in the synthesis of ergosterol by the alga *O danica*, poriferasterol by *O malhamensis*[179] and of cholesta-$\Delta^{5,7,22}$-trienol by *T pyriformis* [176] The point during the sterol biosynthesis reaction sequence that the Δ^{22}-desaturation occurs is uncertain

Structural requirements for introduction of the Δ^{22} double bond are not known but this bond is found in sterols having C-24 saturated or unsaturated alkyl groups and it is also found in non-alkylated sterol side chains Sterols having the conjugated double bond system $\Delta^{22,24(25)}$ had not been identified from a plant or fungal source Until recently, fungal sterols having the Δ^{22} double bond were in the later stages of nuclear double bond rearrangement, i e double bonds in the Δ^{7}, $\Delta^{5,7}$, $\Delta^{5,7,24(28)}$ positions Parks *et al* [155] have isolated the previously unidentified sterol ergosta-$\Delta^{8(9),22}$-dienol from a polyene-resistant mutant of *S cerevisiae* which can be converted to ergosterol by wild-type yeasts It is unknown whether the desaturation occurs early in the formation of ergosterol or if this is a unique feature of the particular yeast strain

Biosynthesis of C_{27} *and* C_{29} *Sterols by the Fungi*

As more fungal species are studied, it is becoming increasingly apparent that cholesterol and other Δ^{5}-C_{27} and C_{29} sterols characteristic of animal and higher plant organisms are more common to the fungi than was first realized. This is particularly true for the aquatic phycomycetes and rust (spores) fungi which have been examined Only recently have reports on the sterol composition appeared in the literature and no studies have been carried out on the biosynthesis of these sterols using fungal systems. For reference, the transalkylation reaction leading to the formation of C_{29} sterols is given in Scheme 1 and a general pathway of cholesterol biosynthesis is given in Scheme 3

[176] Zander, J M and Caspi, E (1970) *J Biol Chem* **245**, 1682
[177] Bimpson, T (1970) Ph D Thesis, University of Liverpool
[178] Smith, A R H , Goad, L J and Goodwin, T W (1968) *Chem Commun* 926
[179] Smith, A R H , Goad, L J and Goodwin, T W (1968) *Chem Commun* 1259

SUMMARY

Relatively few fungal species have been examined for their sterol constituents and the different analytical methods employed almost equal the investigations in number There appears to be considerable variation in the sterol composition of fungal organisms, but certain generalizations concerning their distribution between the various taxa can be made Indeed, the more recent studies involving the identification of fungal sterols supports the contention that ergosterol is the most frequently encountered sterol in these primative organisms Since the more advanced instruments for separating and identifying complex mixtures of organic compounds have been applied in this area, it is more apparent that ergosterol is not the sole sterol in most fungal species and in many cases not the predominant one as previously believed Where ergosterol is present, but not predominant, other C_{28} sterols which are presumably ergosterol precursors accumulate to varying degrees depending on the species and conditions of growth The extent to which ergosterol and its precursors are further metabolized by fungi is largely unknown, but Turner[29] lists almost thirty fungal metabolites of this nature which have been structurally identified Moreover, it appears that ergosterol is totally absent from the aquatic phytomycetes and Δ^5 derivatives of C_{27}, C_{28}, and/or C_{29} sterols are present Desmosterol has also been identified in these species Although most of the homobasidiomycete fungi appear to produce ergosterol, this compound has not been reported for the few heterobasidiomycete (rust spores) fungi examined

As we learn more about the distribution of a particular class of compounds produced by biological systems, their role as a chemotaxonomic character must be considered It is questionable, however, whether lipids represent an important taxonomic tool in any system at specific or sub-specific levels After all, the principal value of chemotaxonomy is to provide additional characters to aid in the distinction of closely related species or subspecies which cannot be readily delimited on a morphological basis and to aid in establishing phylogenetic relationships Secondary metabolites or end-products of metabolism which generally have little known primary function have provided the most important chemotaxonomic characters in plant systems The fungi have a complex genetic constitution and exhibit a great inter- and intra-specific morphological variability which is influenced considerably by environmental factors and, from the few species examined, it appears that this variability is also reflected in their qualitative and particularly their quantitative distribution of sterols Thus, it is doubtful that sterols will emerge as an important character for distinguishing fungal species It must not be overlooked, however, that sterol distribution may prove helpful as an additional tool in organizing fungal taxa at higher taxonomic levels Patterson[180] has found that this to be true for the major algal divisions Rhodophyta (red algae) and Phaeophyta (brown algae) and suggests that sterol distributions may be of value in the systematics of the Chlorophyta (green algae)

Nes[181] points out that with the information now available on the *de novo* synthesis of sterols, it is apparent that these compounds are produced by the same general pathway regardless of the organism and that this pathway evolved early in the evolutionary development of biological systems In the formation of squalene from acetate, for example, the entire sterol producing biosphere appears to have a series of specific enzymes which catalyze reactions involving common substrates In the conversion of squalene to the principal 4-desmethyl sterols the same general steps are also present in most organisms, but the quantitatively important intermediates and the sequence in which certain reactions occur differs

[180] PATTERSON, G W (1971) *Lipids* **6**, 120
[181] NES, W R (1971) *Lipids* **6**, 219

between major taxonomic groups. A large number of natural and synthetic compounds which appear to be structurally intermediate in the formation of sterols have been identified and many of these can be biologically converted to the principal sterols such as cholesterol, ergosterol, and sitosterol Schroepfer *et al*[75] have listed forty-two sterol derivatives which can be converted to cholesterol in mammalian systems Although less attention has been given to phytosterol biosynthesis, it appears that the conversion of cycloartenol into principal 4-desmethyl phytosterols may occur through a number of possible intermediates which vary according to the plant system This also appears to be true in the formation of ergosterol by fungi The number of reports on the biosynthesis of fungal sterols is moderate and most of them involve the yeasts In many cases, these studies were not carried out with the aim of showing relevance to sterol formation by fungi but the yeast were used only as a tool for the study of analogous reactions occurring in plant and particularly mammalian systems. At this time, the information on the mode of ergosterol formation by both yeast and mycelial fungi is too fragmentary to determine if significant differences occur between these morphological forms As shown in Schemes 2 and 3, there are a number of sterols which represent potential intermediates in the formation of ergosterol by fungi These include 24-methyl and 24-methylene sterols and their Δ^{14} isomers The relative participation of 24-methyl and 24-methylene intermediates is determined by the point in the basic reaction sequence that that $\Delta^{24(28)}$-reductase is operative As noted before, introduction of the Δ^{14} double bond is independent of the 24-alkyl group and may be present in 24-demethyl, 24-methyl, and 24-methylene isomers However, only 24-methyl sterols having the Δ^{14} double bond have been isolated from fungi (see Table 1)

The quantitatively important intermediates in sterol biosynthesis differ not only between species of major taxa but they may also vary between tissues and developmental stages of the same organism These intermediates cannot always be accurately determined by product analysis since those which are quantitatively important may exist in low steady-state concentrations The high relative concentrations of potential intermediates which are often detected may only be an expression of the less favored pathways operating simultaneously with the predominant one The accumulation of these compounds may also reflect their inability to be further metabolized to the predominant sterol product or a low rate of turnover It has not been established whether the enzymes responsible for the conversion of 4,4-dimethyl to principal 4-desmethyl sterols are relatively non-specific or, if a number of enzymes specific for each intermediate exists Now that the basic steps in this conversion are generally known, the two principal problems which remain are determining the sequence of these reactions involving the quantitatively significant intermediates and, perhaps more important, is understanding the regulatory mechanisms which determine the reaction sequence [180]

The degree to which major groups of organisms vary in their ability to produce their predominant sterols by alternate routes appears to be in the order plants > fungi > animals However, with more investigation it is probable that considerably more variability may be found in the fungi The fungi are a diverse group of organisms which have a complex genetic potential Throughout the fungal life cycle, the two generations which differ in ploidy levels are further complicated by the dikaryotic and heterokaryotic conditions These conditions could lead to additional inter- and intra-specific variation in the sterol composition of this heterogeneous group of organisms For this reason, generalizations concerning the sterol distributions in a fungal species should be based on analyses of more than a single isolate In only a few instances has more than one isolate of a single mycelial species

been examined for its sterol composition and significant differences were noted (see Table 1) Phenotypic variations at the molecular level may also vary within the same culture, as is often illustrated by differences in pigmentation

Acknowledgements—Special thanks to Mrs Lila Mager for her secretarial assistance and Tony Paschall for his technical assistance