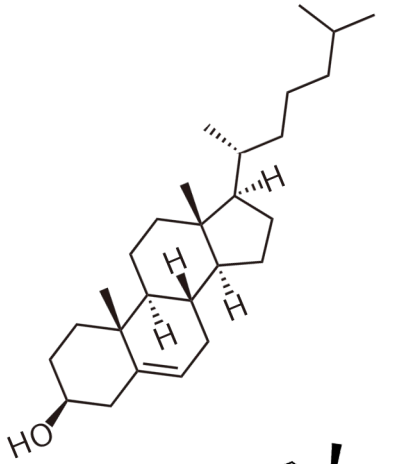
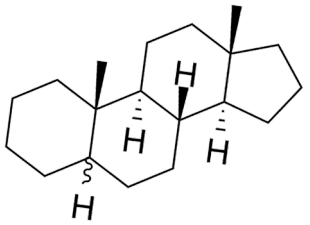
Protein Mechanics

1st Seminar

Discovery and Study of Metabolic Pathway





Lanosterol Biosynthesis

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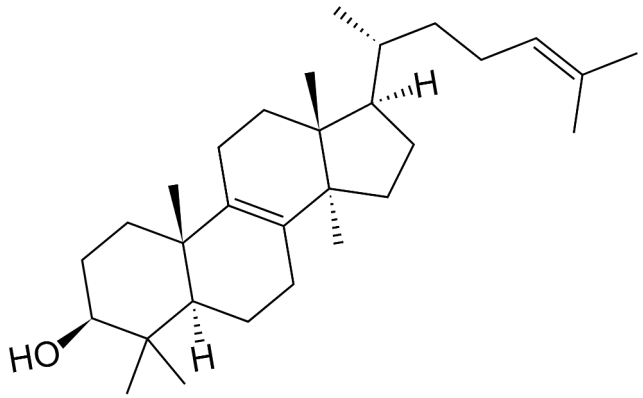

April 2024

ΠΑΝΕΠΙΣΤΗΜΙΟ ΚΡΗΤΗΣ
UNIVERSITY OF CRETE

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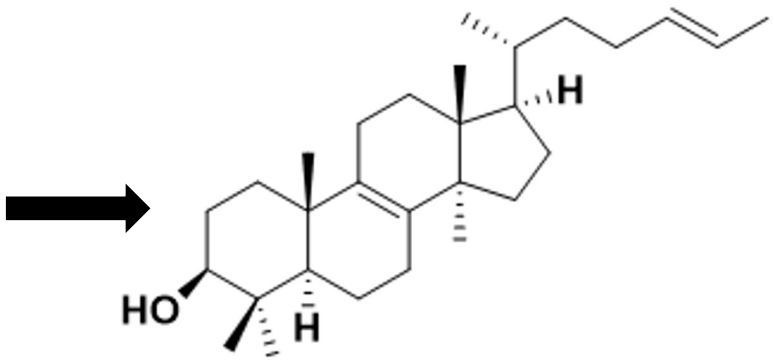
Introduction

Lanosterol was first isolated from sheep's wool in 1930 by German researchers A. Windhaus and R. Tschesche

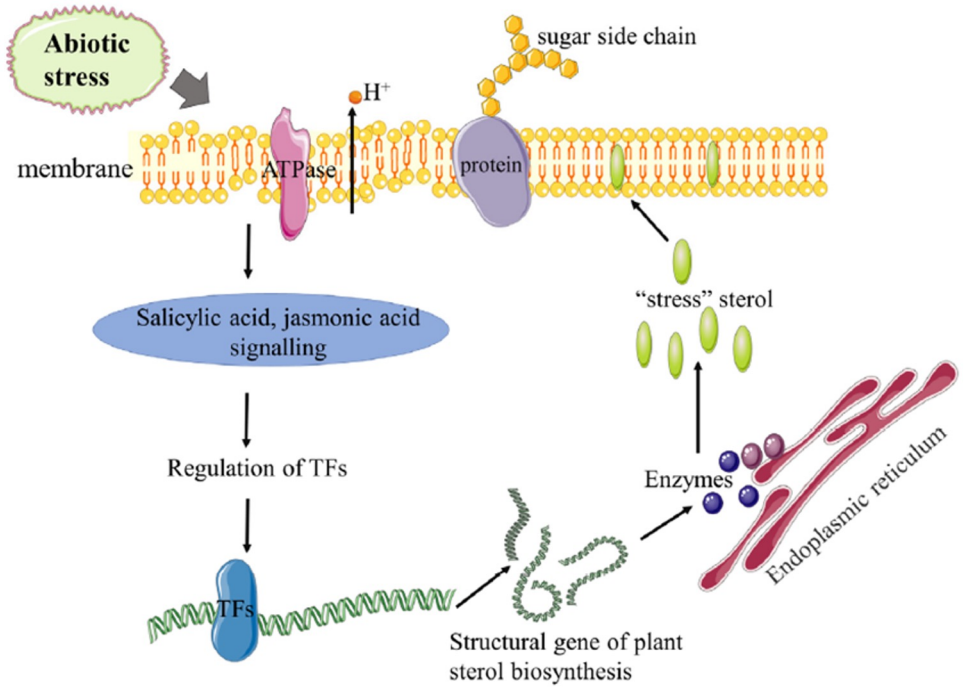
Lanolin



lanosterol

In **photosynthetic organisms** (plants, algae) the role of lanosterol is taken by cycloartenol, a **cyclopropane triterpenoid**.

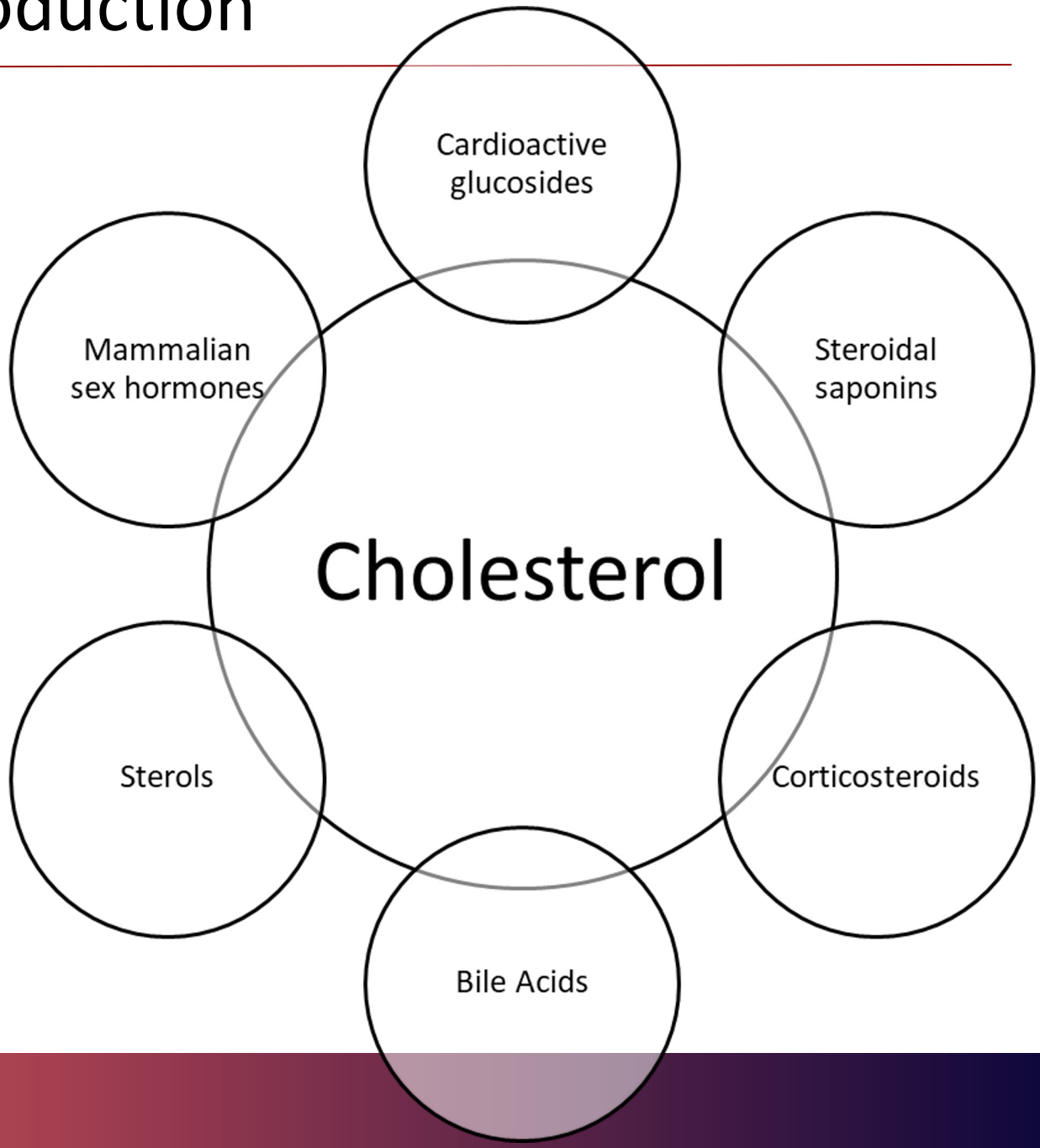
Cycloartenol is the precursor of all sterols in plants and is also derived from squalene oxide.



Introduction

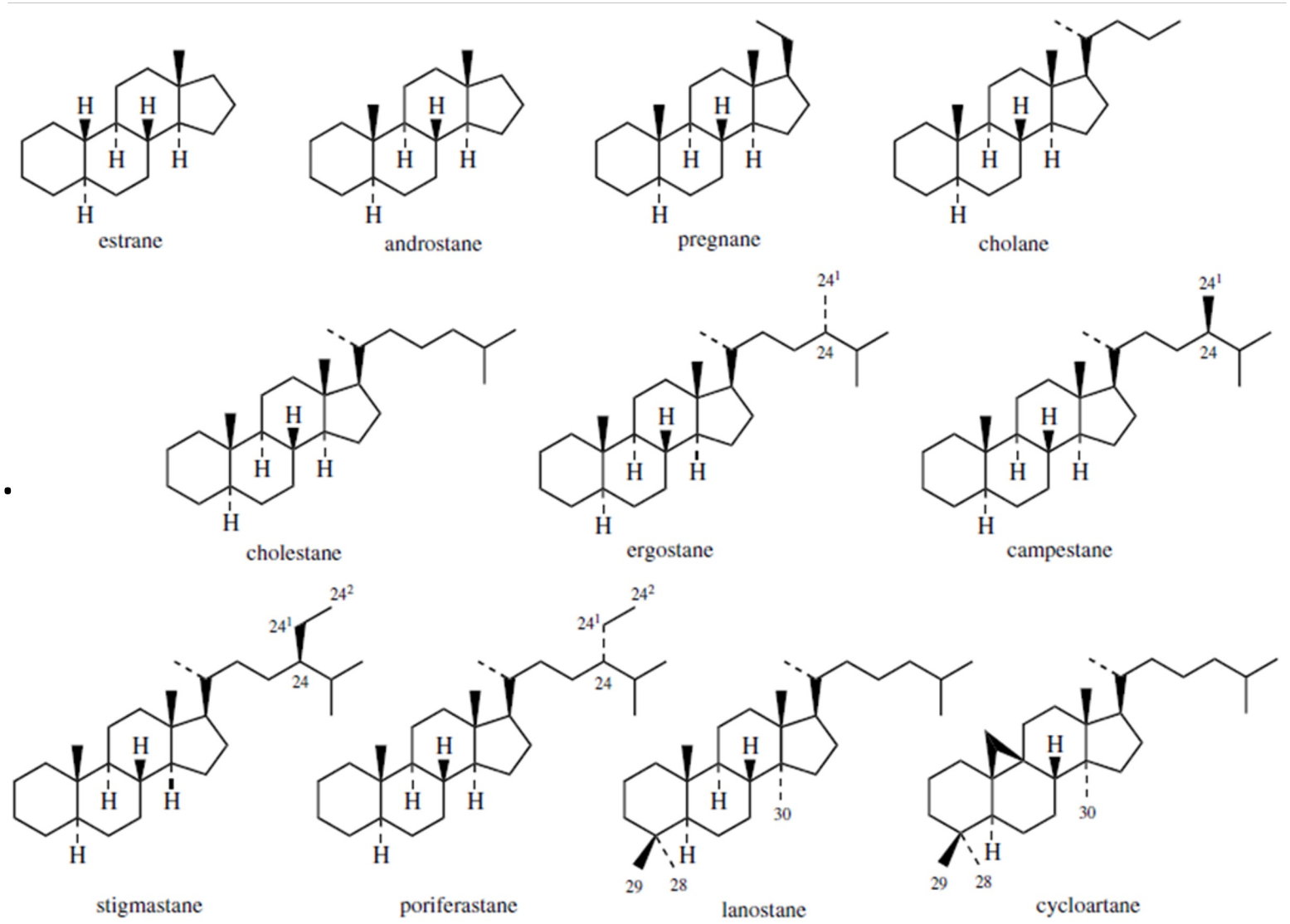
•**Steroids** are triterpenoid derivatives that contain the tetracyclic ring system of **lanosterol** but lack the three methyl groups in C-4 and C-14.

"the core structure from which all others are derived by biological modification"
(Merck Index)



Introduction

Cholesterol derived steroids…



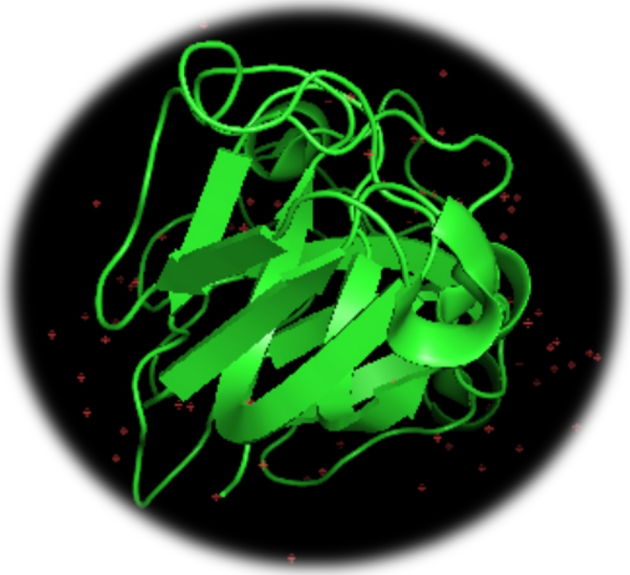
Introduction

- **Lanosterol & Cataract Treatment**

What's cataract?

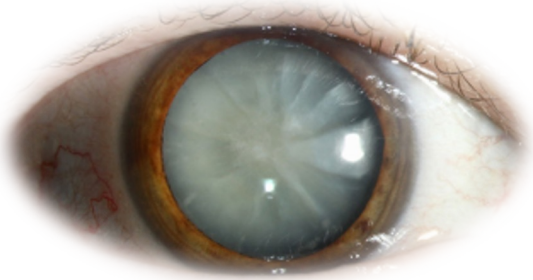
Crystalline proteins form a strict macro-structure responsible for: i) less transparency and ii) refractive index

Lanosterol synthase (LSS) is expressed in the eye lens!



PDB entry: 2G98

Disruption of 3D structure → Protein Aggregation



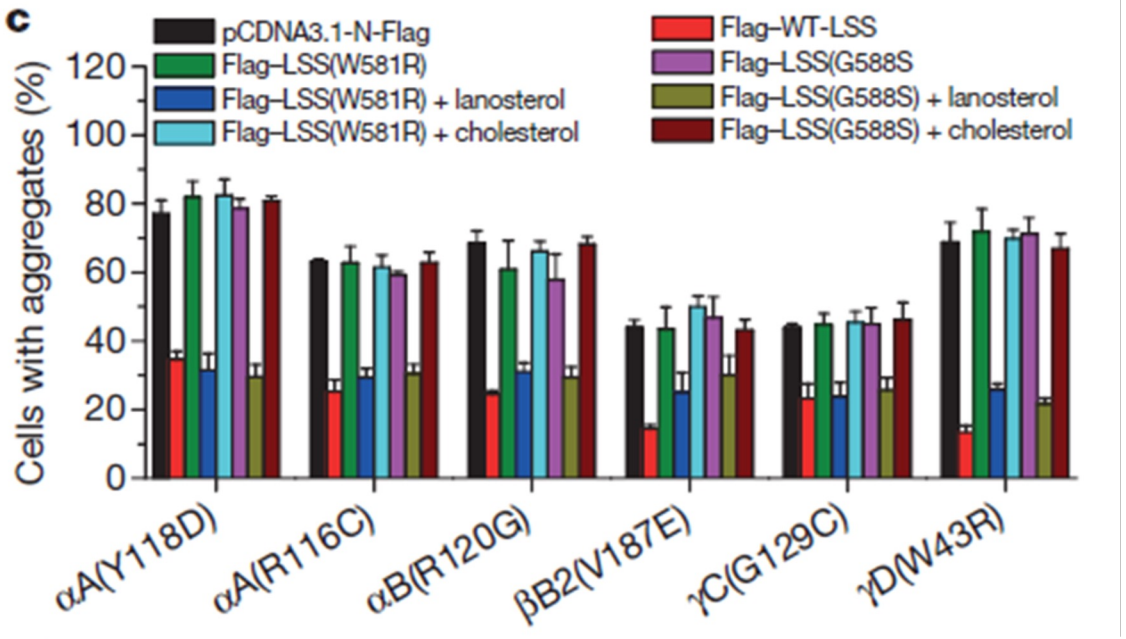
"Aggregation is an intrinsic property of mutant crystallines."(Zhao et al. 2015)

Introduction

W581R, G588S

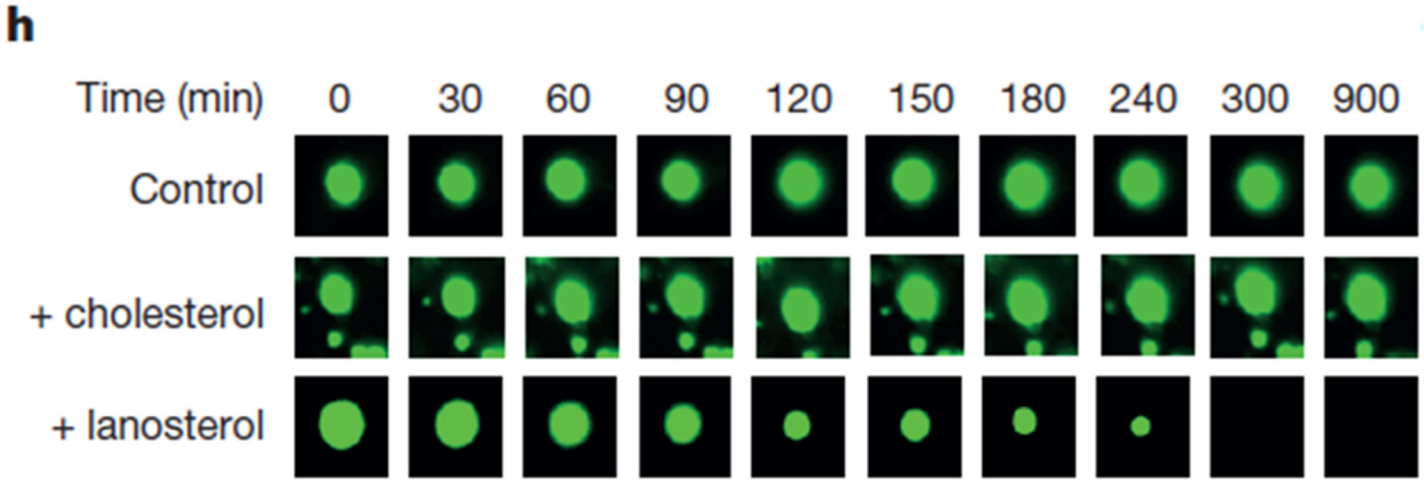
↓

Loss of LSS cyclase activity



Mutant crystallines

h, Effects of DMSO, cholesterol or lanosterol on αA-crystallin(Y118D) aggregates in human lens progenitor cells by serial live-cell imaging. Progression of crystallin aggregation dissolution by lanosterol can be observed, as evidenced by decreased green fluorescence following the time-course.



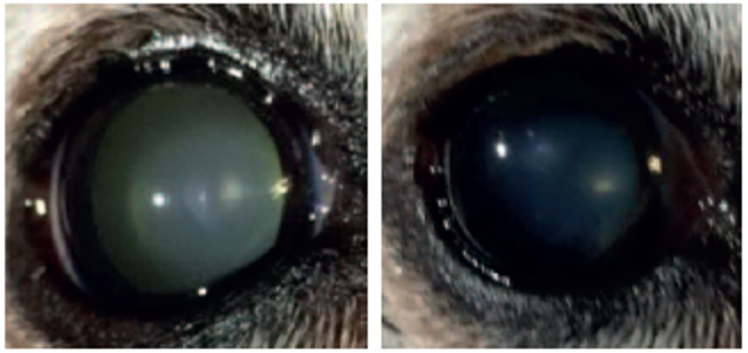
Conclusion:

- **Lanosterol**, not cholesterol, and
- **WT-LSS**

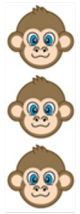
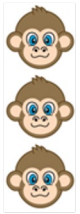
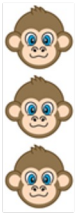
can decrease preformed crystalline aggregates

Introduction

•Dogs treated with lanosterol showed increased lens clarity

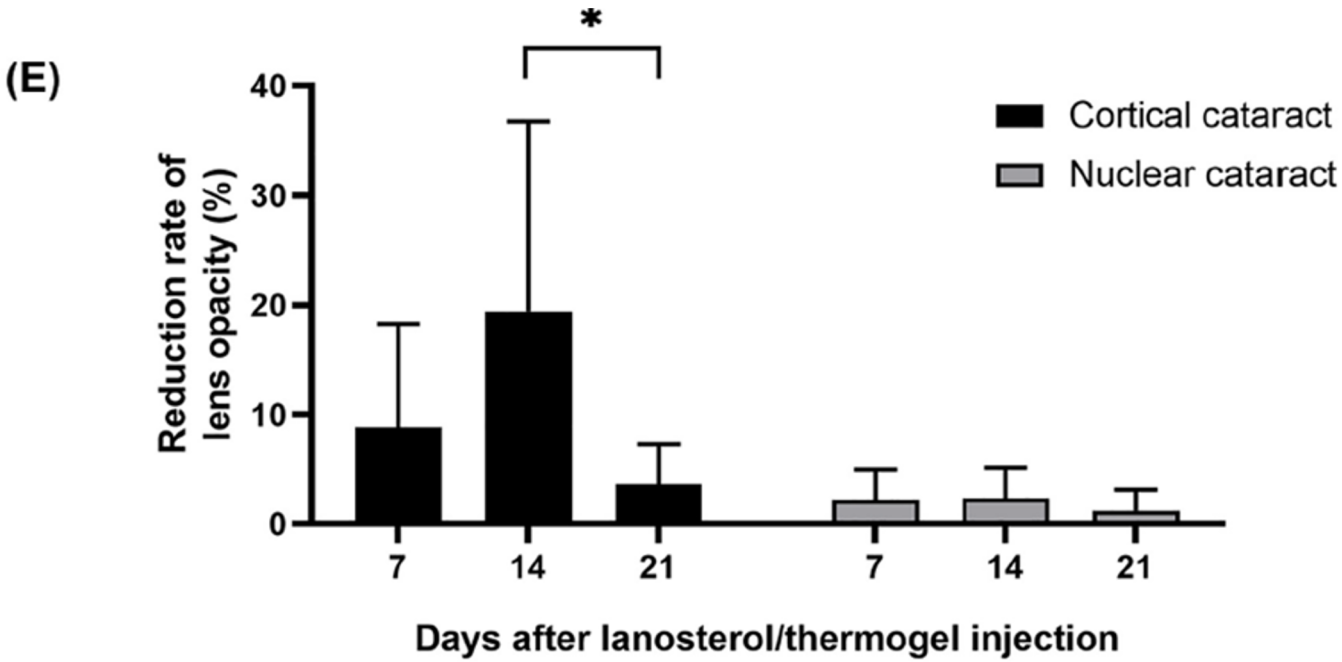


•**2022**: Effect of lanosterol concentration in lens opacification and proteins in cynomolgus monkeys

CONTROL	NATURAL CORTICOID CATARACT	NUCLEAR CATARACT
		

•**First in vivo model on primates!**

•Thermogel injection



Introduction

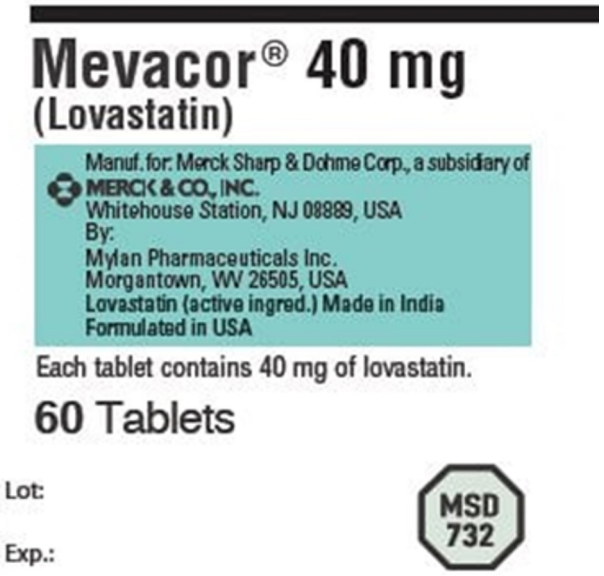
Increased LDL cholesterol levels are associated with coronary heart disease (CHD)

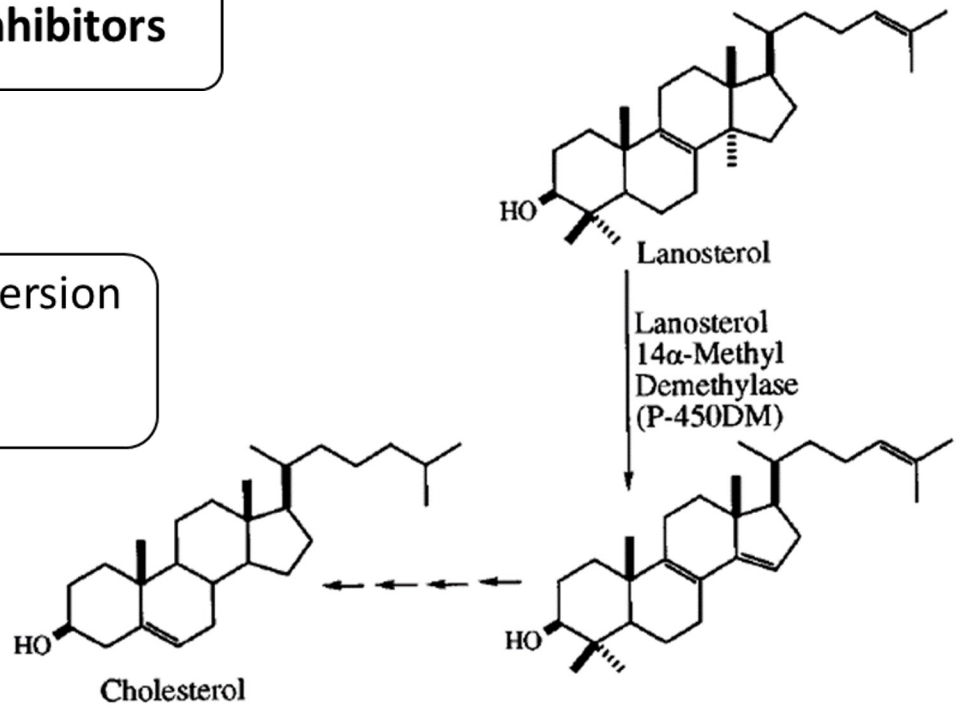
Development of cholesterol biosynthesis **inhibitors**

Inhibition of rate limiting step of the conversion of lanosterol to cholesterol (catalyzed by **P-450DM**)

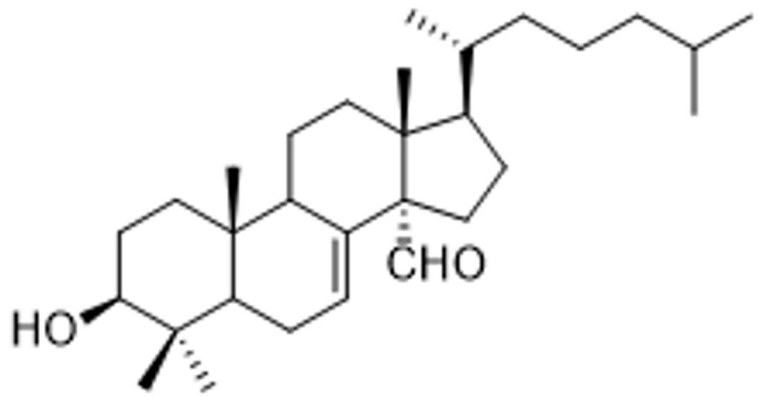
Lanosterol analogs as inhibitors

Competitive inhibitor of HMGR (hypercholesterolemia)





Introduction



9

Lanosterol analog 9:

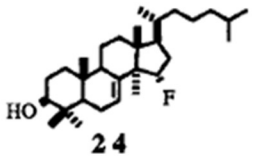
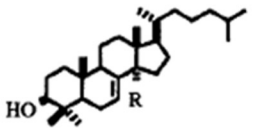
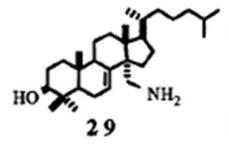
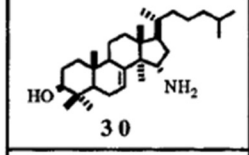
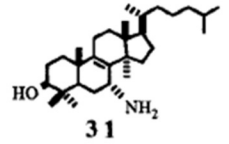
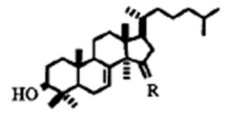
- Binds more tightly to P-450DM compared to 24,25-dihydrolanosterol (6) which is one of the natural substrates
- Competitive inhibitor
- Endogenous suppressor of HMGR

Inhibitors

- **Type 1**: 25a, 25b, 26, similar structure to natural intermediates (i.e 9)
- **Type 2**: Aminolanosterols
- **Type 3**: Ketone or oxime analogs of lanosterol (C-15)

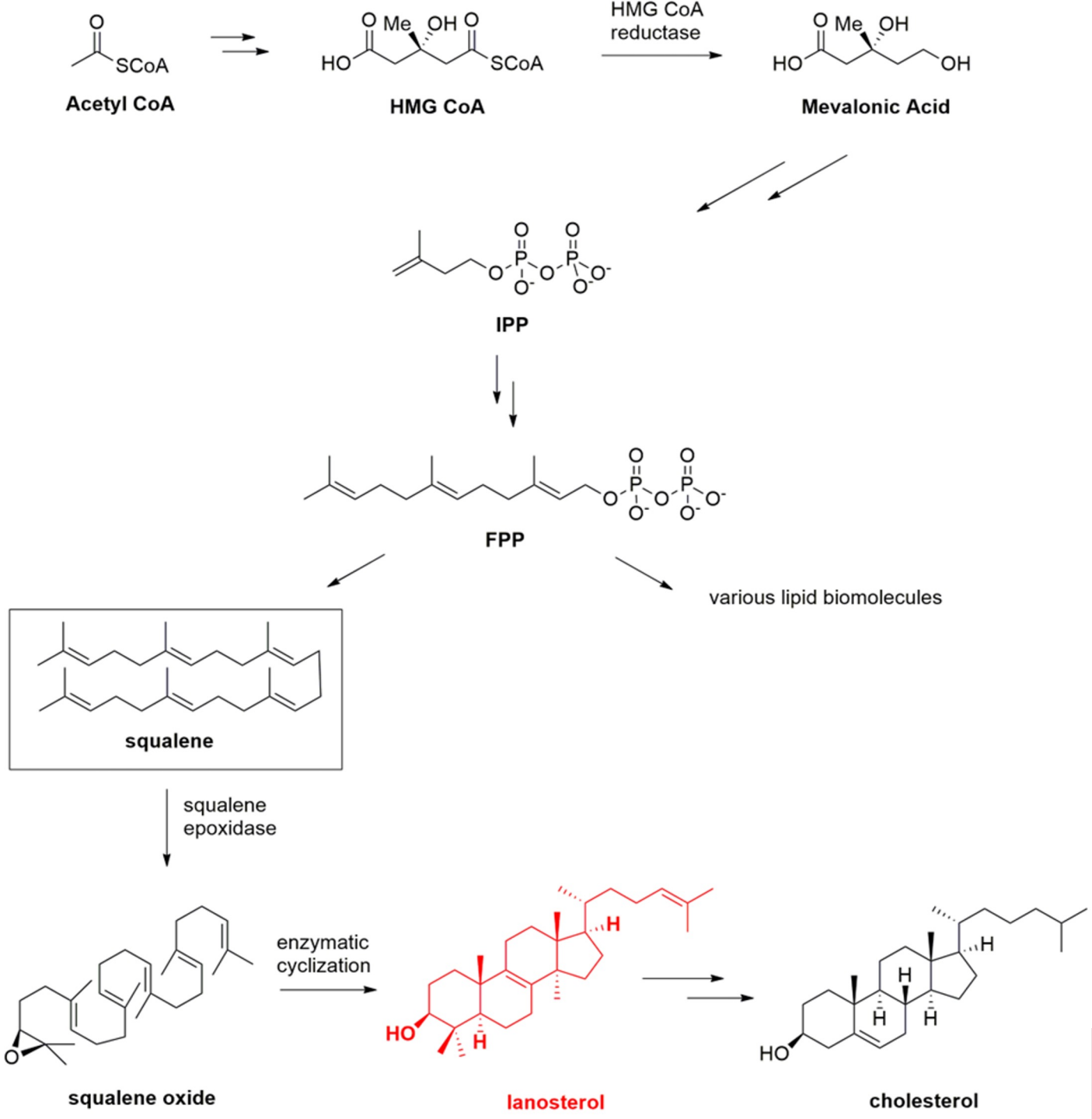
Introduction

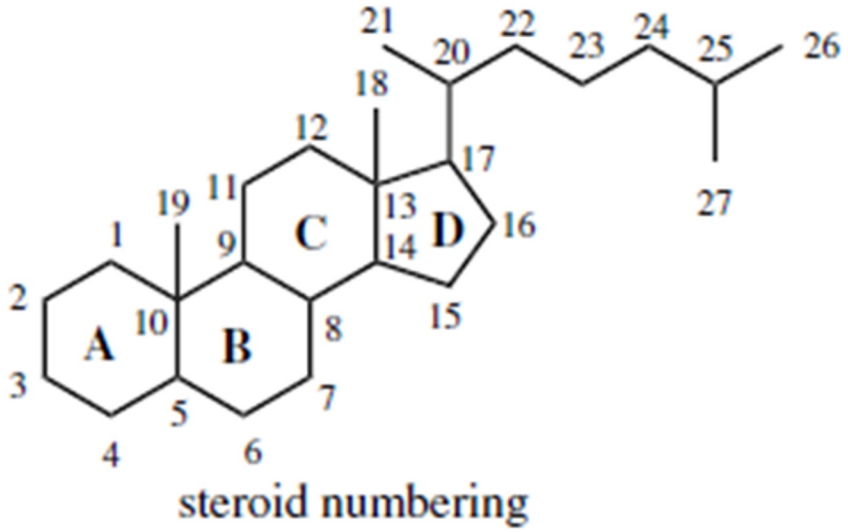
Table 1 Summary of the Biochemical Evaluation of Selected Potential Dual-Action Inhibitors of Cholesterol Biosynthesis

	Inhibitor	IC_{50} (μM) P-450DM *in vitro* [a]	P-450DM CHO	HMGR CHO	HMGR AR45	Reference
TYPE 1	 **24**	>100	5.0	1.0	>20	31, 46
TYPE 1	 **9** R=CHO	NA[b]	1.9	3.0	2.0	16, 32
TYPE 1	**25a** R=CH(OH)CH_3 (less polar diastereomer)	0.33	0.3	3.0	>20	7, 39
TYPE 1	**25b** R=CH(OH)CH_3 (more polar diastereomer)	1.6	0.3	1.5	>20	7, 39
TYPE 1	**26** R=C(CH_3)O	9.1	2.7	1.0	>20	7, 39
TYPE 1	**27** R=NOH	8.9	4.5	1.0	2.0	40
TYPE 1	**28** R=$NOCH_3$	100	>20	10	>20	40
TYPE 2	 **29**	4.0	>20	1.5	>20	
TYPE 2	 **30**	1.5	0.15	6.0	>20	
TYPE 2	 **31**	60	1.0	2.0	5.0	
TYPE 3	 **32** R=O	7.1	1.0	0.5	1.8	40
TYPE 3	**33** R=NOH	3.0	2.0	0.6	1.5	40
TYPE 3	**34** R=$NOCH_3$	77	>20	15	>20	40
TYPE 3	**35** R=NOBn	100	>20	15	NA	40

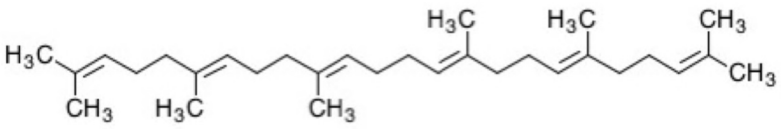
Experiments carried out using rat liver microsomal preparations.
IC50 value is not available.
See Reference 16 for details of inhibition of P-450DM by compound **9**.

Steroid Biosynthesis





Squalene

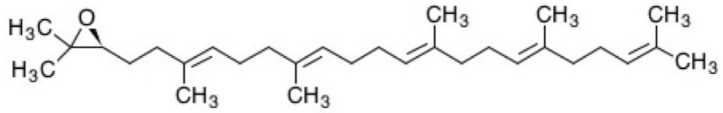


C00751

Squalene monooxygenase

Alternative squalene epoxidase

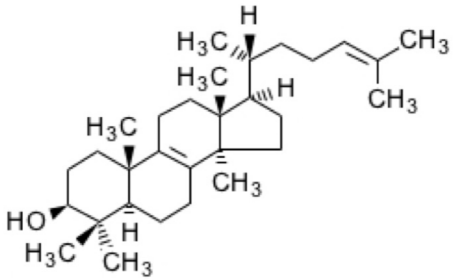
(S)—Squalene—2,3—epoxide



C01054

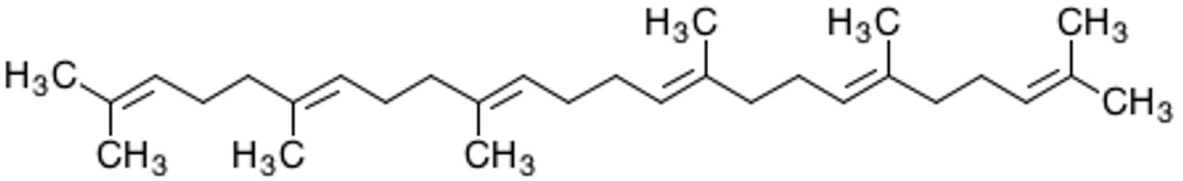
Lanosterol synthase

Lanosterol

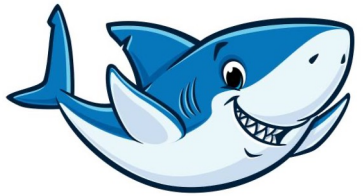


C01724

Squalene



C00751



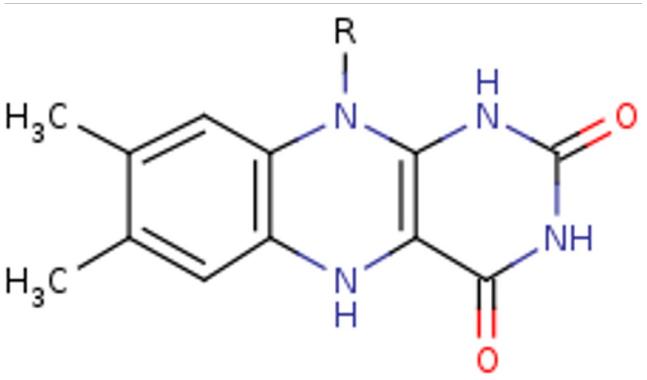
- Formula: $C_{30}H_{50}$
- Alternative names: spinacene, supraene
- KEGG entry: C00751
- CAS number: 111-02-4
- Price per 1 gram: €301*

*As analytical standard from Sigma Aldrich

Squalene monooxygenase	Alternative squalene oxidase
▪ EC number: 1.14.14.17 1. Oxidoreductases 1.14 Acting on paired donors with incorporations or reduction of molecular O_2 1.14.14 With reduced flavin or flavoprotein as one donor, and incorporation of one atom of oxygen into the other donor 1.14.14.17 Squalene monooxygenase	▪EC number: 1.14.19.- 1. Oxidoreductases 1.14 Acting on paired donors with incorporations or reduction of molecular O_2 1.14.19 With oxidation of a pair of donors resulting in the reduction of O_2 to two molecules of water

Reaction catalyzed by squalene monooxygenase:

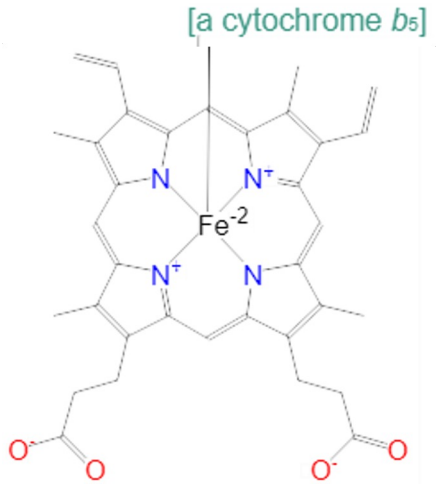
Squalene + reduced NADPH-hemoprotein reductase + O_2 ⟶ (3S)-2,3-epoxy-2,3-dihydrosqualene + oxidized NADPH + H_2O



Cofactor: NADPH + FAD

Reaction catalyzed by alternative squalene oxidase:

Squalene + 2 Ferrocytochrome b5 + O_2 + 2H ⟶ (S)-2,3-epoxysqualene + 2 Ferricytochrome b5 + H_2O

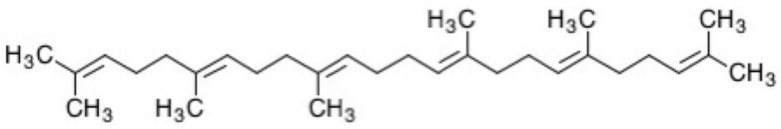


Cofactor: Fe*

*Ferrocytochrome b5 pubchem substance SID: 4245

Ferricytochrome b5 pubchem substance SID: 4242

Squalene

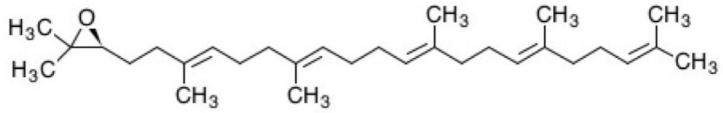


C00751

Squalene monooxygenase

Alternative squalene epoxidase

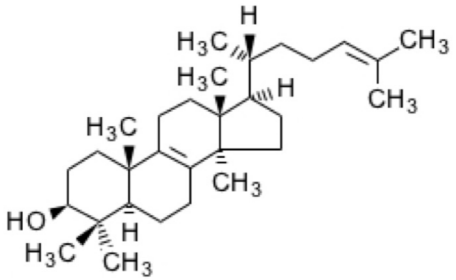
(S)—Squalene—2,3—epoxide



C01054

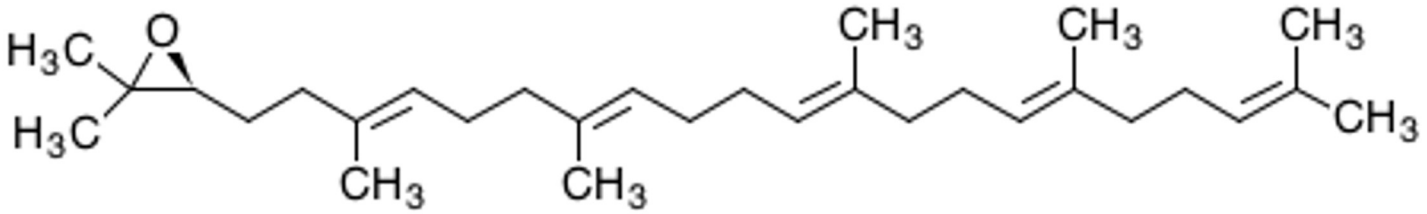
Lanosterol synthase

Lanosterol



C01724

(S)-Squalene-2,3-epoxide



C01054

- Formula: $C_{30}H_{50}O$
- Alternative names: (S)-2,3-epoxisqualene, Squalene-2,3-oxide, 2,3-oxidosqualene, (3S)-2,3-epoxy-2,3-dihydrosqualene
- CAS number: 54910-48-4
- KEGG entry: C01054

Lanosterol Synthase

- EC number: 5.4.99.7

  5. Isomerases

  5.4 Intramolecular transferases

  5.4.99 Transferring other groups

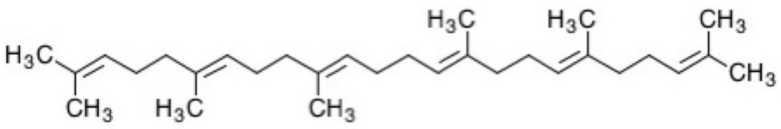
  5.4.99.7 Lanosterol synthase

- Cofactor: None
- It catalyzes the cyclization of (S)-Squalene-2,3-epoxide

Reaction:

(S)-Squalene-2,3-epoxide  Lanosterol

Squalene

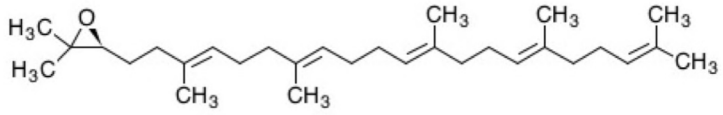


C00751

Squalene monooxygenase

Alternative squalene epoxidase

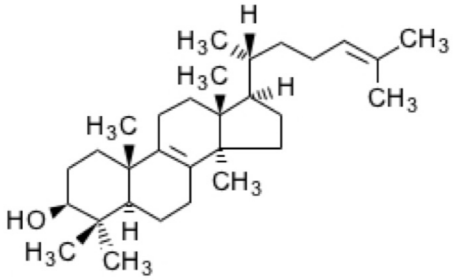
(S)—Squalene—2,3—epoxide



C01054

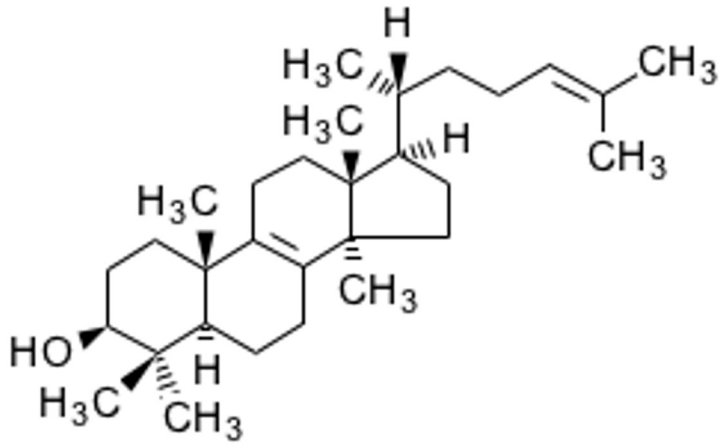
Lanosterol synthase

Lanosterol



C01724

Lanosterol

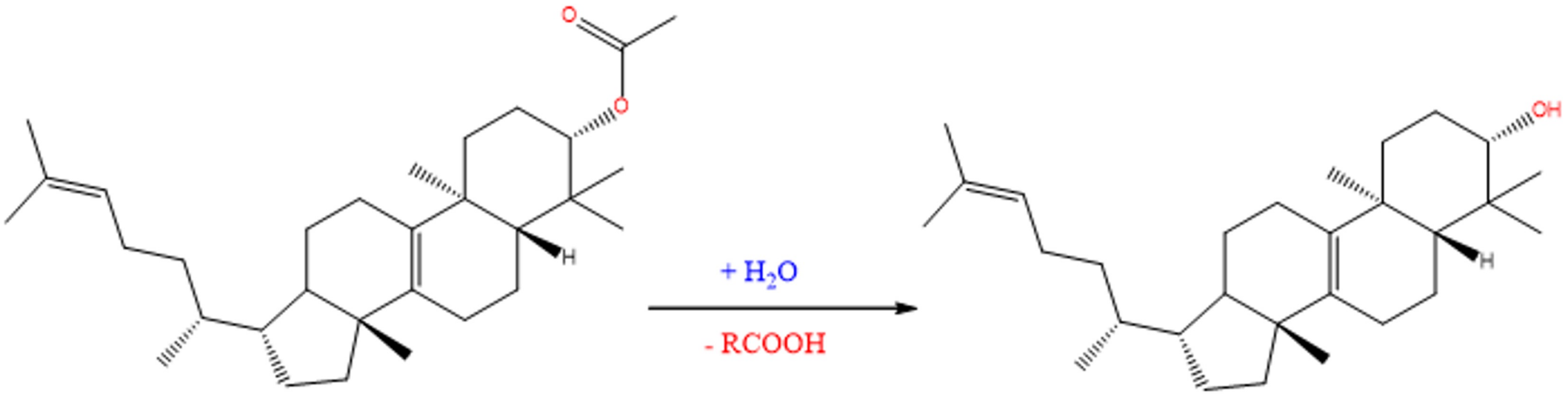


C01724

- Formula: C30H50O
- Alternative names: 4,4’,14-alpha-trimethyl-5alpha-cholesta-8,24-dien-3beta-ol
- KEGG entry: C01724
- CAS number: 79-63-0
- Price per 5 mg: €821*

*>=93%, powder. From Sigma Aldrich

Alternative Pathway: Lanosteryl acetate



- Formula: $C_{32}H_{52}O_2$
- Alternative names: Lanosta-8,24-dien-3β-ol, acetate/ Lanosta-8,24-dien-3β-yl acetate/ Lanosterol acetate/ Lanosteryl acetate/ 3β-Acetoxylanosta-8,24-diene

- CAS number: 2671-68-3
- Molecular weight: 468.7541
- Price per 5 mg: €464.48*

* 95.00% Lanosteryl acetate from ChemicalBook

Why Lanosterol Synthase?

Involved in the rate-determining step of the early cholesterol and other sterol biosynthesis pathways

Lanosterol Synthase

Attractive drug target → Interest in lanosterol synthase inhibitors as potential cholesterol-reducing drugs

Involved in one of the most remarkable one-step constructions → Cationic polycyclization reaction of (3S)-2,3-oxidosqualene into tetracyclic lanosterol

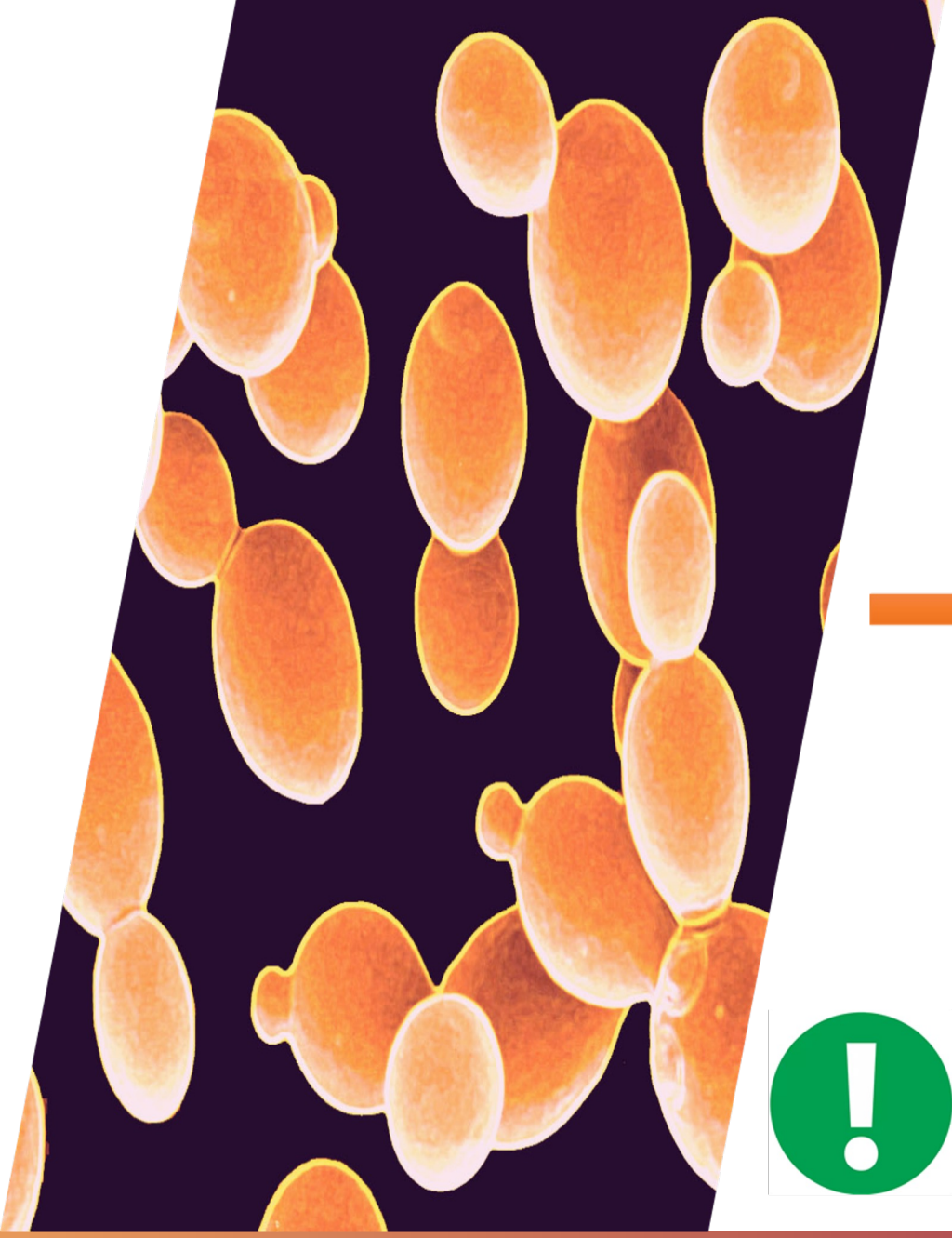
- Alteration of 20 bonds
- Formation of 4 rings
- It sets 7 stereocenters

The Origin of Lanosterol Synthase

Genes Encoding Lanosterol Synthase

- Animals & Humans
  - Lanosterol Synthase gene (LSS gene)
    or
    human Oxidosqualene Cyclase gene (hOSC)
- Fungi
  - *S. Cerevisiae* (SceERG7 gene)
  - *Candida albicans*
  - *Ganoderma lucidum*
- Plants
  - *Arabidopsis thaliana* (At3g45130)

***Saccharomyces cerevisiae*: The best studied eukaryote**



brewer's yeast

baker's yeast

- ✓ It has been extensively studied → Model unicellular organism
- ✓ Genetic manipulation → Easy overexpression or deletion of genes
- ✓ High expression levels
- ✓ Stable and scalable → cost-effectiveness
- ✓ Reduced risk of contamination

Lanosterol synthase is a preferred target of antifungal drugs

Nucleotide Sequence of the *SceERG7* Gene

ATGACAGAATTTTATTCTGACACAATCGGTCTACCAAAGACAGATCCACGTCTTTGGAGACTGAGAACTGATGAGCTAGGCCGAGAAAGCTGGGAATATTTAACCCCTCAGCAAGCCGCA
AACGACCCACCATCCACTTTCACGCAGTGGCTTCTTCAAGATCCCAAATTTCCTCAACCTCATCCAGAAAGAAATAAGCATTCACCAGATTTTTCAGCCTTCGATGCGTGTCATAATGGT
GCATCTTTTTTCAAACTGCTTCAAGAGCCTGACTCAGGTATTTTTCCGTGTCAATATAAAGGACCCATGTTCATGACAATCGGTTACGTAGCCGTAAACTATATCGCCGGTATTGAAATT
CCTGAGCATGAGAGAATAGAATTAATTAGATACATCGTCAATACAGCACATCCGGTTGATGGTGGCTGGGGTCTACATTCTGTTGACAAATCCACCGTGTTTGGTACAGTATTGAACTAT
GTAATCTTACGTTTATTGGGTCTACCCAAGGACCACCCGGTTTGCGCCAAGGCAAGAAGCACATTGTTAAGGTTAGGCGGTGCTATTGGATCCCCTCACTGGGGAAAAATTTGGCTAAGT
GCACTAAACTTGTATAAATGGGAAGGTGTGAACCCTGCCCCTCCTGAAACTTGGTTACTTCCATATTCACTGCCCATGCATCCGGGGAGATGGTGGGTTCATACTAGAGGTGTTTACATT
CCGGTCAGTTACCTGTCATTGGTCAAATTTTCTTGCCCAATGACTCCTCTTCTTGAAGAACTGAGGAATGAAATTTACACTAAACCGTTTGACAAGATTAACTTCTCCAAGAACAGGAAT
ACCGTATGTGGAGTAGACCTATATTACCCCCATTCTACTACTTTGAATATTGCGAACAGCCTTGTAGTATTTTACGAAAAATACCTAAGAAACCGGTTCATTTACTCTCTATCCAAGAAG
AAGGTTTATGATCTAATCAAAACGGAGTTACAGAATACTGATTCCTTGTGTATAGCACCTGTTAACCAGGCGTTTTGCGCACTTGTCACTCTTATTGAAGAAGGGGTAGACTCGGAAGCG
TTCCAGCGTCTCCAATATAGGTTCAAGGATGCATTGTTCCATGGTCCACAGGGTATGACCATTATGGGAACAAATGGTGTGCAAACCTGGGATTGTGCGTTTGCCATTCAATACTTTTTC
GTCGCAGGCCTCGCAGAAAGACCTGAATTCTATAACACAATTGTCTCTGCCTATAAATTCTTGTGTCATGCTCAATTTGACACCGAGTGCGTTCCAGGTAGTTATAGGGATAAGAGAAAG
GGGGCTTGGGGCTTCTCAACAAAAACACAGGGCTATACAGTGGCAGATTGCACTGCAGAAGCAATTAAAGCCATCATCATGGTGAAAAACTCTCCCGTCTTTAGTGAAGTACACCATATG
ATTAGCAGTGAACGTTTATTTGAAGGCATTGATGTGTTATTGAACCTACAAAACATCGGATCTTTTGAATATGGTTCCTTTGCAACCTATGAAAAAATCAAGGCCCCACTAGCAATGGAA
ACCTTGAATCCTGCTGAAGTTTTTGGTAACATAATGGTAGAATACCCATACGTGGAATGTACTGATTCATCCGTTCTGGGGTTGACATATTTTCACAAGTACTTCGACTATAGGAAAGAG
GAAATACGTACACGCATCAGAATCGCCATCGAATTCATAAAAAAATCTCAATTACCAGATGGAAGTTGGTATGGAAGCTGGGGTATTTGTTTTACATATGCCGGTATGTTTGCATTGGAG
GCATTACACACCGTGGGGGAGACCTATGAGAATTCCTCAACGGTAAGAAAAGGTTGCGACTTCTTGGTCAGTAAACAGATGAAGGATGGCGGTTGGGGGGAATCAATGAAGTCCAGTGAA
TTACATAGTTATGTGGATAGTGAAAAATCGCTAGTCGTTCAAACCGCATGGGCGCTAATTGCACTTCTTTTCGCTGAATATCCTAATAAAGAAGTCATCGACCGCGGTATTGACCTTTTA
AAAAATAGACAAGAAGAATCCGGGGAATGGAAATTTGAAAGTGTAGAAGGTGTTTTCAACCACTCTTGTGCAATTGAATACCCAAGTTATCGATTCTTATTCCCTATTAAGGCATTAGGT
ATGTACAGCAGGGCATATGAAACACATACGCTTTAA

2196 nucleotides (bp)

Amino Acid Sequence of the *SceERG7* Gene

MTEFYSDTIGLPKTDPRLWRLRTDELGRESWEYLTPQQAANDPPSTFTQWLLQDPKFPQP
HPERNKHSPDFSAFDACHNGASFFKLLQEPDSGIFPCQYKGPMFMTIGYVAVNYIAGIEI
PEHERIELIRYIVNTAHPVDGGWGLHSVDKSTVFGTVLNYVILRLLGLPKDHPVCAKARS
TLLRLGGAIGSPHWGKIWLSALNLYKWEGVNPAPPETWLLPYSLPMHPGRWWVHTRGVYI
PVSYLSLVKFSCPMTPLLEELRNEIYTKPFDKINFSKNRNTVCGVDLYYPHSTTLNIANS
LVVFYEKYLRNRFIYSLSKKKVYDLIKTELQNTDSLCIAPVNQAFCALVTLIEEGVDSEA
FQRLQYRFKDALFHGPQGMTIMGTNGVQTWDCAFAIQYFFVAGLAERPEFYNTIVSAYKF
LCHAQFDTECVPGSYRDKRKGAWGFSTKTQGYTVADCTAEAIKAIIMVKNSPVFSEVHHM
ISSERLFEGIDVLLNLQNIGSFEYGSFATYEKIKAPLAMETLNPAEVFGNIMVEYPYVEC
TDSSVLGLTYFHKYFDYRKEEIRTRIRIAIEFIKKSQLPDGSWYGSWGICFTYAGMFALE
ALHTVGETYENSSTVRKGCDFLVSKQMKDGGWGESMKSSELHSYVDSEKSLVVQTAWALI
ALLFAEYPNKEVIDRGIDLLKNRQEESGEWKFESVEGVFNHSCAIEYPSYRFLFPIKALG
MYSRAYETHTL

731 amino acids

Expasy
Swiss Bioinformatics Resource Portal

: Characteristics of the fungal Lanosterol Synthase (*SceERG7*)

Stable protein

MOLECULAR WEIGHT	pI (Theoretical)	INSTABILITY INDEX (II)	EXTINCTION COEFFICIENT (M^{-1} cm^{-1} at 280 nm)		ABSORBANCE 0.1%(=1 g L^{-1}, OPTICAL DENSITY)	
			With cystines:	*Without cystines*:	*With cystines*:	*Without cystines*:
83460.42 g mol^{-1}	6.16	39.76	153975	153100	1.845	1.834

$$E = \#(Tyr) \times Ext(Tyr) + \#(Trp) \times Ext(Trp) + \#(Cystine) \times Ext(Cystine),$$

Where: #(Tyr)=40, Ext(Tyr)=1490, #(Trp)=17, Ext(Trp)=5500, #(Cystine)=7, Ext(Cystine)=125

$$Absorb = \mathrm{E} \div Molecular\ weight$$

Where: Molecular weight= 83460.42 g mol^{-1}

SOLUPROT

Protein	Solubility
sce:YHR072W	0.447

The Lanosterol synthase song

1

OS-Cyclase, OS-Cyclase, a great enzyme are you
OS-Cyclase, OS-Cyclase, the sterol pathway needs you
You take open squalene oxide,And quickly form four rings from it
OS-Cyclase, OS-Cyclase, you shape the steroid backbone.

2

OS-Cyclase, OS-Cyclase, in eukaryotes you spread out
OS-Cyclase, OS-Cyclase, bacteria cells do not lodge you
In fungi and animals you form
Just triterpene lanosterol,
In plants your creativity bursts into plenty of compounds.

3

Lord of the Rings, Lord of the Rings, your mechanism is magic
Your action starts from (an) aspartate, that protonates the substrate
The target is its epoxide,
Which opens forming alcohol group,
From then no one, from then no one, can stop cyclizing process.

4

Electron cloud of double bond starts soon to be excited
And as a hungry dog to a bone it throws itself on C-2
Forming the first of the four rings,
Leaving a new plus charge behind,
A thrill knocks on the door of all the other double bond clouds.

...

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