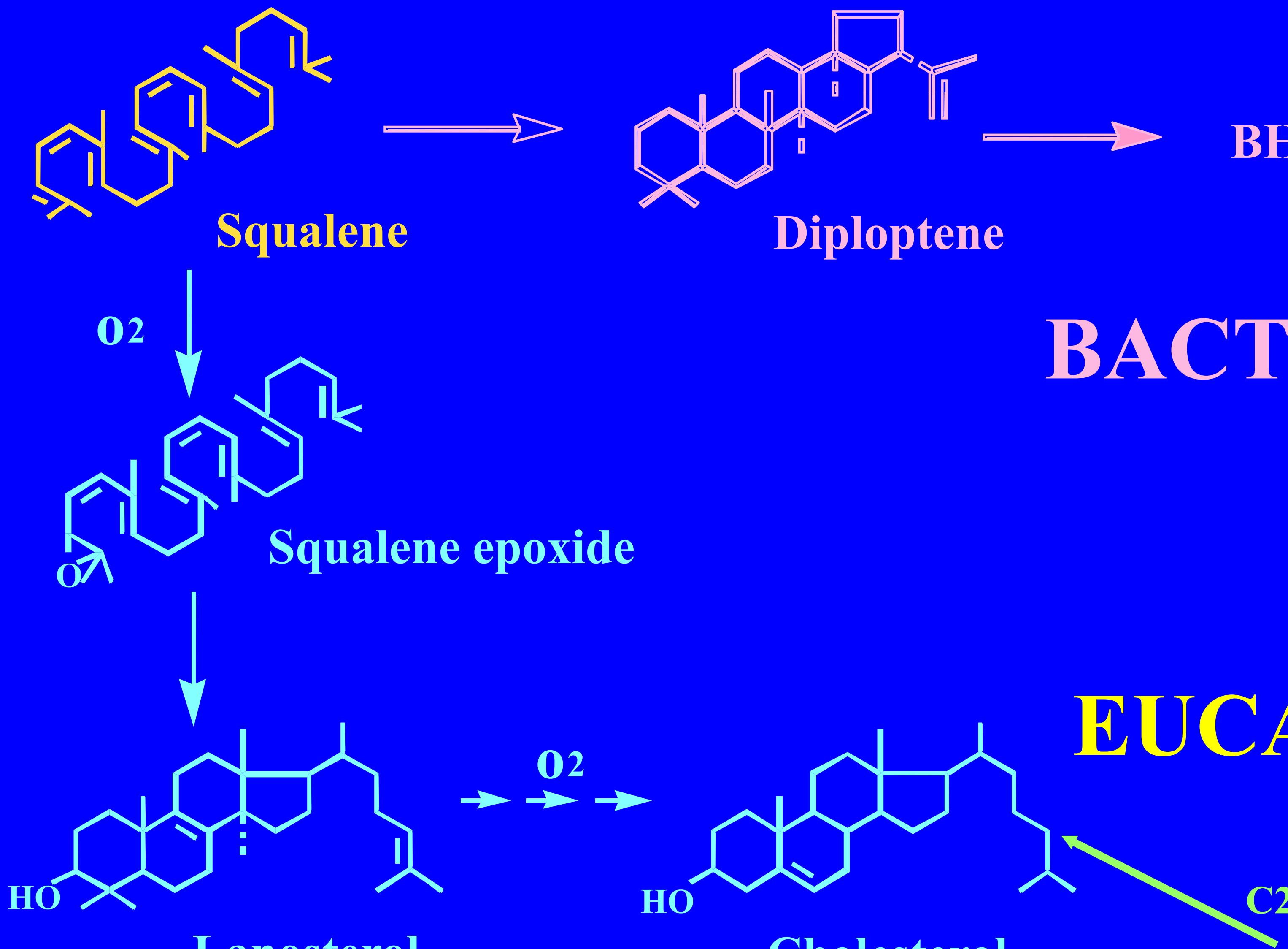
12.158 Lecture 6

- Steroids
  - Structures and biosynthesis
  - Diagenesis
  - Steroidal hydrocarbons; stereochemistry vs maturity
  - Steroids as age and environment indicators
  - Enigmatic steroids 2- and 3-alkyl and carboxysteroids

Evolution of Hopane & Sterol Bioynthesis



Squalene

Diploptene

BHP

BACTERIA

Squalene epoxide

EUCARYA

Lanosterol

Cholesterol

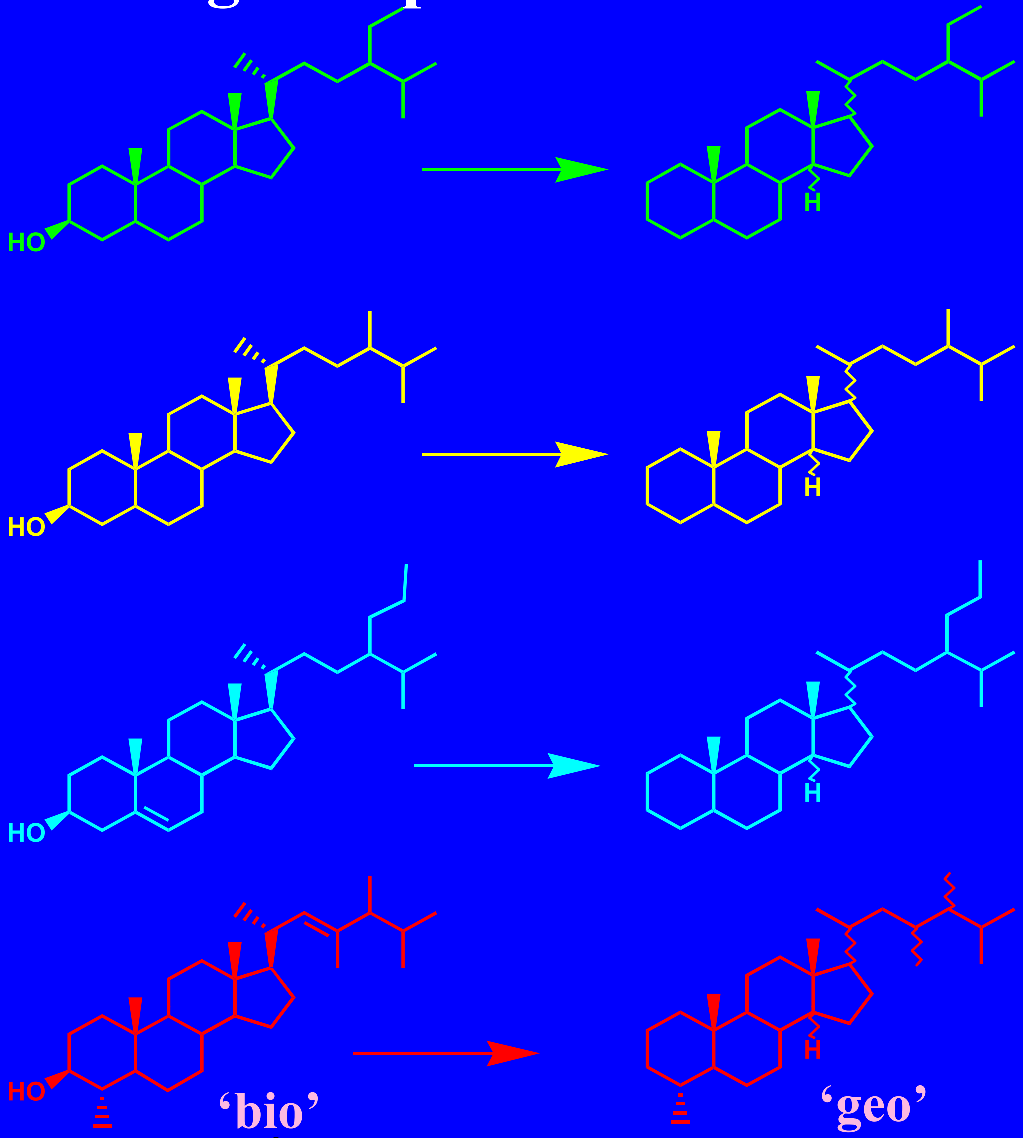
C24 substitution by algae

some bacteria - Methylococcus

Mycobacteria, Myxobacteria

Algal Steroids

- **Encode a variety of age-diagnostic signatures –**
  C-isotopes + steroids from algae & plants



chlorophyceans C_{29}

diatoms C_{28}

chrysophytes C_{30}

dinoflagellates C_{30}

Functional Role of Sterols

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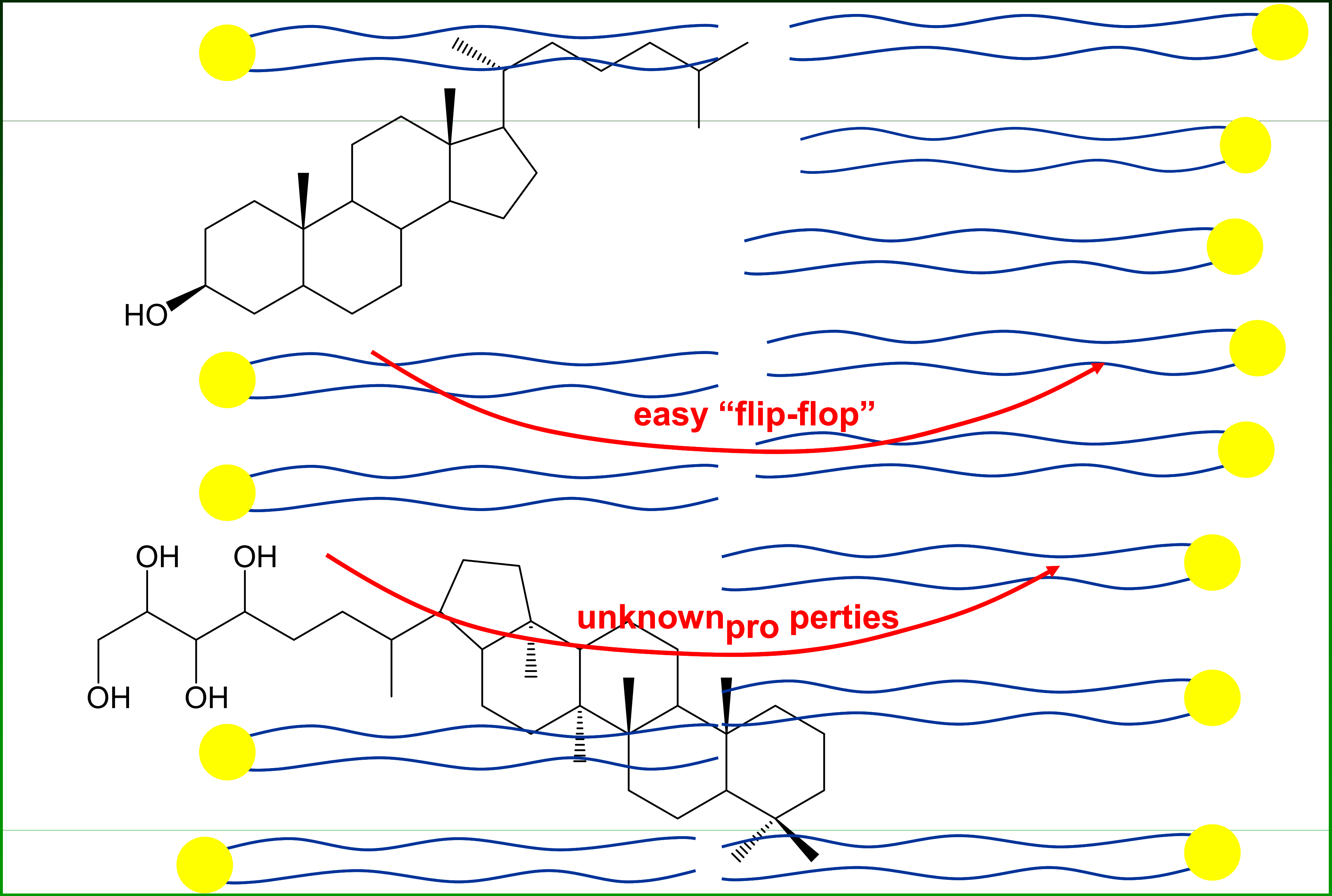
While it became clear very early that cholesterol plays an important role in controlling cell membrane permeability by reducing average fluidity, it appears now that it has a key role in the lateral organization of membranes and free volume distribution. These two parameters seem to be involved in controlling membrane protein activity and "raft" formation (review in *Barenholz Y, Prog Lipid Res 2002, 41, 1*).

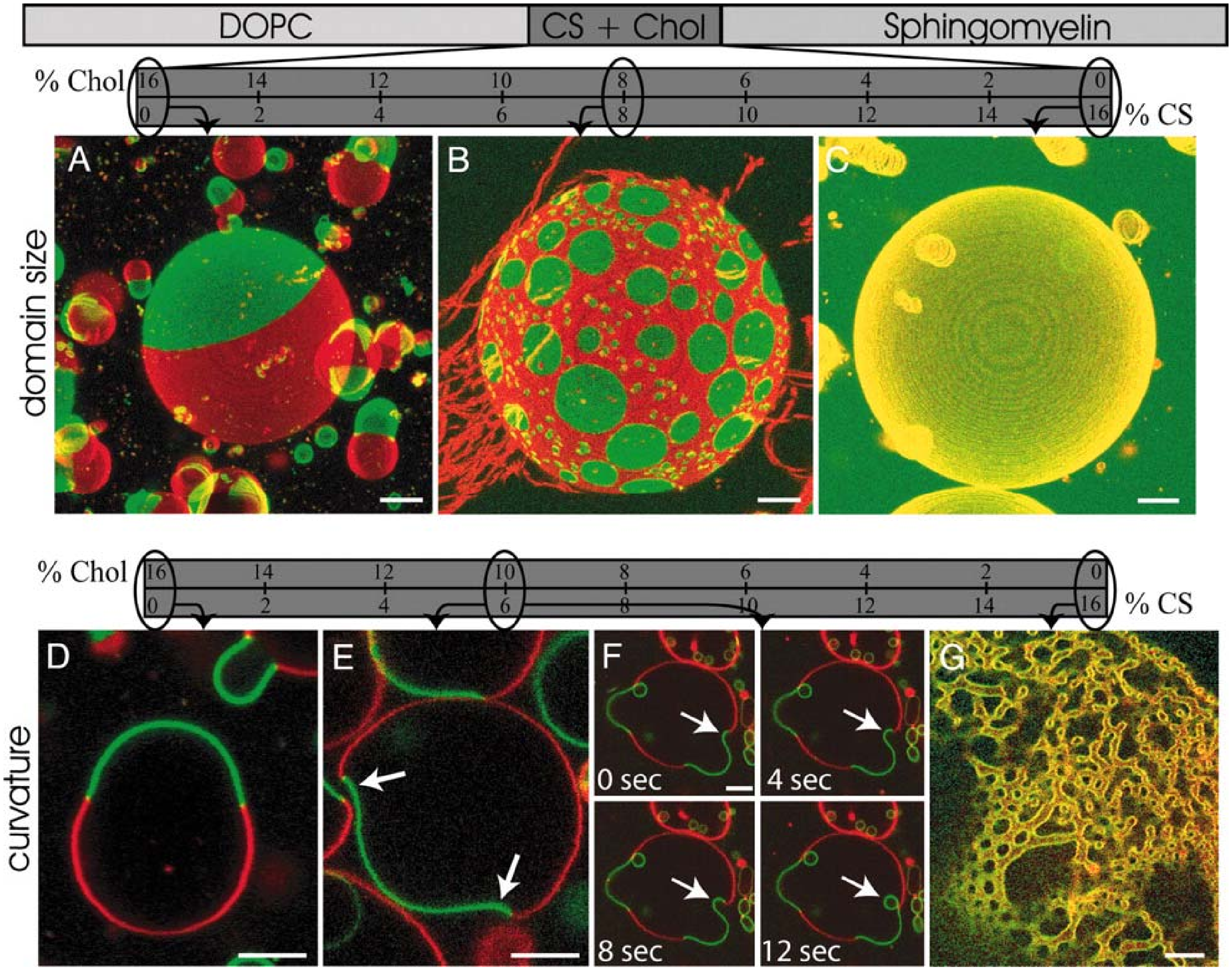
Do sterols & hopanoids serve the same membrane function?



easy “flip-flop”

unknown properties

Fig. 4. Different proportions of cholesterol and CS in GUVs modulate domain size, domain curvatures, budding, and the formation of tubular structures



Bacia, Kirsten et al. (2005) Proc. Natl. Acad. Sci. USA 102, 3272-3277

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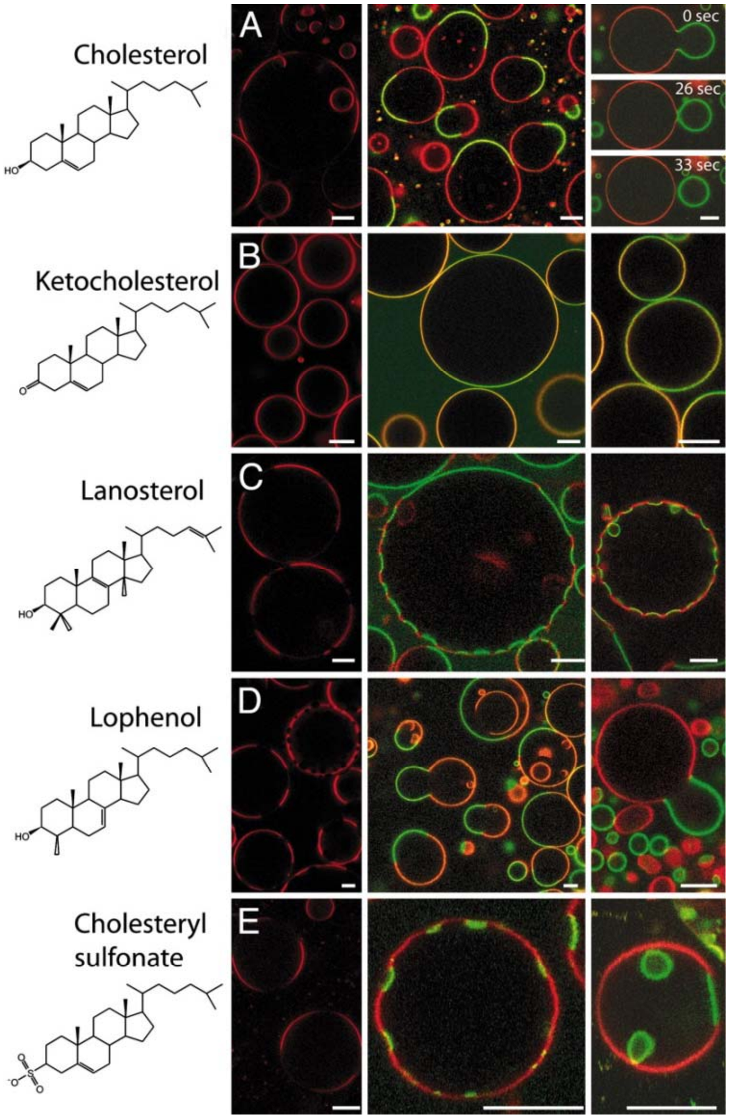
USA 102, 3272-3277. Copyright (c) 2005, National Academy of Sciences, U.S.A.

Fig. 2. The molecular structure of a sterol determines separation of phases in GUVs and the curvature of the lo phase



Bacia, Kirsten et al. (2005) Proc. Natl. Acad. Sci. USA 102, 3272-3277

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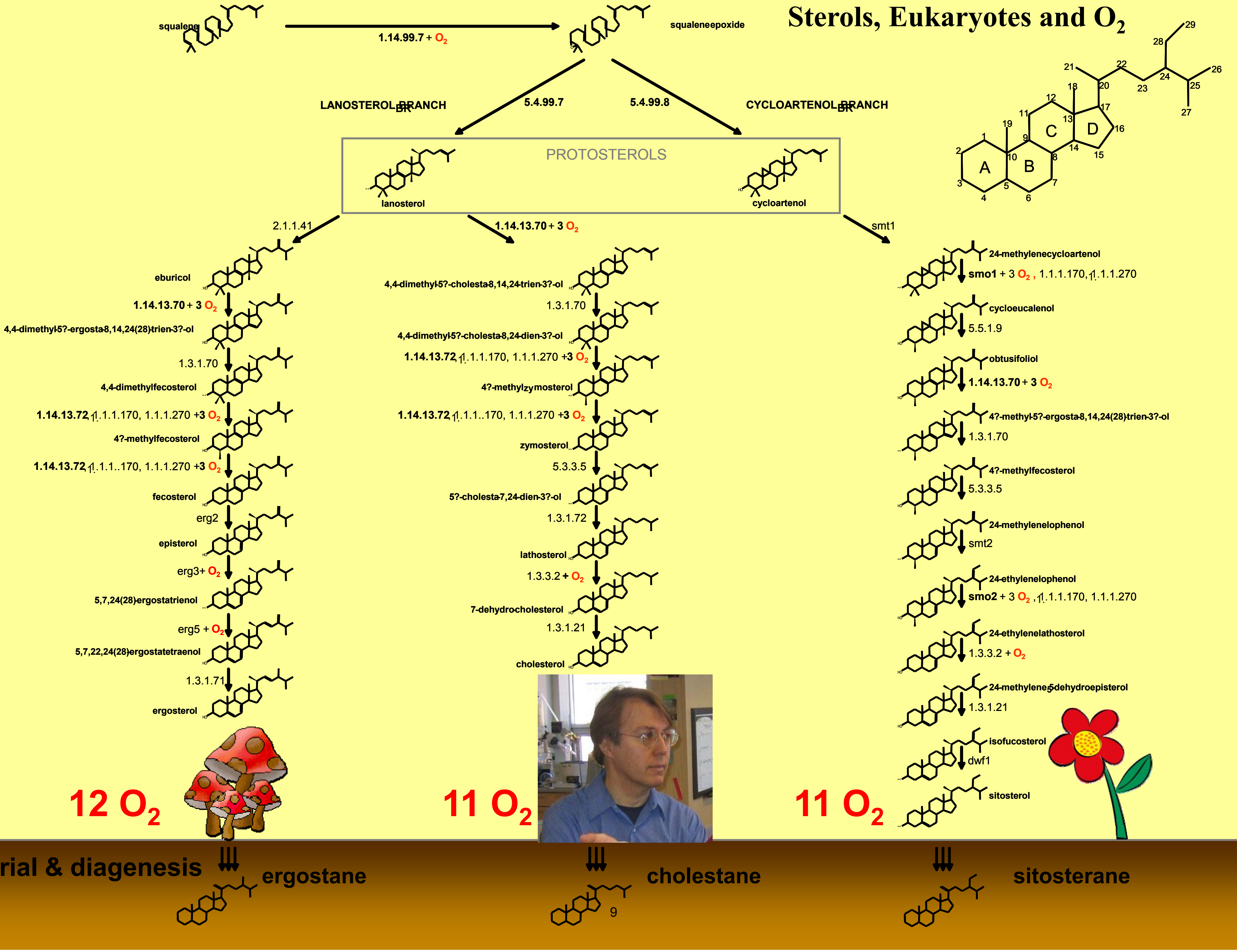
USA 102, 3272-3277. Copyright (c) 2005, National Academy of Sciences, U.S.A.

Trivia

- Structural studies from 1900 to 1932, mainly by H.O. Wieland "on the constitution of the bile acids and related substances" (Nobel Prize Chemistry 1927) and by A.O.R. Windaus on "the constitution of sterols and their connection with the vitamins" (Nobel Prize Chemistry 1928), led to the exact structure and stereochemical configuration of cholesterol
- The term "**phytosterol**" was proposed by Thoms in 1897 to denote sterols of plant origin. The principal phytosterols are compounds having 28 to 30 carbon atoms and include campesterol, stigmasterol and β-sitosterol

http://www.cyberlipid.org/sterols/ster0003.htm

Sterols, Eukaryotes and O_2



squalene → squaleneepoxide: 1.14.99.7 + O_2

LANOSTEROL BRANCH 5.4.99.7 | 5.4.99.8 **CYCLOARTENOL BRANCH**

PROTOSTEROLS

lanosterol | cycloartenol

2.1.1.41

eburicol

1.14.13.70 + 3 O_2

4,4-dimethyl-5?-ergosta-8,14,24(28)-trien-3?-ol

1.3.1.70

4,4-dimethylfecosterol

1.14.13.72, 1.1.1.170, 1.1.1.270 +3 O_2

4?-methylfecosterol

1.14.13.72, 1.1.1..170, 1.1.1.270 +3 O_2

fecosterol

erg2

episterol

erg3+ O_2

5,7,24(28)ergostatrienol

erg5 + O_2

5,7,22,24(28)ergostatetraenol

1.3.1.71

ergosterol

1.14.13.70 + 3 O_2

4,4-dimethyl-5?-cholesta-8,14,24-trien-3?-ol

1.3.1.70

4,4-dimethyl-5?-cholesta-8,24-dien-3?-ol

1.14.13.72, 1.1.1.170, 1.1.1.270 +3 O_2

4?-methylzymosterol

1.14.13.72, 1.1.1..170, 1.1.1.270 +3 O_2

zymosterol

5.3.3.5

5?-cholesta-7,24-dien-3?-ol

1.3.1.72

lathosterol

1.3.3.2 + O_2

7-dehydrocholesterol

1.3.1.21

cholesterol

smt1

24-methylenecycloartenol

smo1 + 3 O_2, 1.1.1.170, 1.1.1.270

cycloeucalenol

5.5.1.9

obtusifoliol

1.14.13.70 + 3 O_2

4?-methyl-5?-ergosta-8,14,24(28)-trien-3?-ol

1.3.1.70

4?-methylfecosterol

5.3.3.5

24-methylenelophenol

smt2

24-ethylenelophenol

smo2 + 3 O_2, 1.1.1.170, 1.1.1.270

24-ethylenelathosterol

1.3.3.2 + O_2

24-methylene-5-dehydroepisterol

1.3.1.21

isofucosterol

dwf1

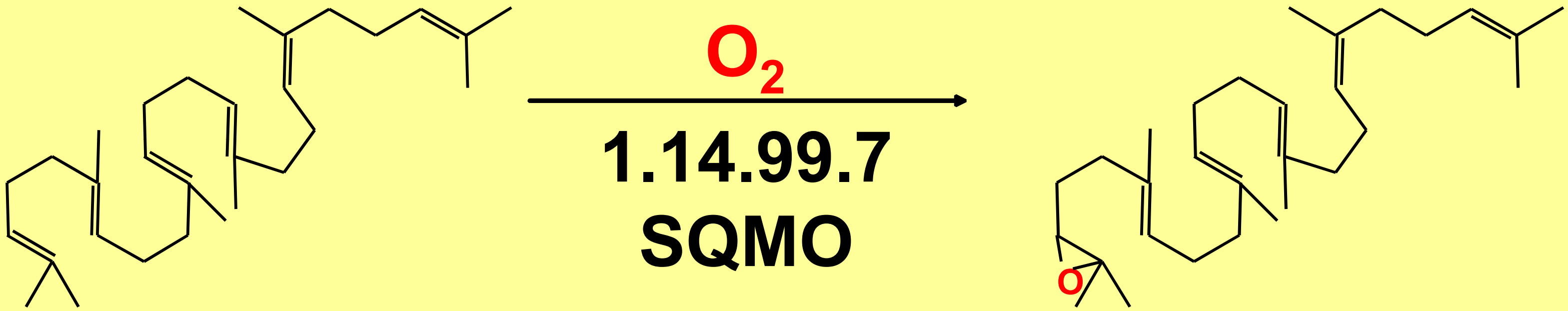
sitosterol

12 O_2 | **11 O_2** | **11 O_2**

Burial & diagenesis

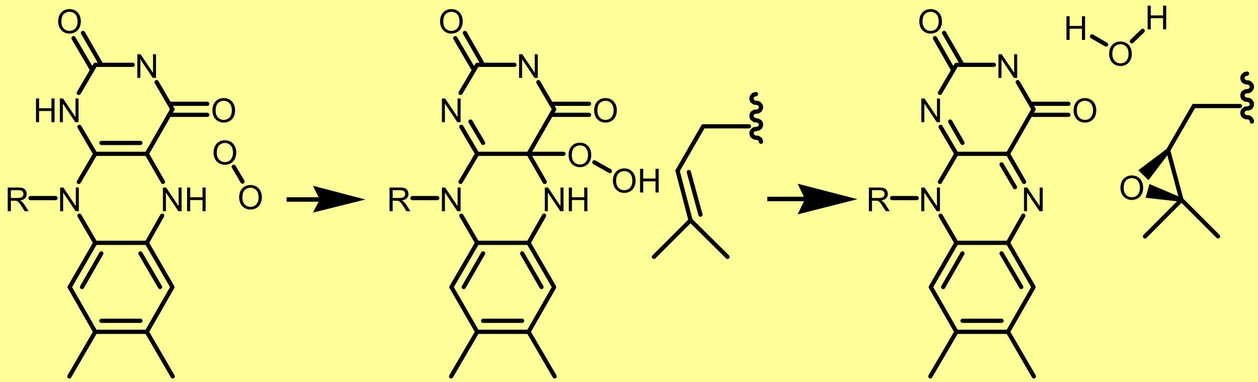
ergostane | **cholestane** | **sitosterane**

Squalene Epoxidase



squalene

squalene epoxide

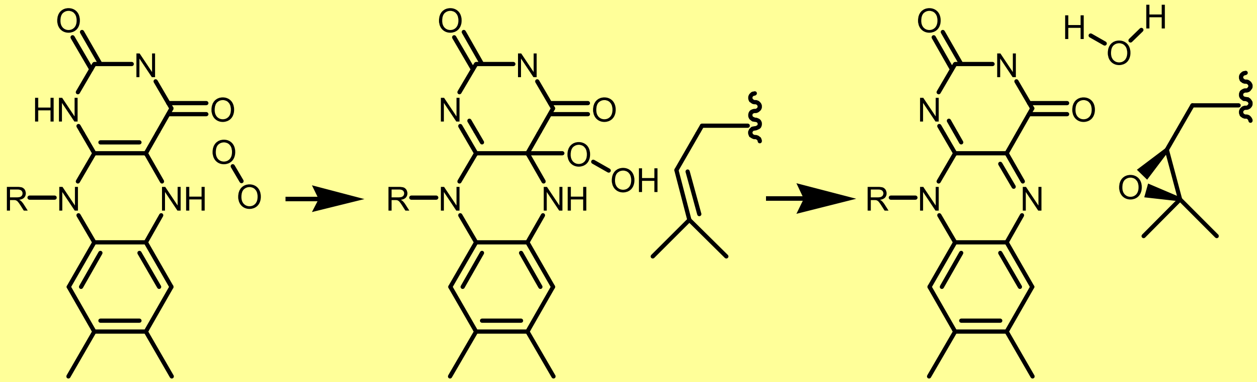


$Fl_{red}H2 + O_2$ FlH(4a)-OOH + Squalene $_2O$ + (3S)-2,3-Oxidosqualene

Regenerated by NADPH-c450 reductase

Squalene Epoxidase

- SQMO Requires O_2 (and FAD)
- K. Bloch and T.T. Tchen, 1956 with $^{18}O_2$
- Electrophilic hydroperoxide reacts with 2,3-DB of squalene
- Water is not a chemically feasible alternative to O_2
- No evidence of any alternative oxygen-donors
- SQMO functionally conserved in Eukarya and Bacteria



$Fl_{red}H2 + O_2$ **FlH(4a)-OOH** + **Squalene** **$_2O$ + (3S)-2,3-Oxidosqualene**

Flox + H

Regenerated by NADPH-c450 reductase

ENTRY EC 1.14.99.7

NAME squalene monooxygenase
squalene epoxidase
squalene-2,3-epoxide cyclase
squalene 2,3-oxidocyclase squalene hydroxylase
squalene oxydocyclase squalene-2,3-epoxidase
CLASS Oxidoreductases Acting on paired donors with incorporation of molecular oxygen Miscellaneous SYSNAME squalene,hydrogen-donor:oxygen oxidoreductase (2,3-epoxidizing) REACTION squalene + AH2 + O2 = (S)-squalene-2,3-epoxide + A + H2O SUBSTRATE squalene AH2 O2 PRODUCT (S)-squalene-2,3-epoxide A H2O COMMENT A flavoprotein (FAD). This enzyme, together with EC 5.4.99.7 lanosterol synthase, was formerly known as squalene oxidocyclase.

http://www.genome.ad.jp/dbget-bin/www_bget?enzyme+1.14.99.7

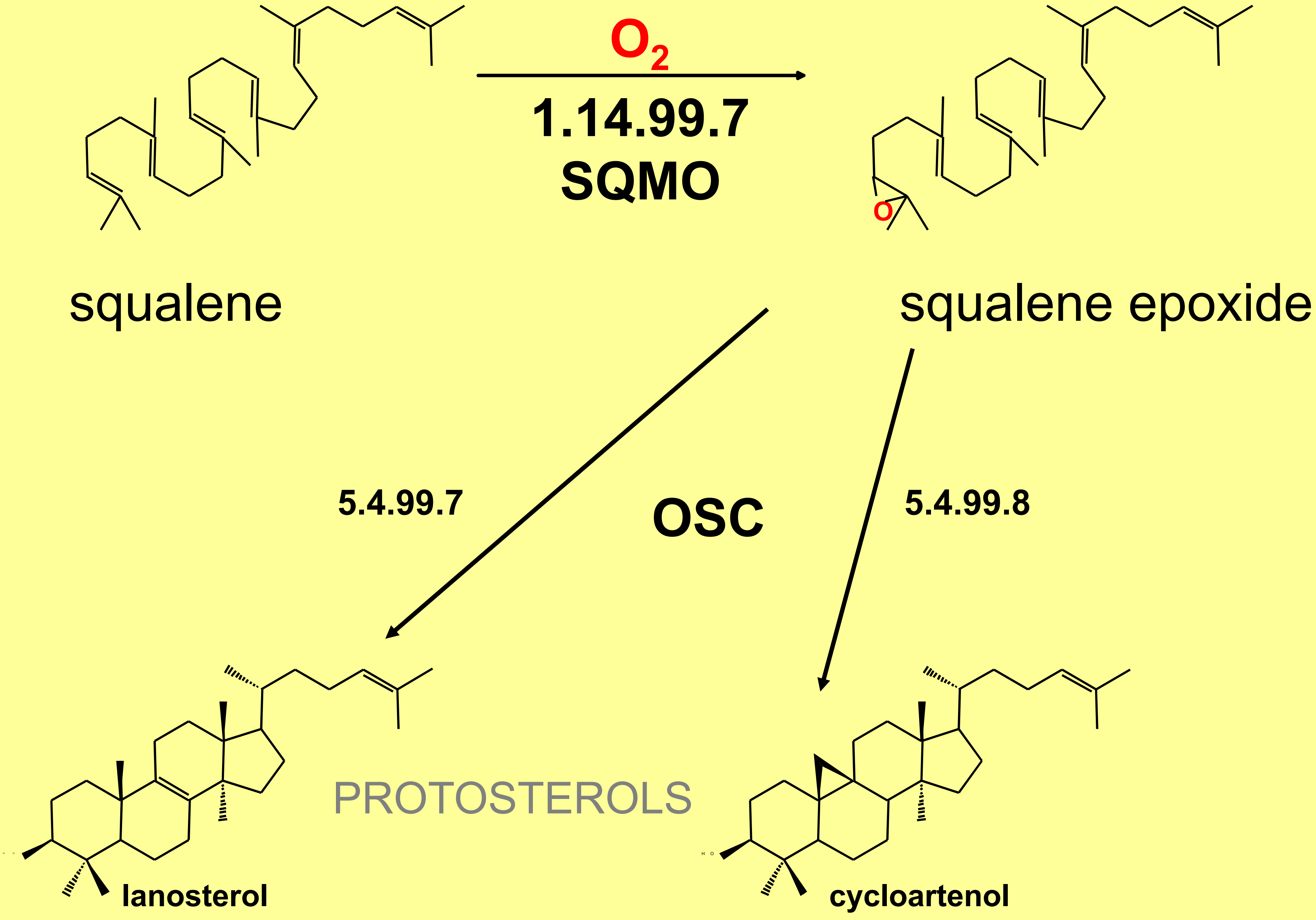
Corey, E.J., Russey, W.E. and Ortiz de Montellano, P.R. 2,3-Oxidosqualene, an intermediate in the biological synthesis of sterols from squalene. J. Am. Chem. Soc. 88 (1966) 4750-4751. 2 Tchen, T.T. and Bloch, K. On the conversion of squalene to lanosterol in vitro. J. Biol. Chem. 226 (1957) 921-930. 3 [UI:67015265] van Tamelen, E.E., Willett, J.D., Clayton, R.B. and Lord, K.E. Enzymic conversion of squalene 2,3-oxide to lanosterol and cholesterol. J. Am. Chem. Soc. 88 (1966) 4752-4754. 4 [UI:70158491] Yamamoto, S. and Bloch, K. Studies on squalene epoxidase of rat liver. J. Biol. Chem. 245 (1970) 1670-1674.

SUMMARY

Rat liver microsomes previously heated to 50” for 5 mm accumulate 2,3-oxidosqualene on incubation with squalene. Squalene epoxidase activity can be assayed either with squalene and heated microsomes or with IO,Il-dihydrosqualene and intact microsomes. **In common with other monooxygenases, the epoxidase requires TPNH and molecular oxygen.** Both a soluble fraction of rat liver and microsomes are necessary for enzyme activity. Carbon monoxide or potassium cyanide fall to inhibit squalene epoxidation. The present paper reports some properties of t,he squalene epoxidase system and describes a heat treatment of rat liver microsomes which abolishes cyclase activity without impairing the enzymatic conversion of squalene to 2,3-oxidosqualene.

Yamamoto, S. and Bloch, K. Studies on squalene epoxidase of rat liver. J. Biol. Chem. 245 (1970) 1670-1674

Oxidosqualene Cyclase



O_2

1.14.99.7

SQMO

squalene

squalene epoxide

5.4.99.7

OSC

5.4.99.8

PROTOSTEROLS

lanosterol

cycloartenol

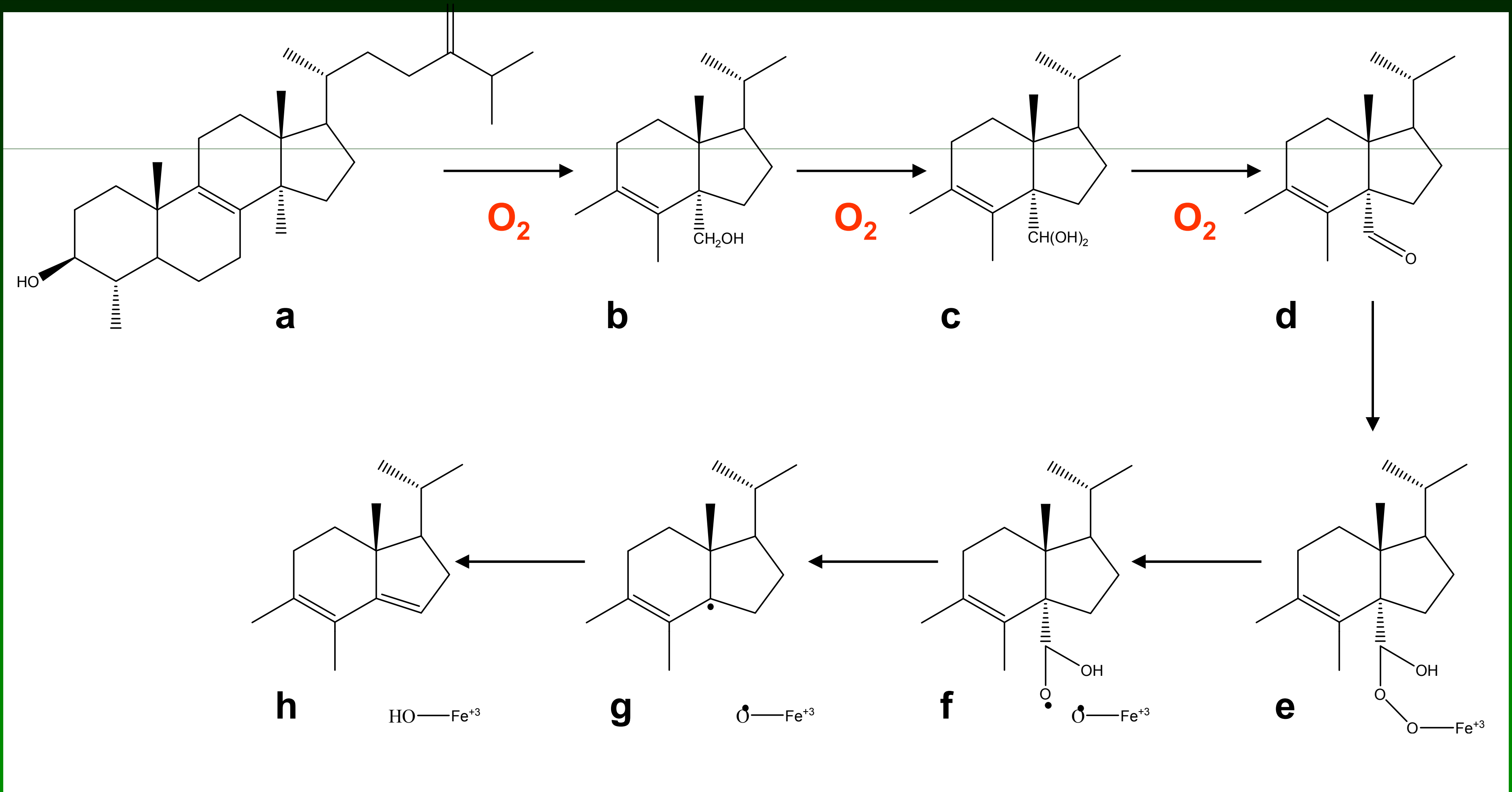
ENTRY EC 5.4.99.7 NAME lanosterol synthase
2,3-epoxysqualene
lanosterol cyclase
squalene-2,3-oxide-lanosterol cyclase lanosterol
2,3-oxidosqualene cyclase squalene 2,3-epoxide:lanosterol cyclase 2,3-oxidosqualene
sterol cyclase oxidosqualene cyclase
2,3-oxidosqualene cyclase
2,3-oxidosqualene-lanosterol cyclase
oxidosqualene-lanosterol cyclase squalene
epoxidase-cyclase
CLASS Isomerases

Dean, P.D.G., Oritz de Montellano, P.R., Bloch, K. and Corey, E.J. A soluble 2,3-oxidosqualene sterol cyclase. J. Biol. Chem. 242 (1967) 3014-3015.

http://www.genome.ad.jp/dbget-bin/www_bget?enzyme+5.4.99.7

http://www.chem.qmul.ac.uk/iubmb/enzyme/reaction/terp/cholesterol.html

Cytochrome CYP51: Sterol 14α-demethylase



- a cyctochrome P450 family enzyme
- ALL P450s have an absolute requirement for molecular O_2
- CYP51 requires 3 molecules of O_2 to activate and remove methyl
- the activated complex contains a porphyrin iron with an iron-oxo species with formal oxidation state of Fe(V)!

The effect of oxygen on biochemical networks and the evolution of complex life.

Jason Raymond and Daniel Segre'   Science 311, 1764-1767 (2006)

Cholesterol

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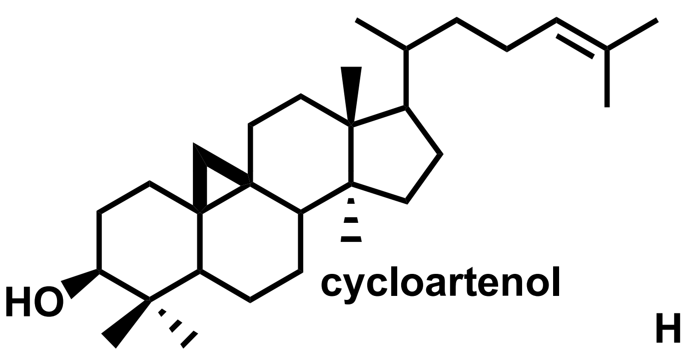
squalene
hopene,
tetrahymanol

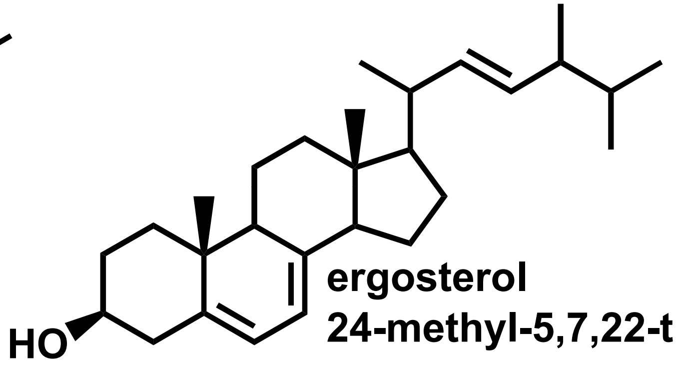
Phytosterols

Whereas vertebrates and fungi synthesize sterols from epoxysqualene through the lanosterol, plants cyclize epoxysqualene to cycloartenol as the initial sterol.

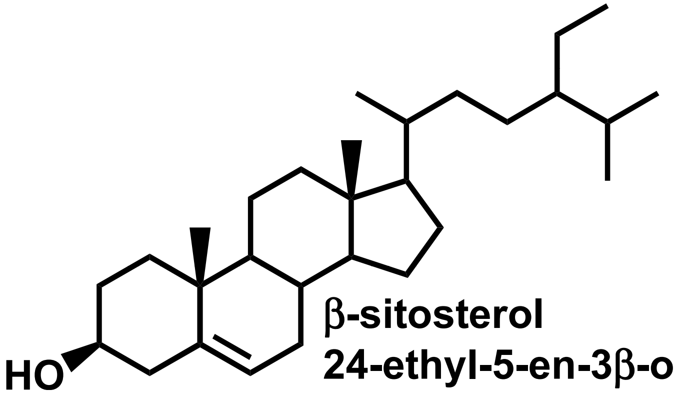
Q. Presumably lanosterol biosynthesis predates cycloartenol biosynthesis? What might have driven the lanosterol-cycloartenol bifurcation?



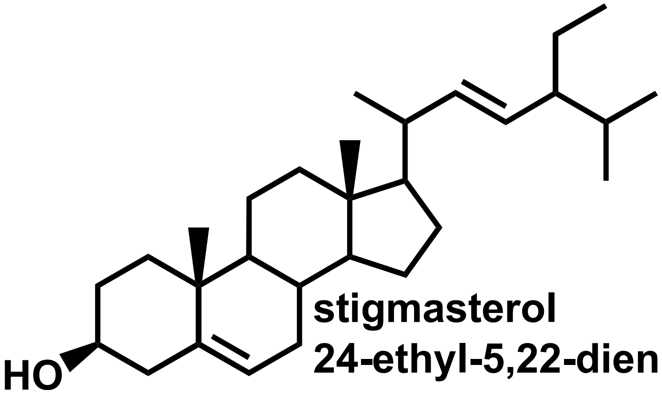
cycloartenol



ergosterol
24-methyl-5,7,22-trien-3β-ol



β-sitosterol
24-ethyl-5-en-3β-ol

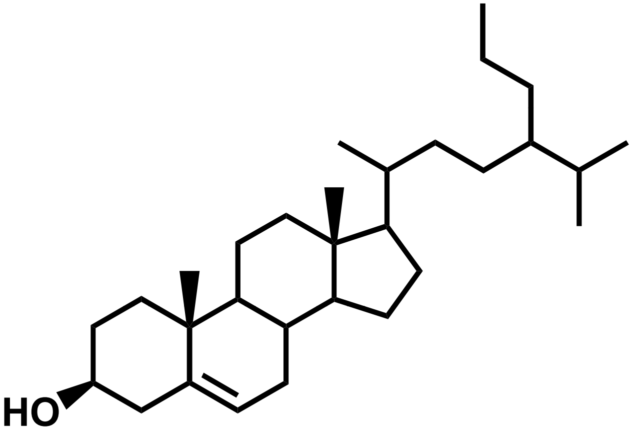


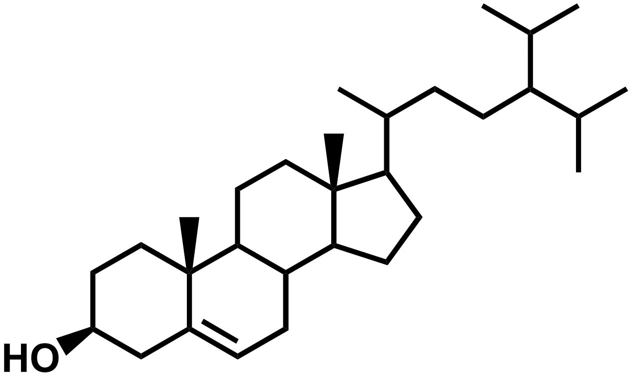
stigmasterol
24-ethyl-5,22-dien-3β-ol

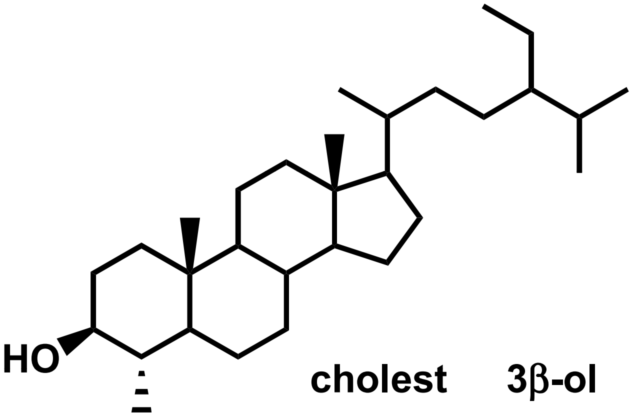
Phytosterols

C_{30}

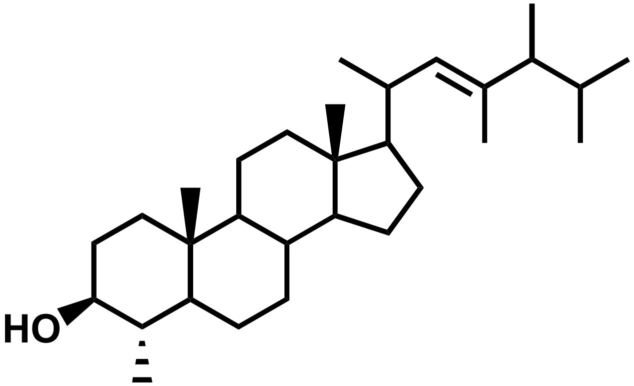
y y







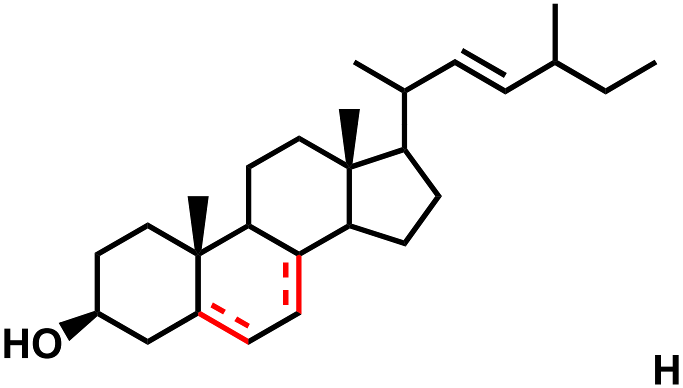
cholest 3β-ol



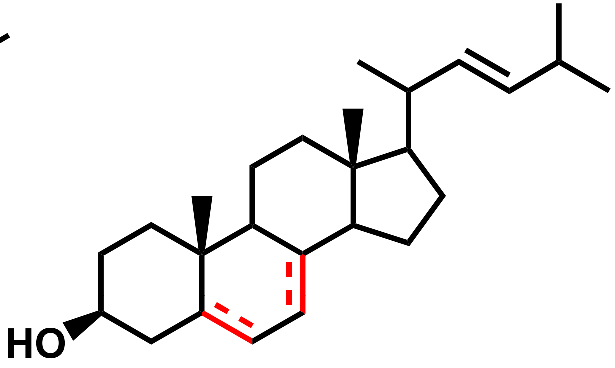
Microorganism	Major or common sterols
Microalgae	
Bacillariophyceae	C28D5,22, C28D5,24(28), C27D5, C29D5, C27D5,22
Bangiophyceae	C27D5, C27D5,22, C28D7,22
Chlorophyceae	C28D5, C28D5,7,22, C28D7,22 C29D5,22, C29D5
Chrysophyceae	C29D5,22, C29D5, C28D5,22
Cryptophyceae	C28D5,22
Dinophyceae	4Me-D0, dinosterol, C27D5, C28D5,24(28)
Euglenophyceae	C28D5,7,22, C29D5, C28D7, C29D5,7, C28D7,22
Eustigmatophyceae	C27D5 (marine) or C29D5 (freshwater)
Haptophyceae	C28D5,22, C27D5, C29D5,22, C29D5
Pelagophyceae	C30 D5,24(28), C29D5,22, C29D5,C28D5 ,24(28)
Prasinophyceae	C28D5, C28D5,24(28), C28D5
Raphidophyceae	C29D5, C28D5,24(28)
Rhodophyceae	C27D5, C27D5,22
Xanthophyceae	C29D5, C27D5
Cyanobacteria:	C27D5, C29D5, C27D0, C29D0 (evidence equivocal)
Methylotrophic bacteria	4Me-D8
Other bacteria	C27D5
Yeasts and fungi	C28D5,7,22, C28D7, C28D7,24(28)
Thraustochytrids	C27D5, C29D5,22, C28D5,22, C29D5,7,22

The nomenclature is CxDy where x is the total number of carbon atoms and y indicates the positions of the double bonds. In general, C28 sterols have a methyl group at C-24, and C29 sterols have a 24-ethyl substituent. Table adapted from data in Volkman (1986); Jones et al. (1994) and Volkman et al.

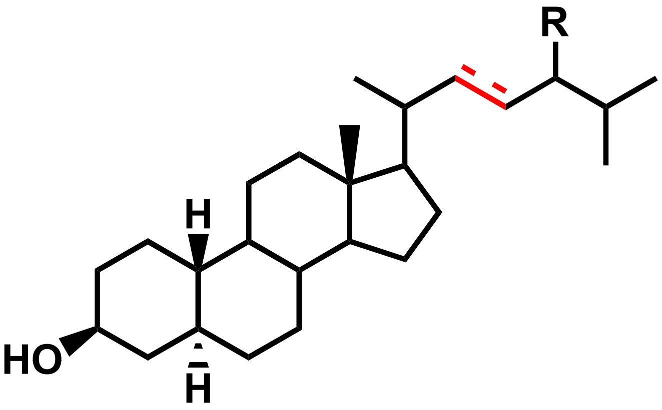
Uncommon Marine Sterols



24-methyl-27-nor sterols and stanol known in sponges: C27 compounds



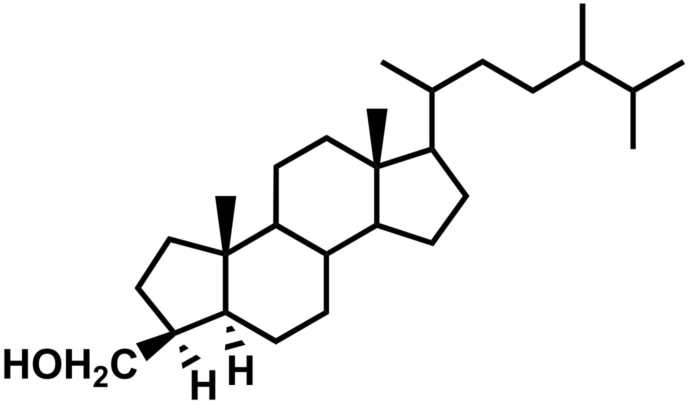
24-nor sterols a range of algae & invertbrates: C26



19-nor sterols

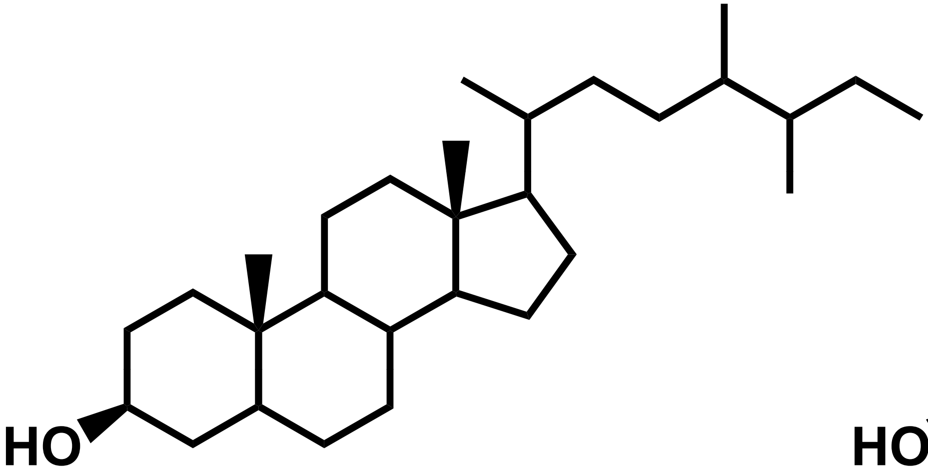
R=H, CH_3, C_2H_5, methylene

Δ22 trans unsaturation probably formed by de-alkylation of algal sterols

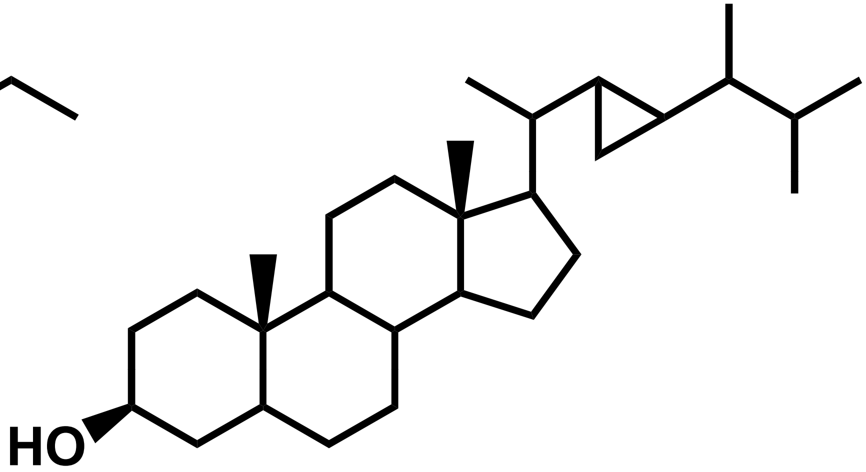


A-nor sterols sponges

Uncommon Marine Sterols (2)



Aplysterol



Gorgosterol

- **1:** Lipids. 1995 Mar;30(3):203-19. Related Articles, Links
  - **Developmental regulation of sterol biosynthesis in Zea mays.**

Guo DA, Venkatramesh M, Nes WD.

Department of Chemistry and Biochemistry, Texas Tech University, Lubbock 79409, USA.

Sixty-one sterols and pentacyclic triterpenes have been isolated and characterized by chromatographic and spectral methods from Zea mays (corn). Several plant parts were examined; seed, pollen, cultured hypocotyl cells, roots, coleoptiles (sheaths), and blades. By studying reaction pathways and mechanisms on plants fed radiotracers ([2-(14)C]mevalonic acid, [2-(14)C]acetate, and [2-(3)H]acetate), and stable isotopes (D2O), we discovered that hydroxymethylgutaryl CoA reductase is not "the" rate-limiting enzyme of sitosterol production. Additionally, we observed an ontogenetic shift and kinetic isotope effect in sterol biosynthesis that was associated with the C-24 alkylation of the sterol side chain. Blades synthesized mainly 24alpha-ethyl-sterols, sheaths synthesized mainly 24-methyl-sterols, pollen possessed an interrupted sterol pathway, accumulating 24(28)-methylene-sterols, and germinating seeds were found to lack an active de novo pathway. Shoots, normally synthesizing (Z)-24(28)-ethylidine-cholesterol, after incubation with deuterated water, synthesized the rearranged double-bond isomer, stigmasta-5,23-dien-3 beta-ol. Examination of the mass spectrum and 1H nuclear magnetic resonance spectrum of the deuterated 24-ethyl-sterol indicated the Bloch-Cornforth route originating with acetyl-CoA and passing through mevalonic acid to sterol was not operative at this stage of development. An alternate pathway giving rise to sterols is proposed.

Sterol ID by GC-MS of TMS and acetate derivatives

- **Relative Retention Times of Nematode Sterols**
- **Mass Spectra of Nematode Sterols**
- Mass Spectral Data for Nematode Sterols, Analyzed as Steryl Acetate Derivatives*a*
  Steryl acetate Mass spectrum (*m/z*, relative intensity to base peak) Cholesta-5,7,9(11)-trienol 424 (5), 364 (100), 349 (33), 251 (31), 209 (64), 197 (43), 195 (52)Cholest-8(14)-enol 428 (100), 413 (18), 368 (6), 353 (13), 315 (16), 288 (7), 273 (6), 255 (21), 229 (42), 213 (43), 81 (80), 55 (83)Cholesterol 368 (100), 353 (14), 260 (15), 255 (13), 247 (18), 213 (14), 147 (48), 145 (37), 81 (74), 55 (71) Cholestanol430 (12), 415 (2), 370 (33), 355 (16), 316 (3), 276 (28), 275 (18), 257 (4), 230 (17), 215 (100), 201 (18), 147 (32), 81 (53)

Sterols in Bacteria

Cyanobacteria - many reports
Methanotrophs - *Methylococcus capsulatus*
Planctomycetes - *Gemmata obscuriglobus*
Myxobacteria - *Nannocyctis exedans*

- Likely false positives - cyanobacteria
- Unusual products - *Gemmata*
- Incomplete pathways *Methylococcus*
- No alkylation at C24
- Potentially obtained from Eukarya by LGT

Sterol Biosynthesis in Bacteria ?

Molecular Microbiology (2003) **47**(2), 471–481

Steroid biosynthesis in prokaryotes: identification of myxobacterial steroids and cloning of the first bacterial 2,3(S)-oxidosqualene cyclase from the myxobacterium*Stigmatella aurantiaca.* Helge Björn Bode, Bernd Zeggel, Barbara Silakowski, Silke C. Wenzel, Hans Reichenbach and Rolf Müller

Summary

Steroids, such as cholesterol, are synthesized in almost all eukaryotic cells, which use these triterpenoid lipids to control the fluidity and flexibility of their cell membranes. Bacteria rarely synthesize such tetracyclic compounds but frequently replace them with a different class of triterpenoids, the pentacyclic hopanoids. The intriguing mechanisms involved in triterpene biosynthesis have attracted much attention, resulting in extensive studies of squalenehopene cyclase in bacteria and (S)-2,3-oxidosqualene cyclases in eukarya. Nevertheless, almost nothing is known about steroid biosynthesis in bacteria. Only three steroid-synthesizing bacterial species have been identified before this study. Here, we report on a variety of sterol-producing myxobacteria. *Stigmatella Aurantiac* is shown to produce cycloartenol, the well-known first cyclization product of steroid biosynthesis in plants and algae. Additionally, we describe the cloning of the first bacterial steroid biosynthesis gene, *cas* , encoding the cycloartenol synthase (Cas) of *S. aurantiaca.* Mutants of *cas* generated via sitedirected mutagenesis do not produce the compound. They show neither growth retardation in comparison with wild type nor any increase in ethanol sensitivity. The protein encoded by *cas* is most similar to the Cas proteins from several plant species, indicating a close evolutionary relationship between myxobacterial and eukaryotic steroid biosynthesis.

Sterol Biosynthesis in Bacteria
Sterols of *Methylococcus capsulatus*

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Bird et al., 1971, Nature 230, 473-4

Phylogenetic and biochemical evidence for an ancient origin of sterol biosynthesis in the Bacteria

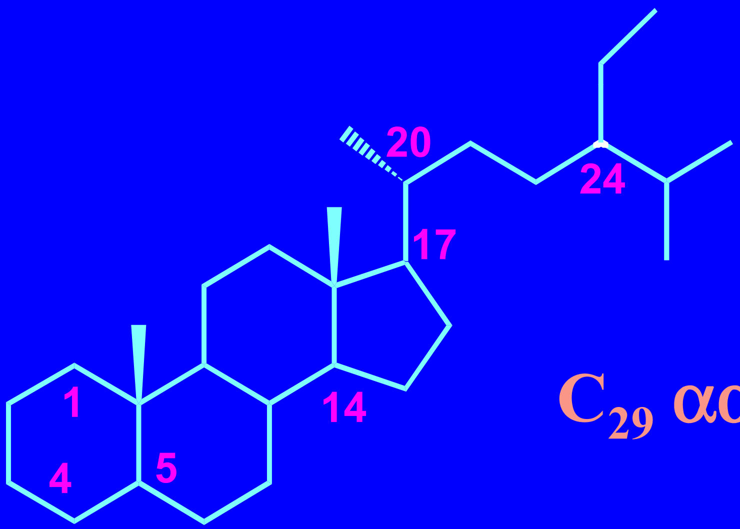
Ann Pearson,* Meytal Budin*, and Jochen J. Brocks† PNAS In Press

- **Abstract**

Sterol biosynthesis is viewed primarily as a eukaryotic process, and the frequency of its occurrence in bacteria long has been a subject of controversy. Two enzymes, squalene monooxygenase (Sqmo) and oxidosqualene cyclase (Osc), are the minimum necessary for initial biosynthesis of sterols from squalene. In this work, 19 protein gene sequences for eukaryotic Sqmo and 12 protein gene sequences for eukaryotic Osc were compared to all available complete and partial prokaryotic genomes. The only unequivocal matches for a sterol biosynthetic pathway were the a-proteobacterium, *Methylococcus capsulatus*, in which sterol biosynthesis already is known, and the planctomycete, *Gemmata obscuriglobus*. The latter species contains the most abbreviated sterol pathway yet identified in any organism. Experiments show that the major sterols in *Gemmata* are lanosterol and its uncommon isomer, parkeol. There are no subsequent modifications of these products. In bacteria, the sterol biosynthesis genes occupy a contiguous coding region and possibly comprise a single operon.

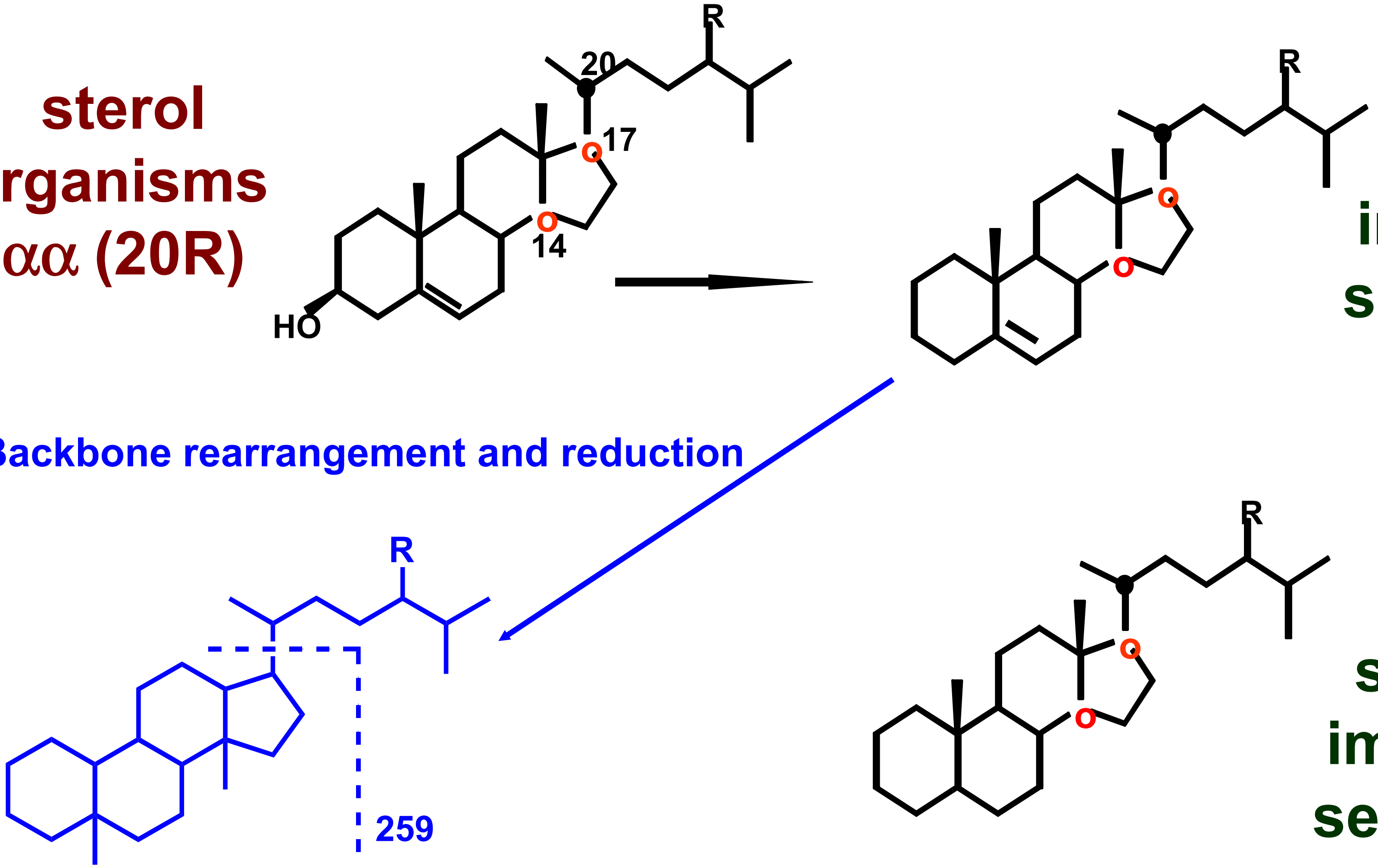
Biomarker Numbering System

C$_{35}$ αβ-hopane



C$_{29}$ ααα-sterane (20R)

Sterol Diagenesis



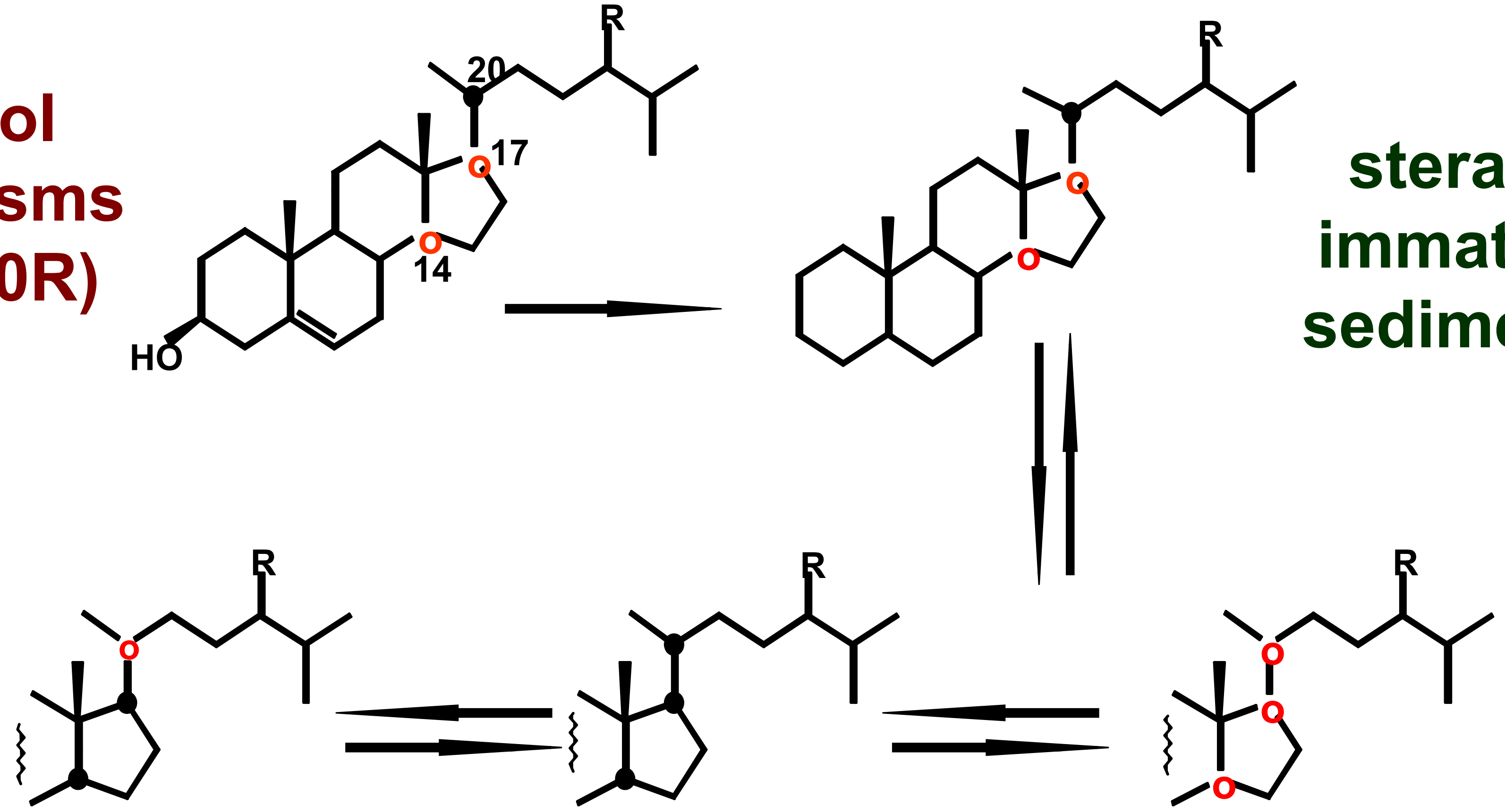
sterol
organisms
αα (20R)

sterene
immature
sediments

Backbone rearrangement and reduction

sterane
immature
sediments

Sterane maturity parameters



sterol
organisms
αα (20R)

sterane
immature
sediments

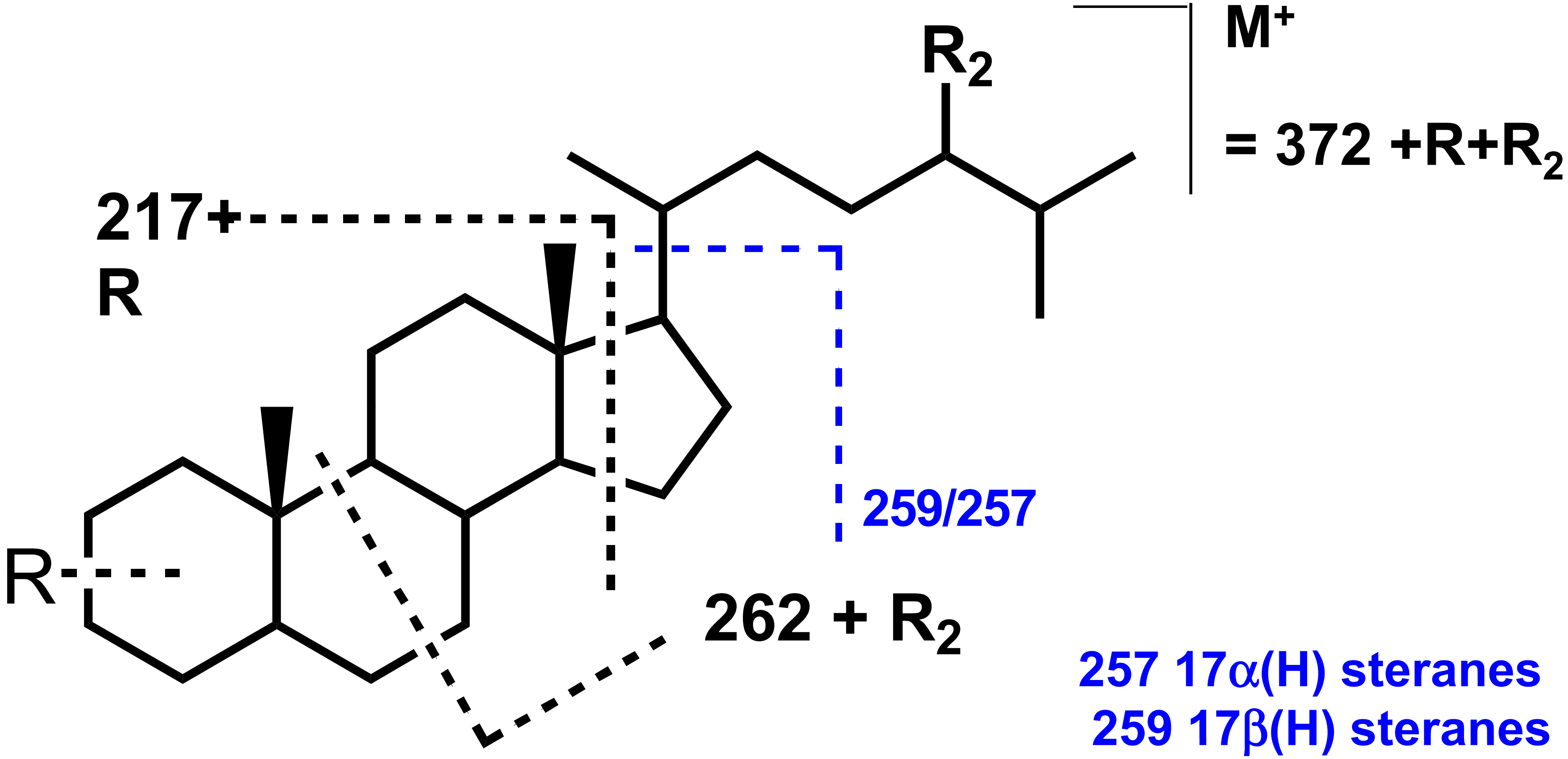
ββ (20S)

ββ (20R)

αα (20S)

mature sediments & oil

Steranes
mass spectral features

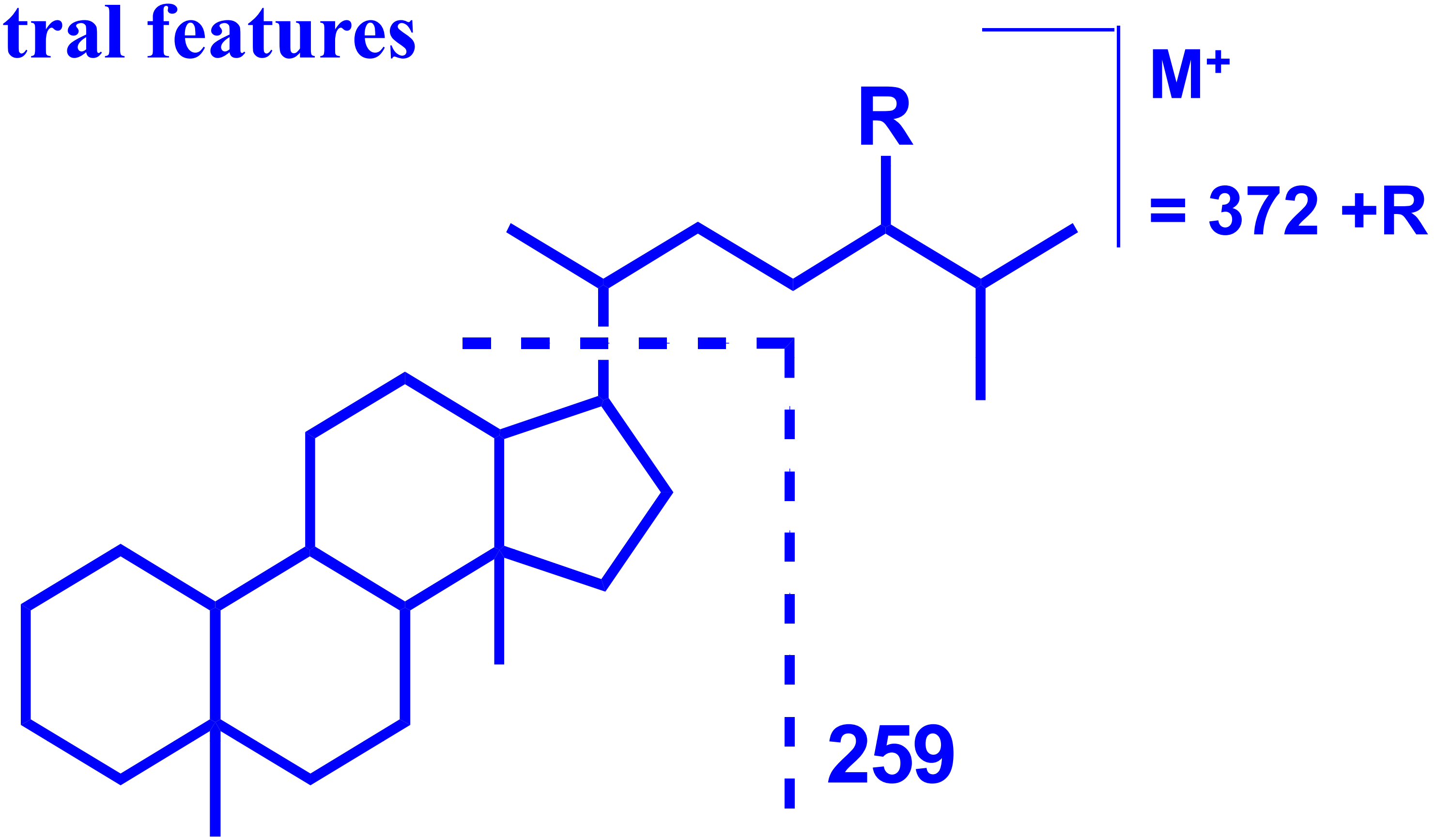


257 17α(H) steranes
259 17β(H) steranes

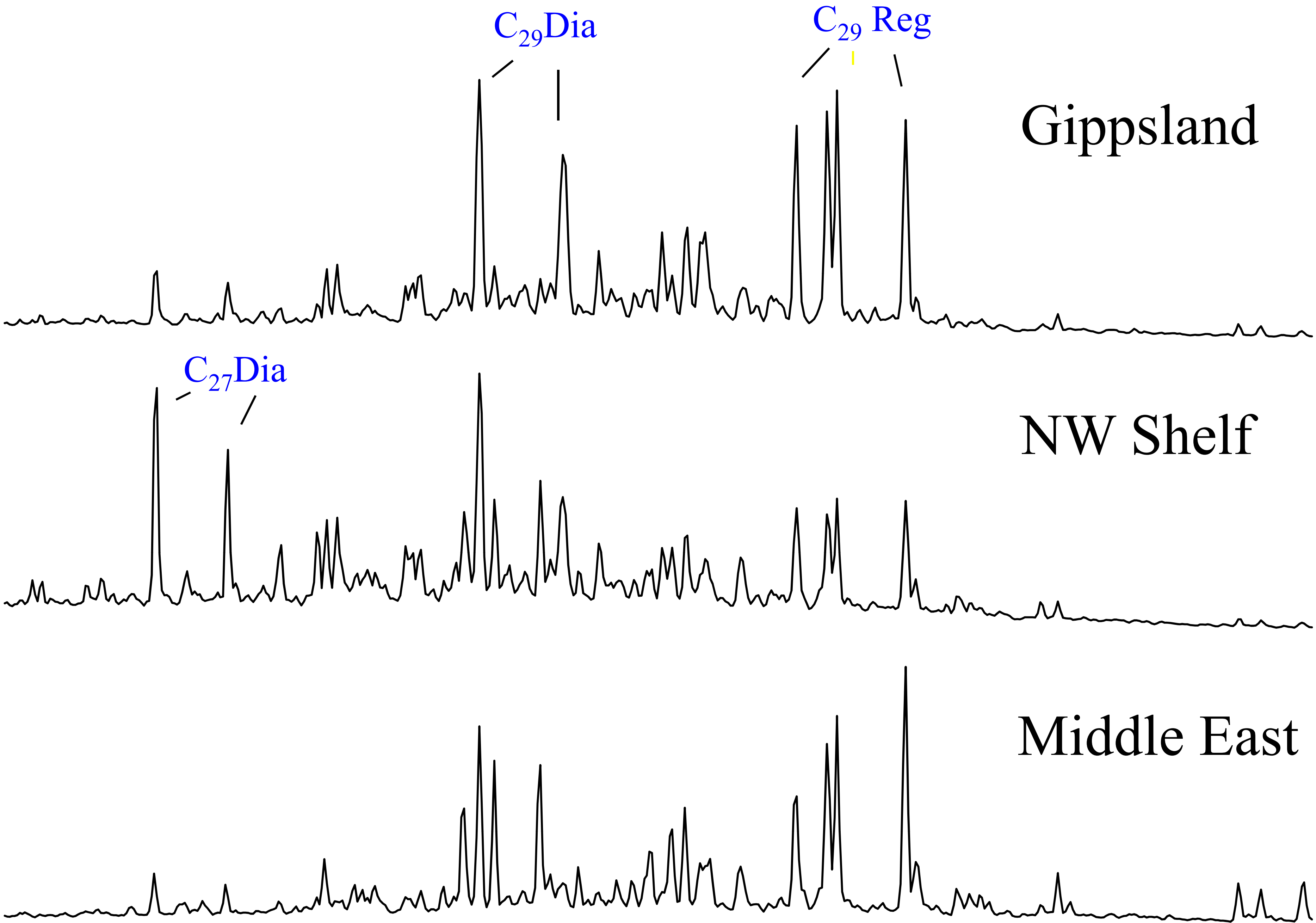
218 > 217 in steranes with ββ config.

149/151 pair also sensitive to stereochemistry

Diasteranes
mass spectral features



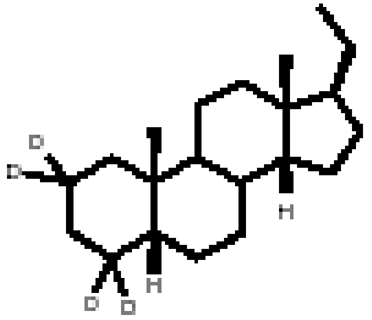
Steranes - 217 Da



Internal Standards for sterane quantitation 217 Da→ 221 Da

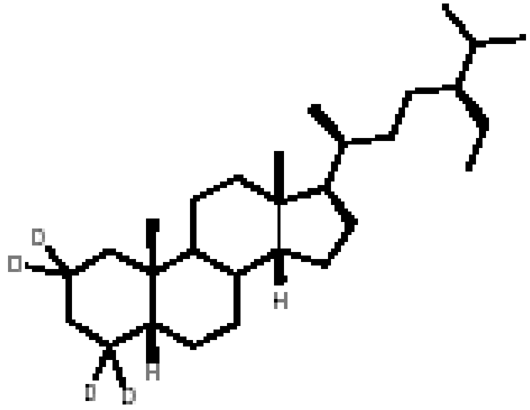
d_4 Pregnane (99%)

$C_{21}H_{32}D_4$	M.W: 292,55
0.01 mg/ml	5 or 10 x 1 ml in isooctane
0.1 mg/ml	5 or 10 x 1 ml in isooctane



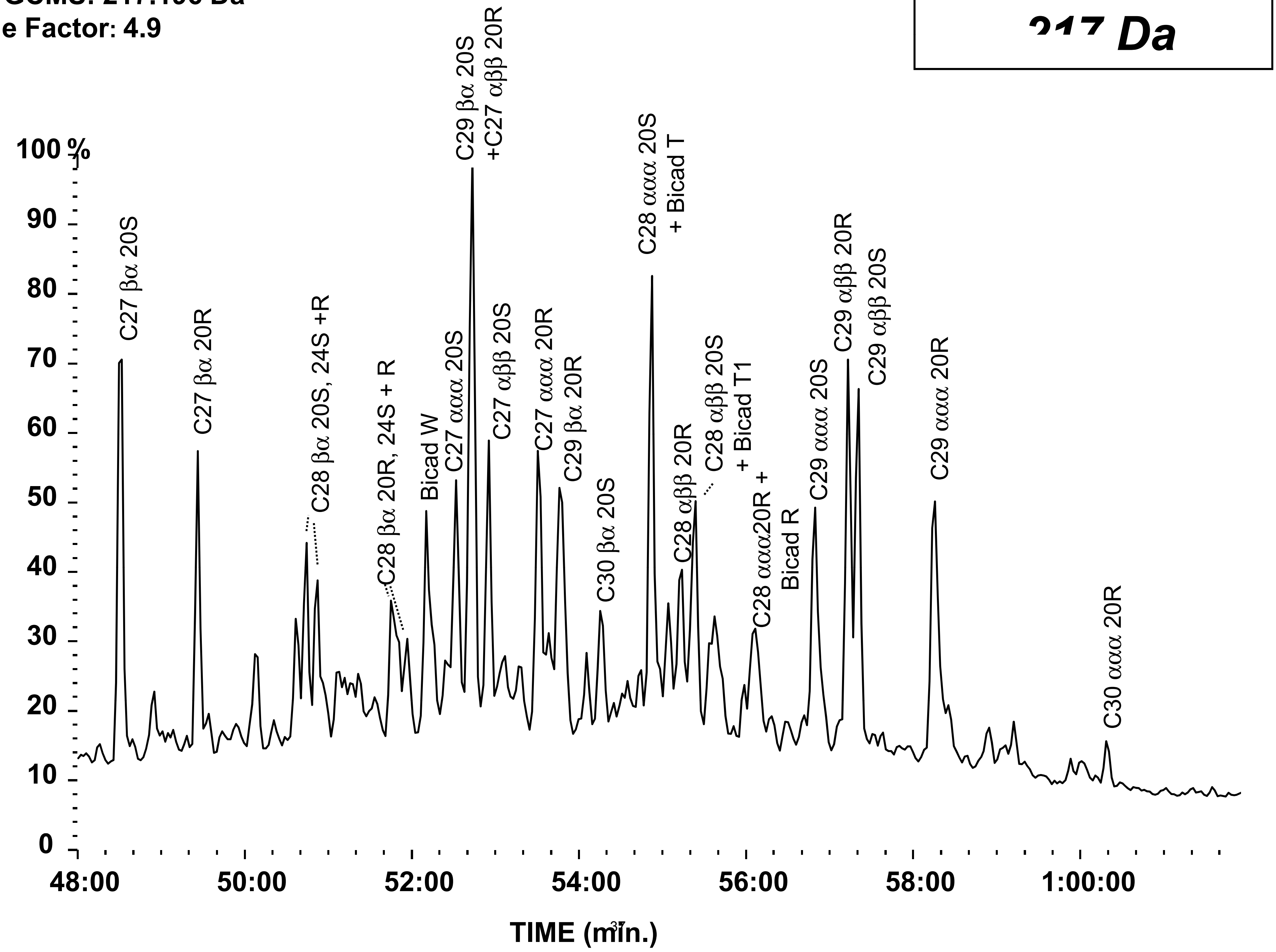
d_4 C_{29} $\alpha\alpha\alpha$(20R) Ethylcholestane (95%)

$C_{29}H_{48}D_4$	M.W: 404,76
0.01 mg/ml	5 or 10 x 1 ml in isooctane
0.1 mg/ml	5 or 10 x 1 ml in isooctane



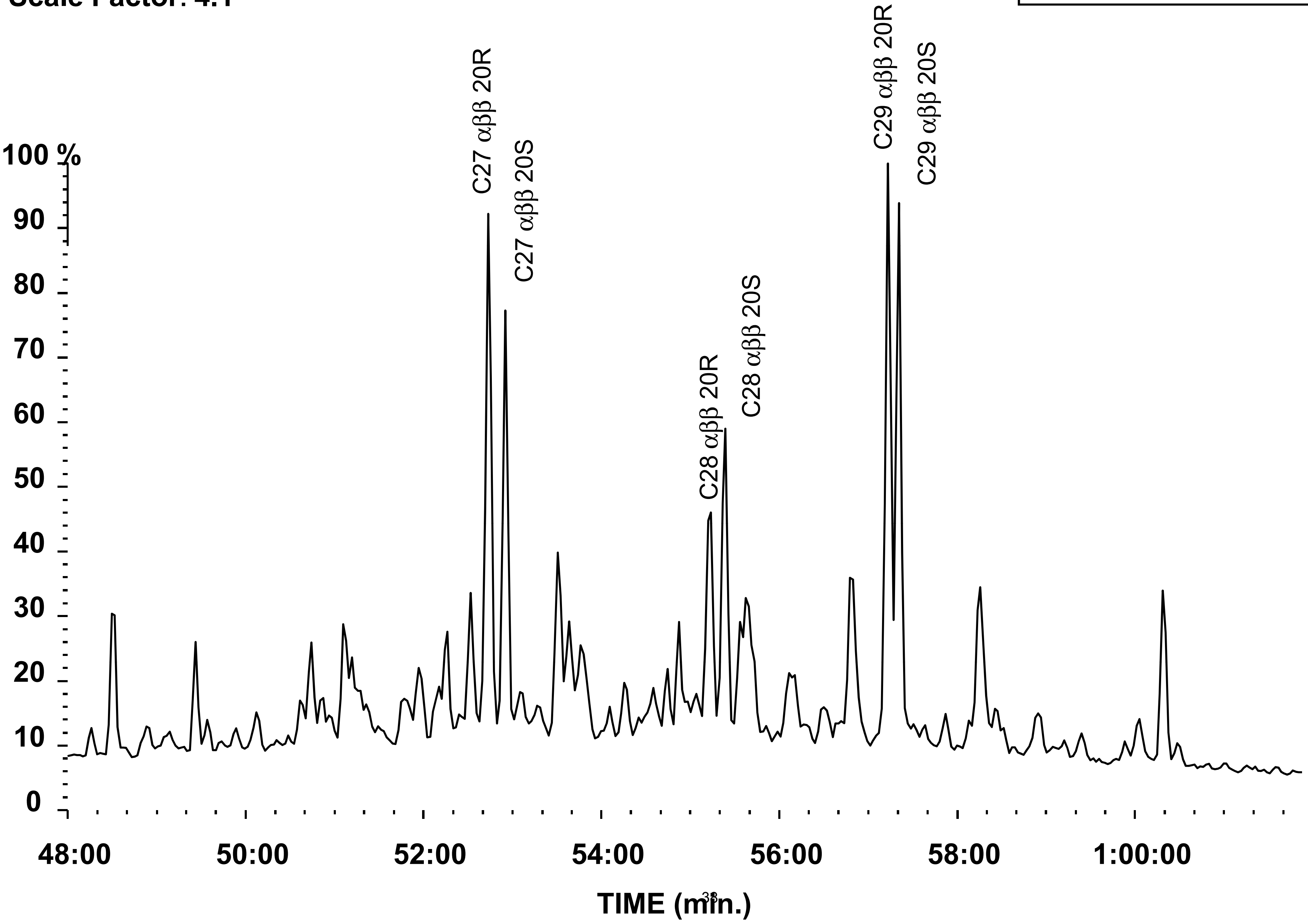
File A6MA15C:4
SIM-GCMS: 217.196 Da
Scale Factor: 4.9

AGSO Standard 217 Da

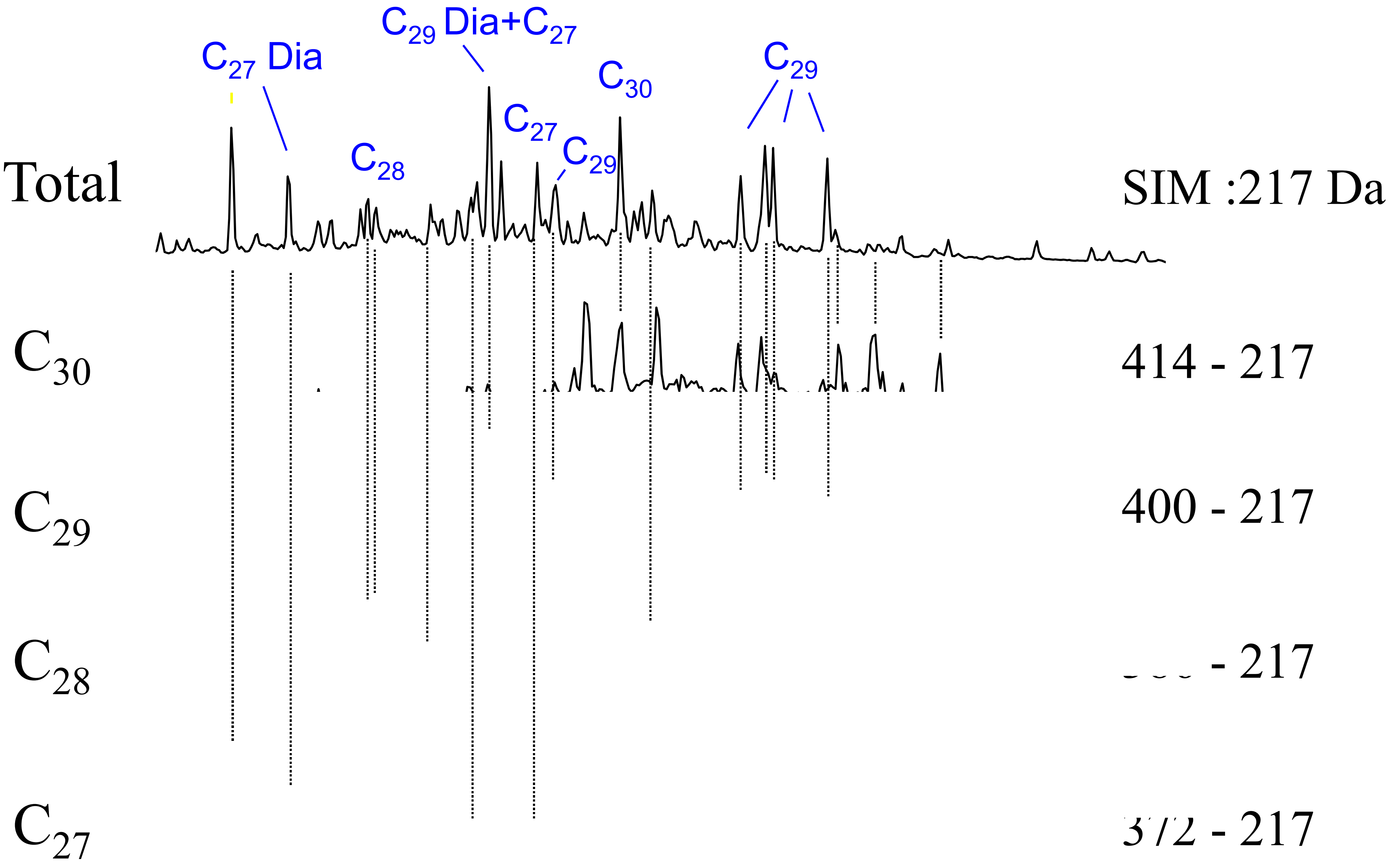


File A6MA15C:4
SIM-GCMS: 218.203
Da
Scale Factor: 4.1

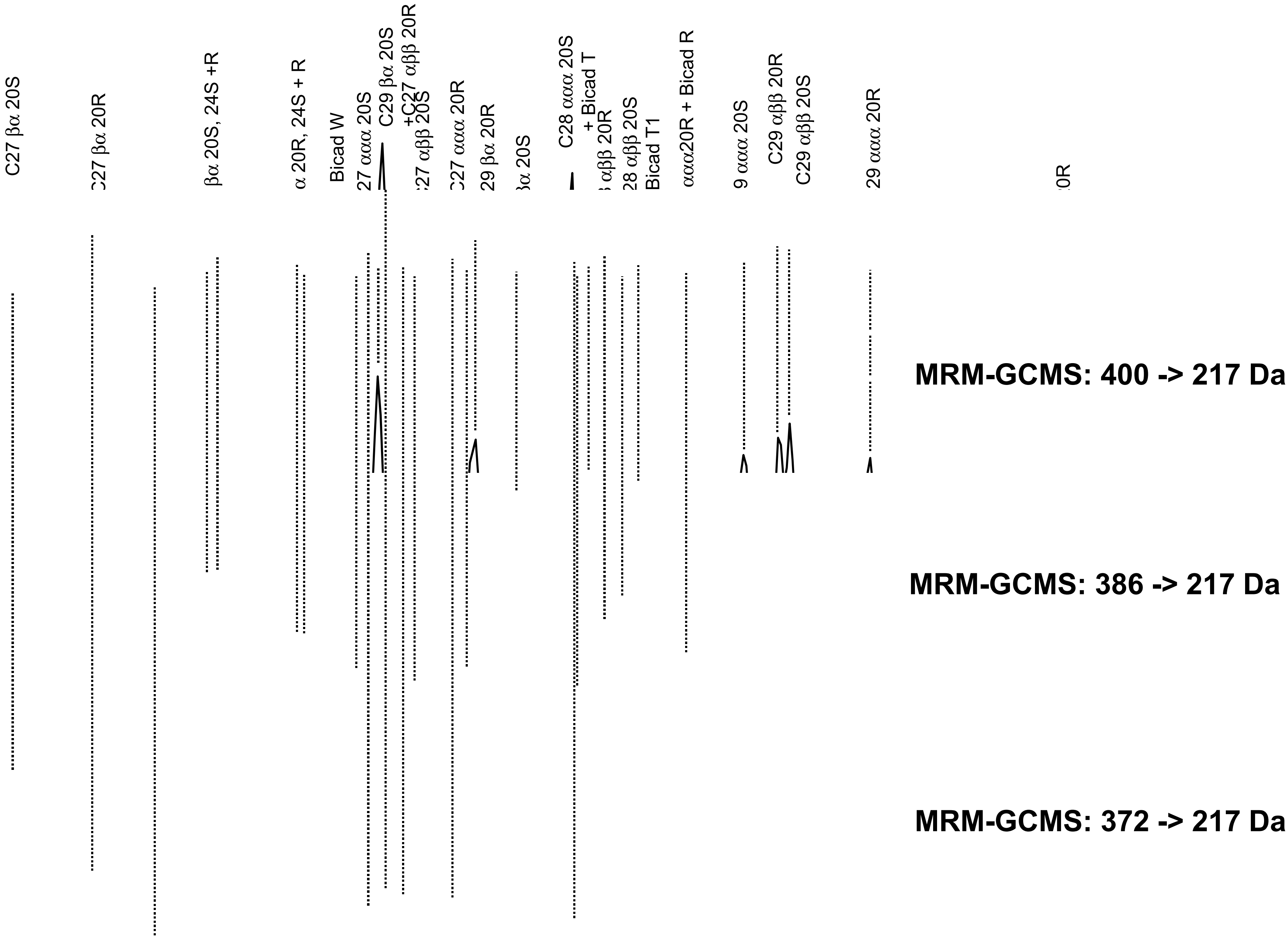
AGSO Standard
218 Da

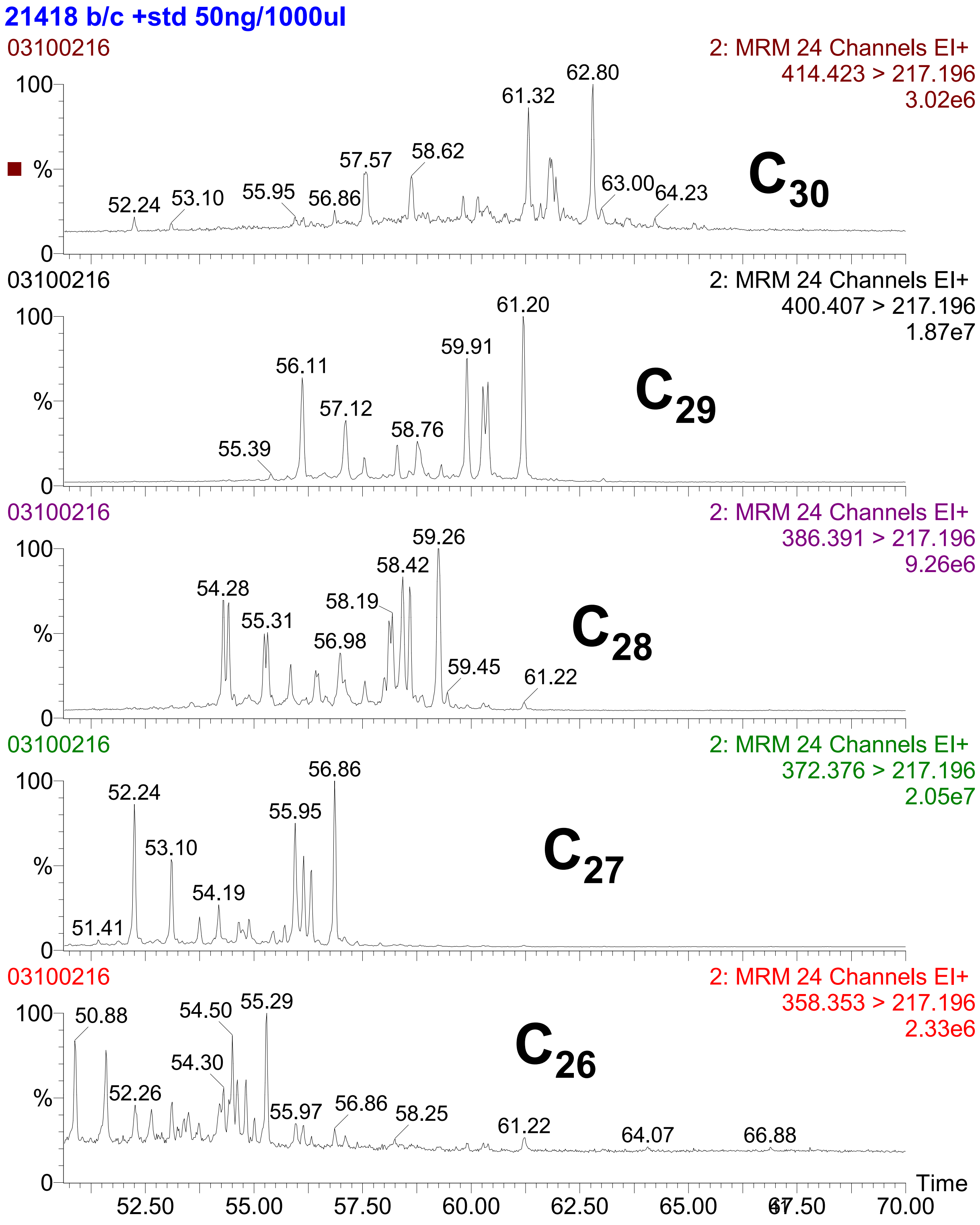


Steranes: SIM -GCMS vs. MRM -GCMS



AGSO Standard: steranes SIM vs. MRM





MRM –GCMS showing steranes from a typical marine sediment L. Permian, Australia

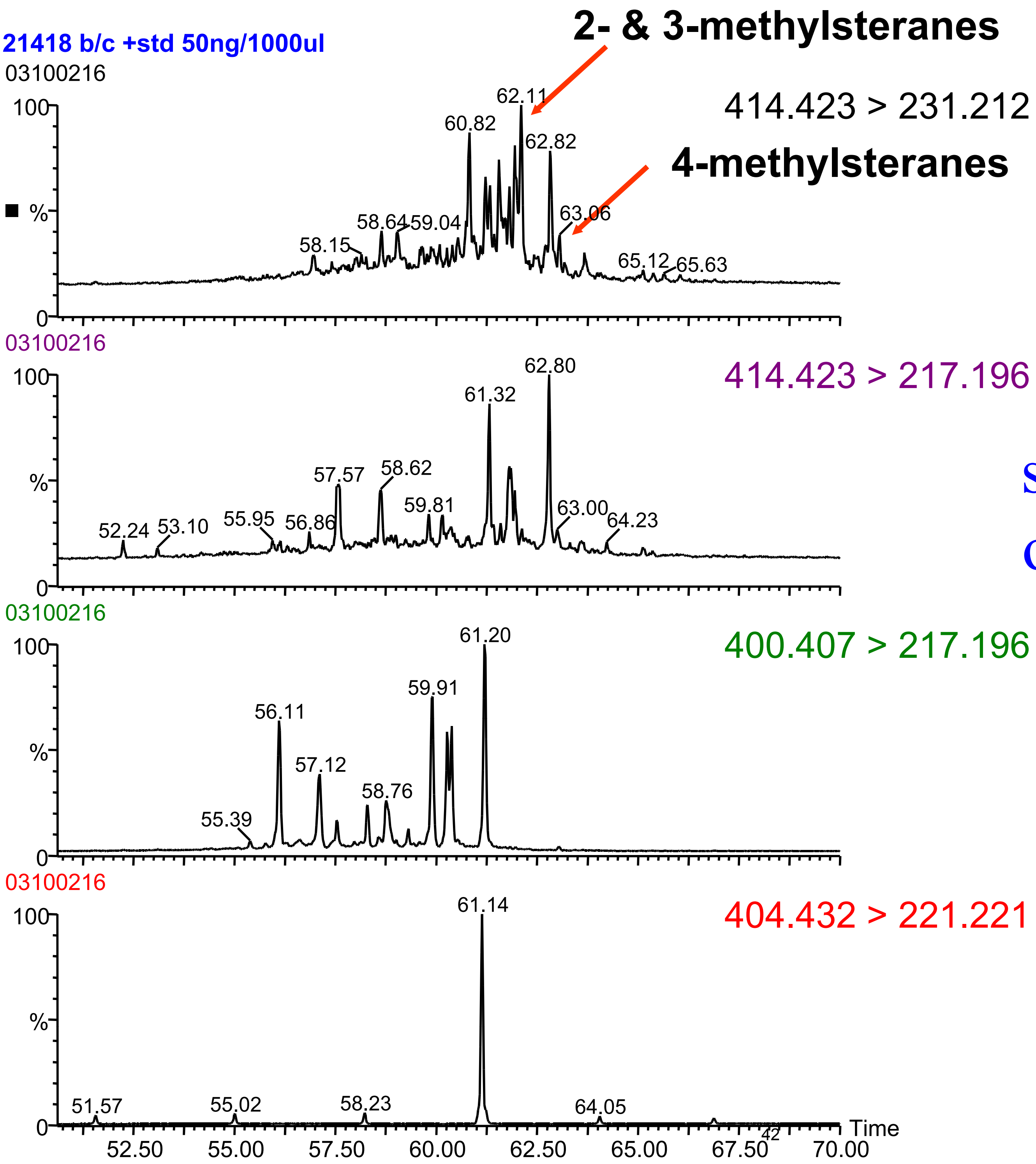
Note similarities in isomer distributions for each homolog

20S/20R

ααα/αββ

βα S+R/ααα+αββ

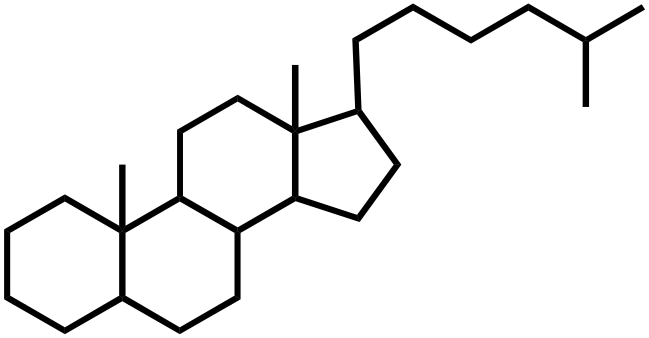
MRM –GCMS showing C_{30} steranes of a marine sediment



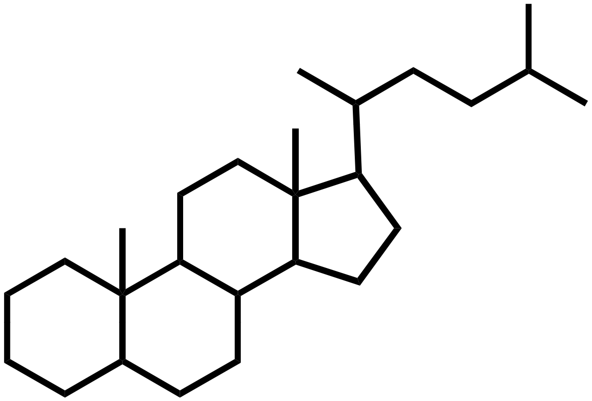
2- & 3-methylsteranes

4-methylsteranes

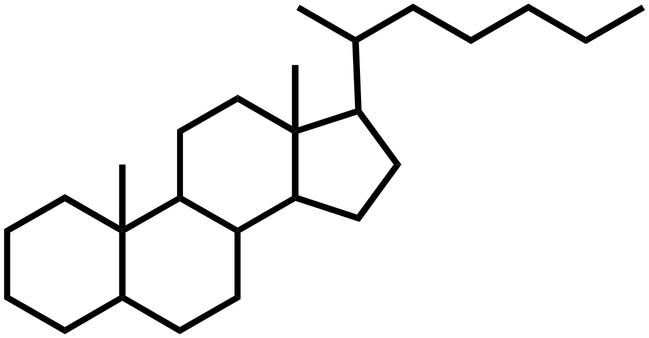
C_{26} steranes



21-nor



24-nor



27-nor

C_{26} steranes elution pattern

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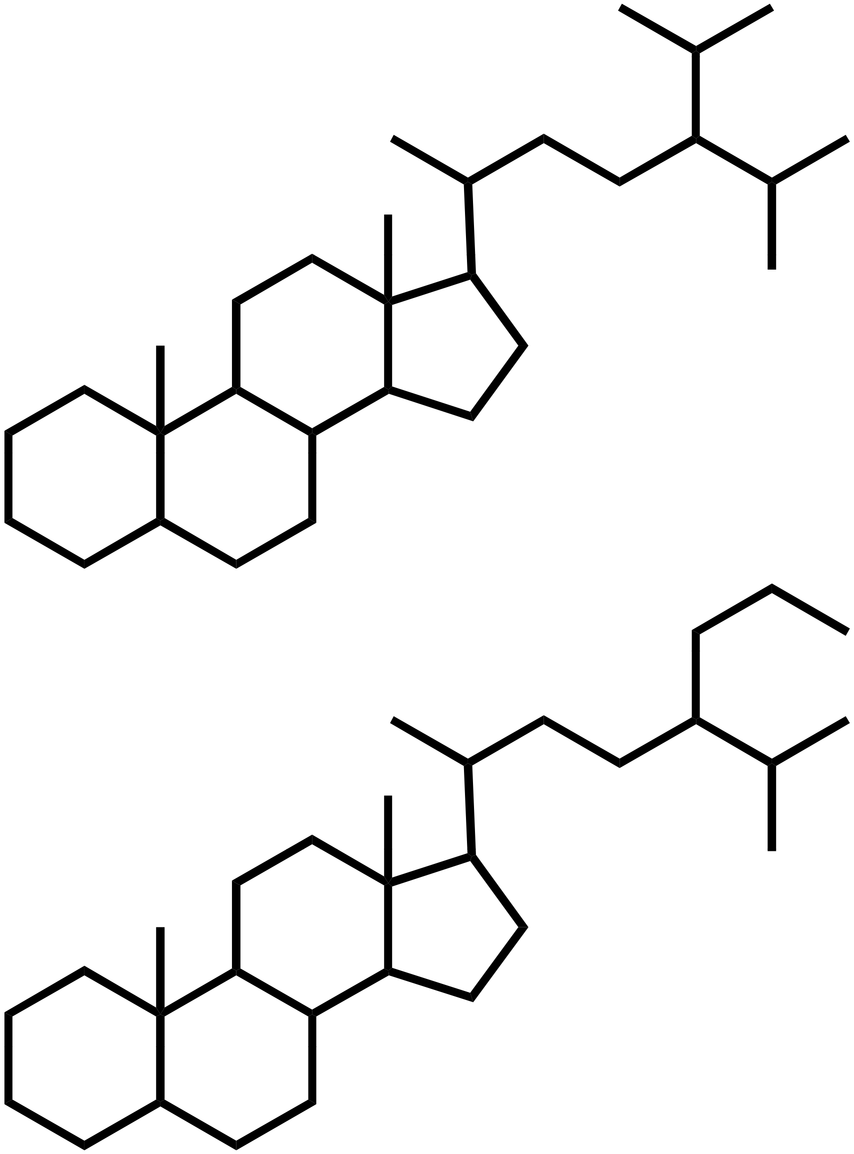
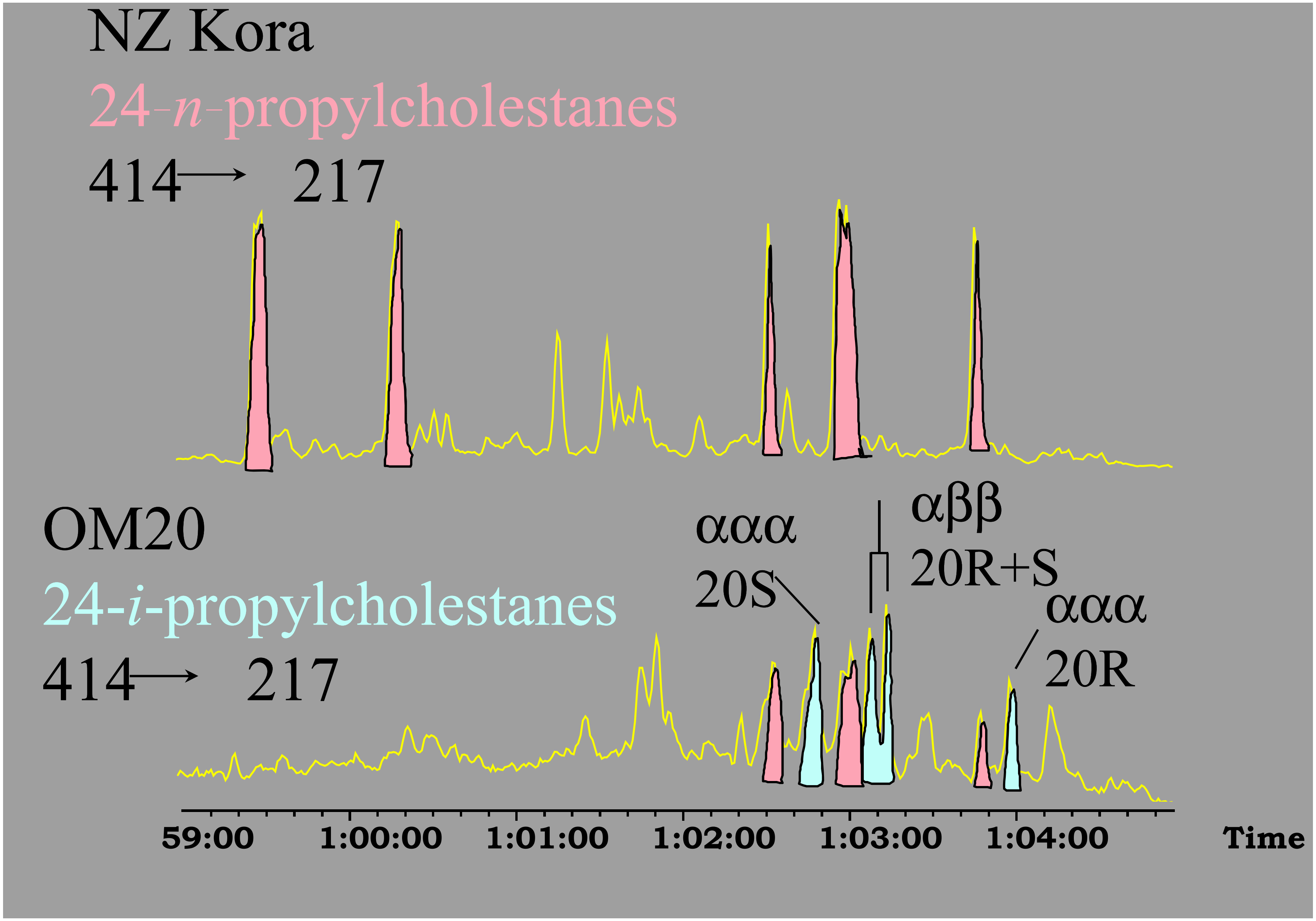
Moldowan et al GCA 55, 1065, 1991

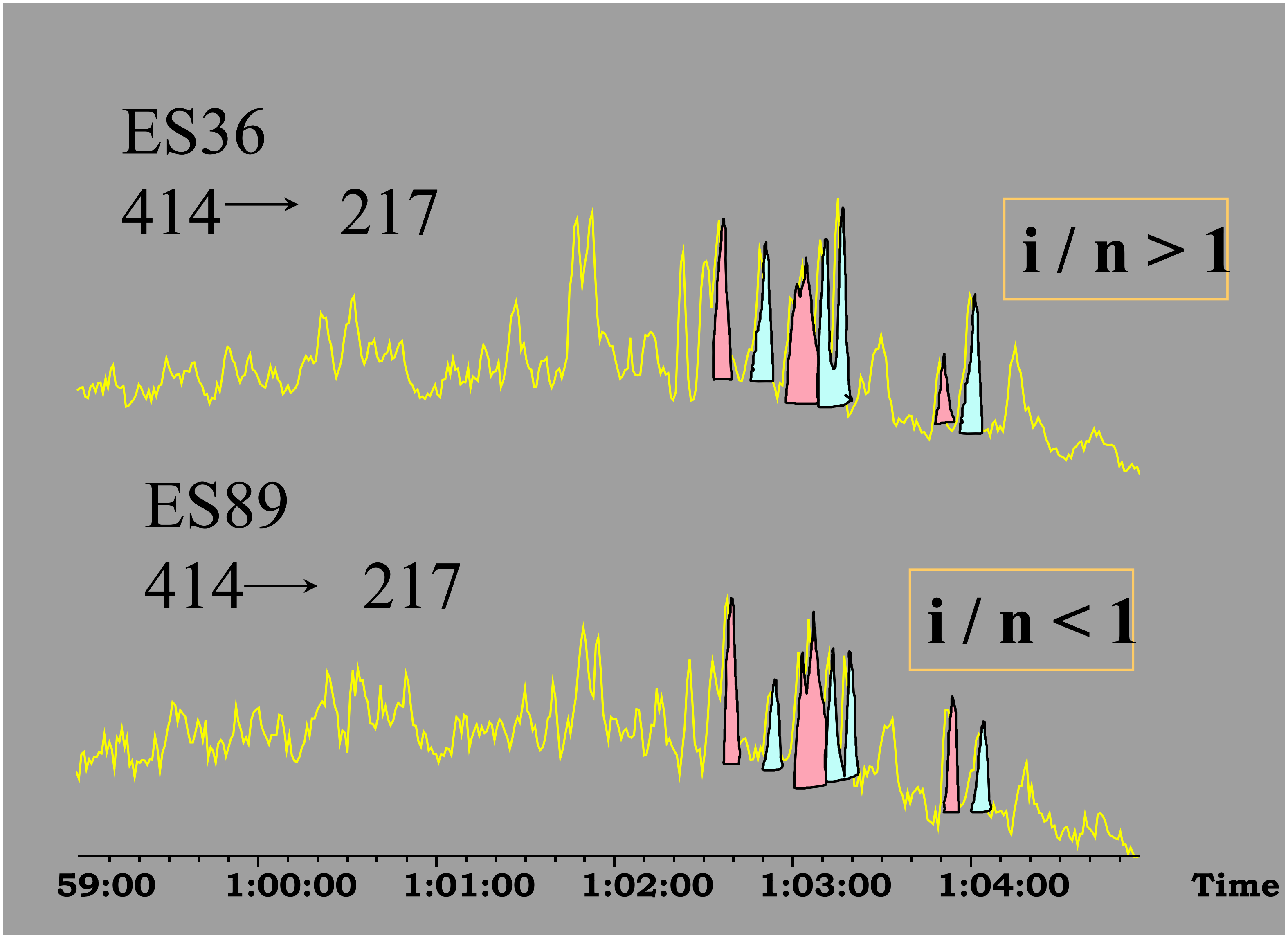
C_{30} Desmethylsteranes

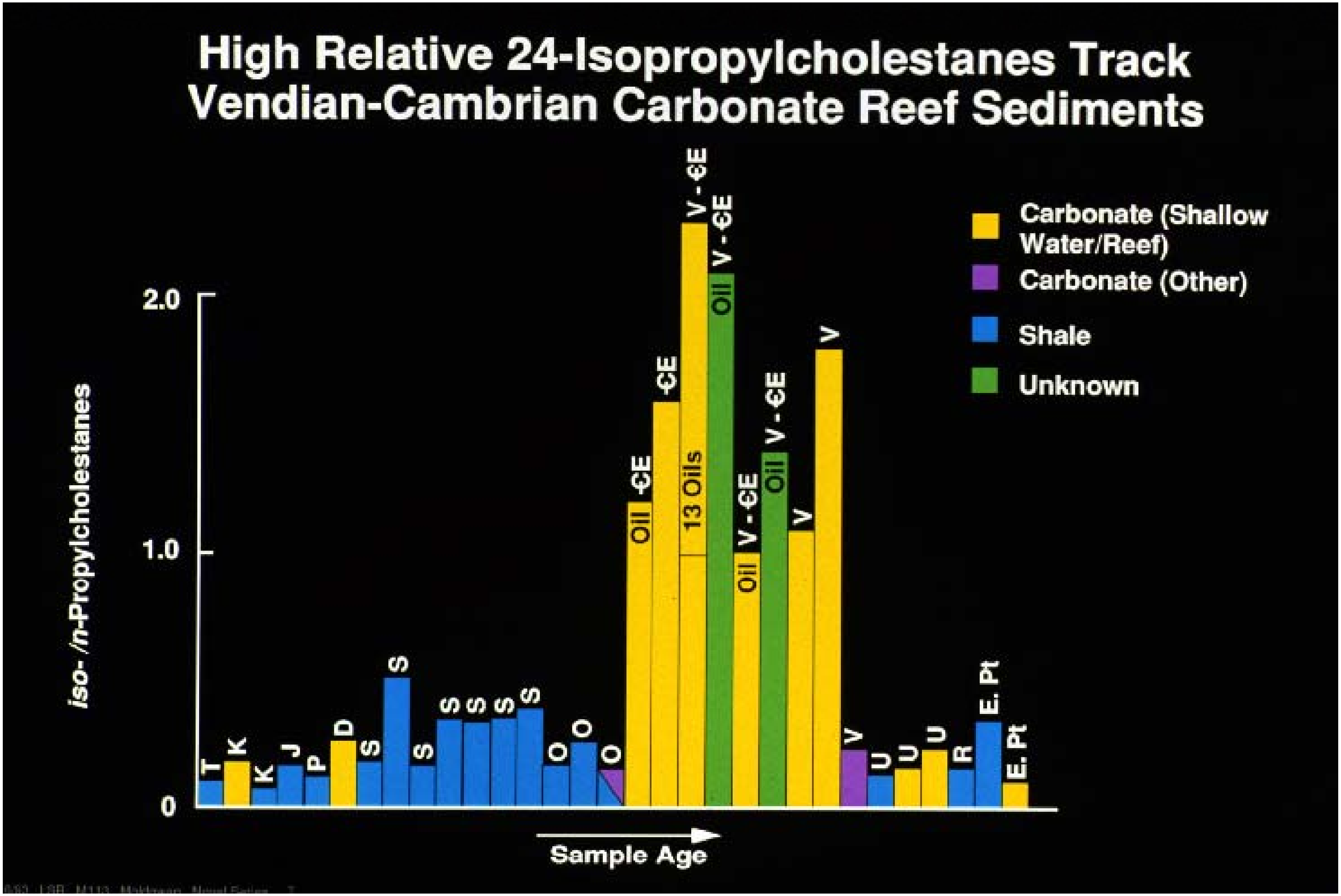


C_{30} Desmethyl Steranes
Oil from Southern Oman



C_{30} Desmethyl Steranes
Eastern Siberia Oils





High Relative 24-Isopropylcholestanes Track Vendian-Cambrian Carbonate Reef Sediments

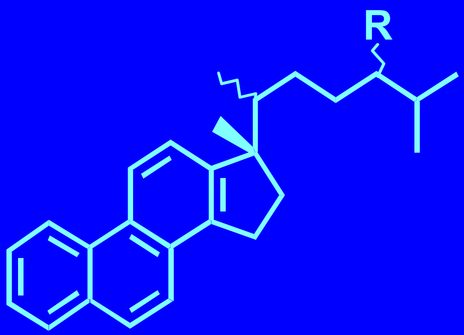
- 🟨 Carbonate (Shallow Water/Reef)
- 🟪 Carbonate (Other)
- 🟦 Shale
- 🟩 Unknown

Aromatic steroids

monoaromatic desmethylsteroid

M^+ --> 253 or m/z 253

maturity = TA/(MA+TA)

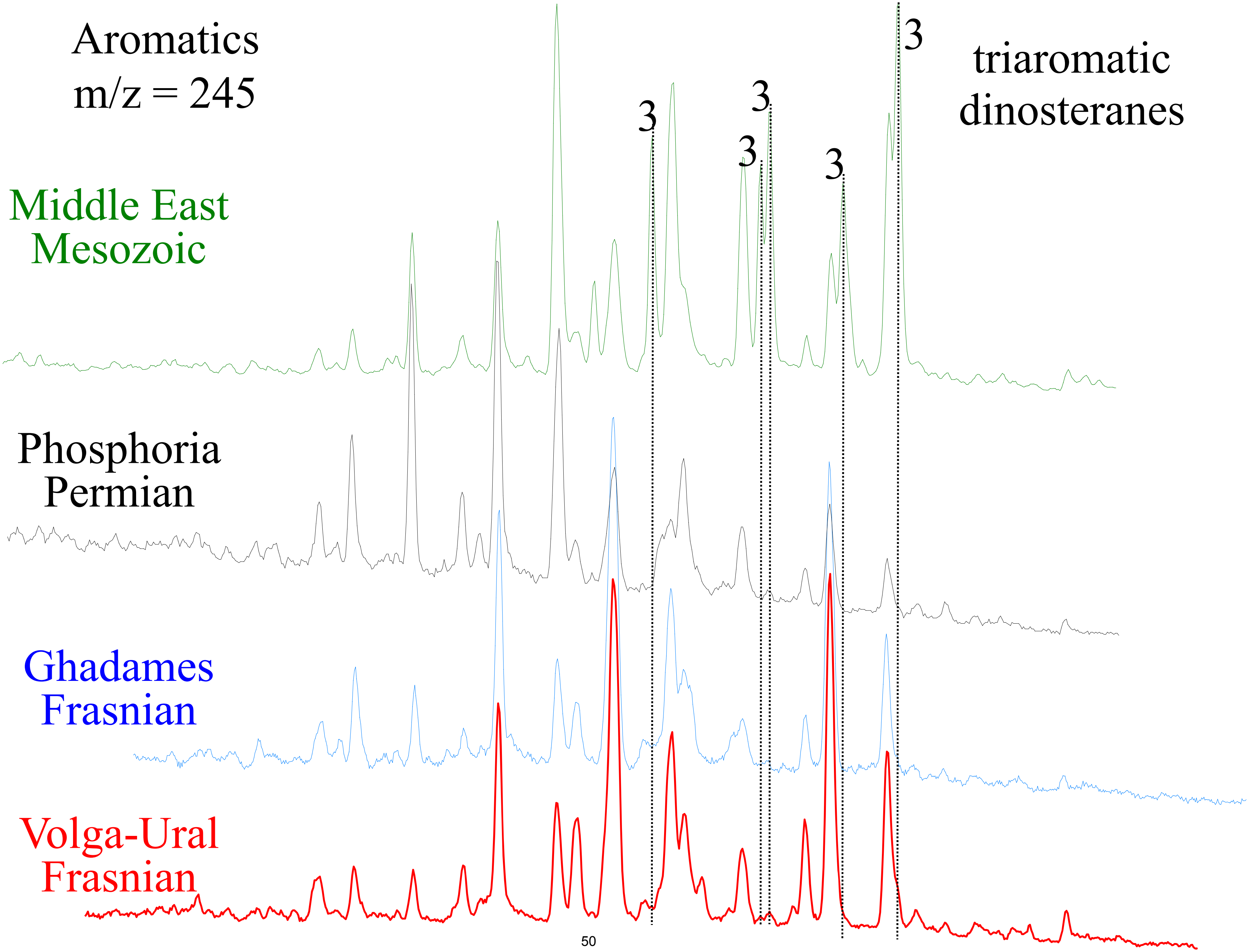


triaromatic desmethylsteroids

m/z 231

triaromatic methylsteroids

m/z 245


Aromatics
m/z = 245
triaromatic
dinosteranes
3
3
3
3
3
3
Middle East
Mesozoic
Phosphoria
Permian
Ghadames
Frasnian
Volga-Ural
Frasnian

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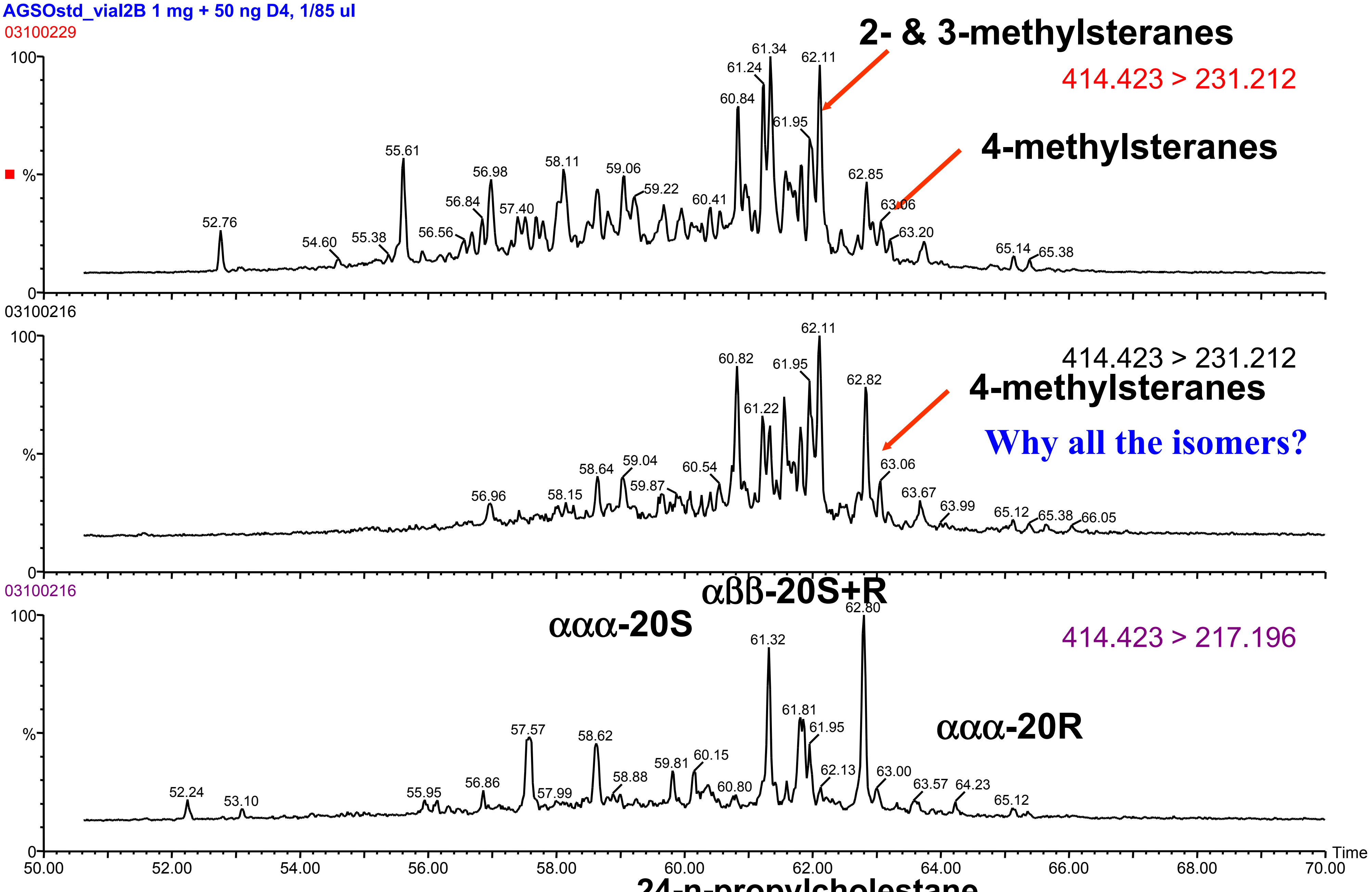
Distribution of dinosteroids in Phanerozoic sediments

Moldowan and Talyzina
Science 281,168-1170, 1998



2- & 3-methylsteranes

414.423 > 231.212

4-methylsteranes

414.423 > 231.212

4-methylsteranes

Why all the isomers?

αββ-20S+R

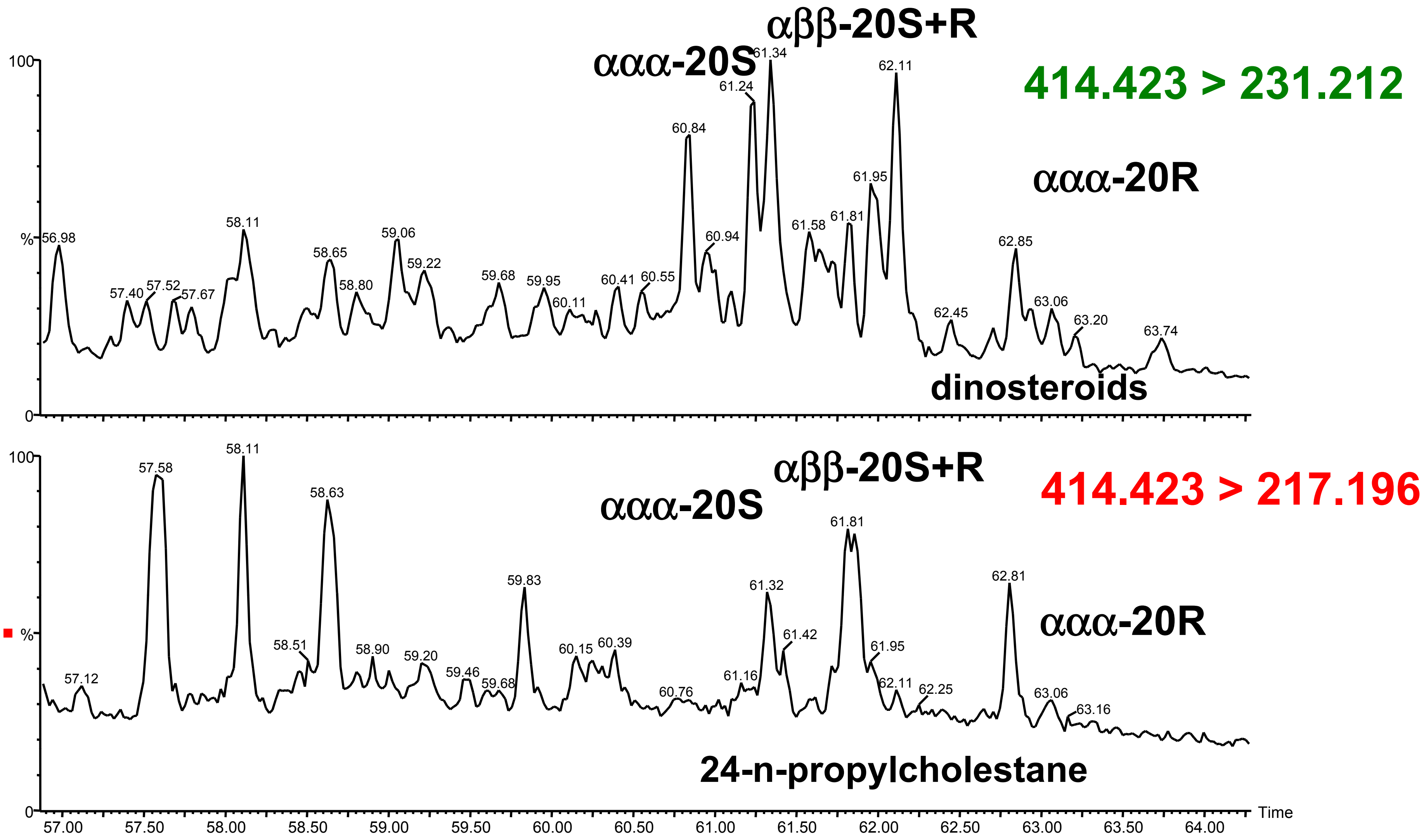
ααα-20S

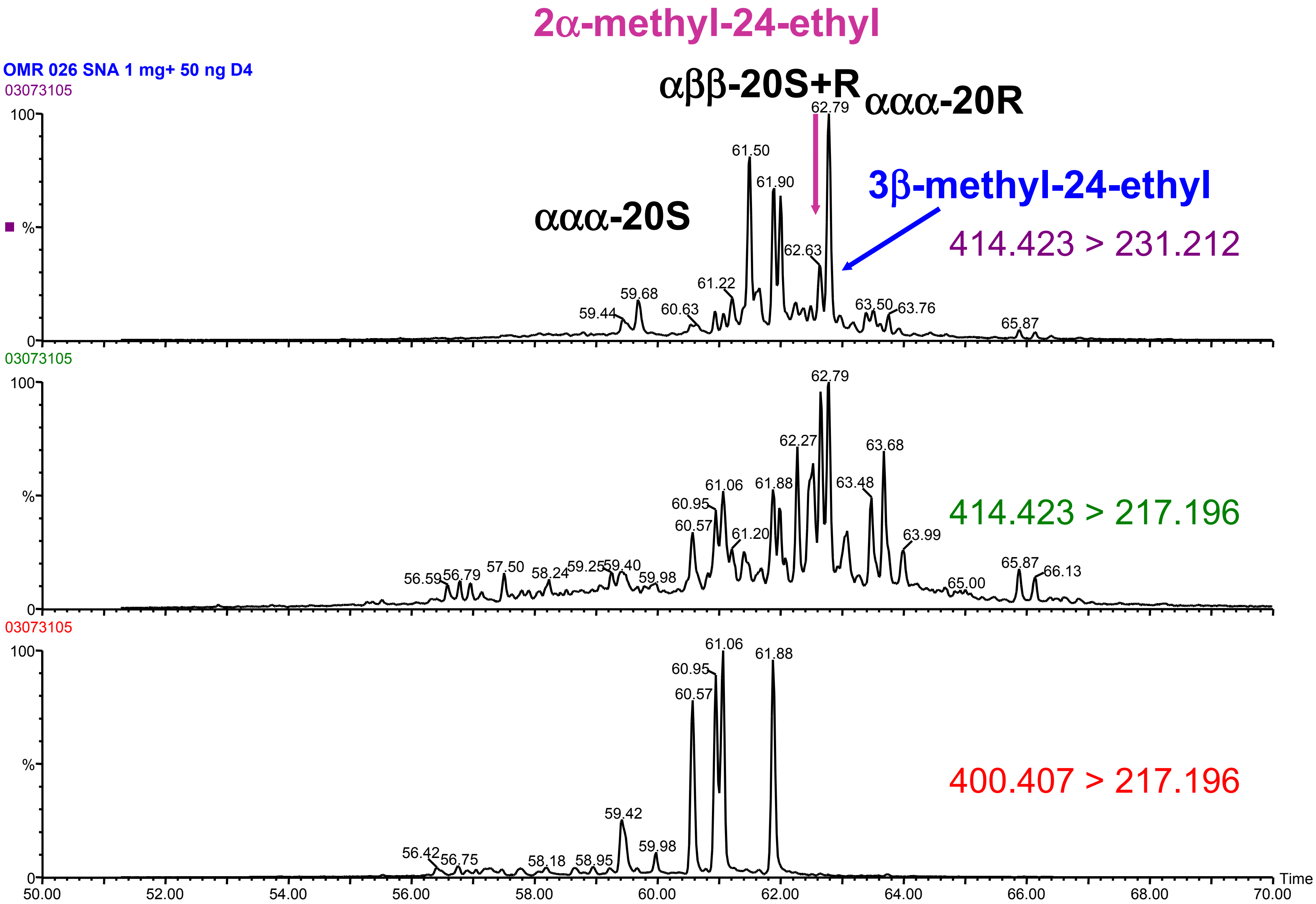
414.423 > 217.196

ααα-20R

24-n-propylcholestane

AGSOstd_vial2B 1 mg + 50 ng D4, 1/85 ul




2α-methyl-24-ethyl
OMR 026 SNA 1 mg+ 50 ng D4
03073105
αββ-20S+R
ααα-20R
62.79
100
61.50
61.90
3β-methyl-24-ethyl
ααα-20S
%
62.63
414.423 > 231.212
61.22
59.68
60.63
63.50
63.76
59.44
65.87
0
03073105
100
62.79
62.27
63.68
61.06
61.88
63.48
60.95
%
414.423 > 217.196
60.57
61.20
63.99
65.87
57.50
59.25
59.40
66.13
56.59
56.79
58.24
59.98
65.00
0
03073105
100
61.06
61.88
60.95
60.57
%
400.407 > 217.196
59.42
59.98
56.42
56.75
58.18
58.95
0
Time
50.00
52.00
54.00
56.00
58.00
60.00
62.00
64.00
66.00
68.00
70.00

2- & 3-alkylsteranes

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Please see: Abstract, JoséA. D. Lopes et al. "Geosteranes: Identification and Synthesis of a Novel Series of 3-Substituted Steranes." *Organic Geochemistry* 26, no. 11-12 (July 1997): 787-790.

Org. Geochem. Vol. 26, Nos. 11–12, pp. 787–790, 1997

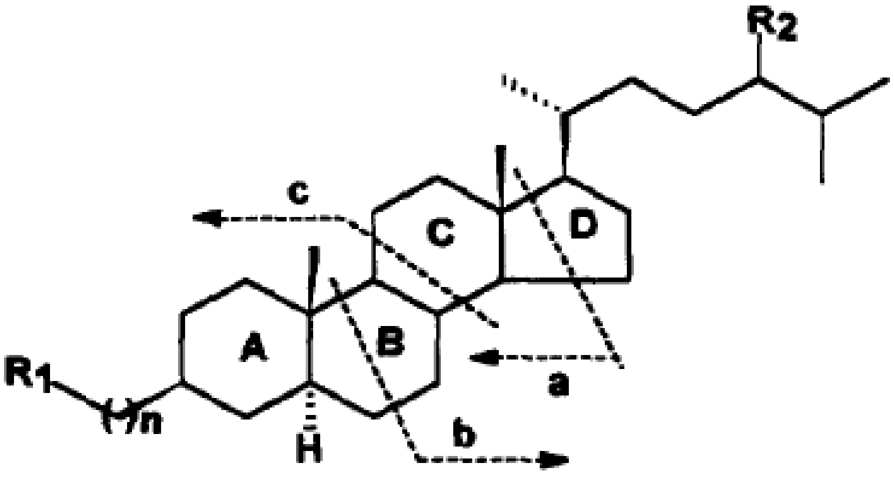
2- & 3-alkylsteranes

INTRODUCTION

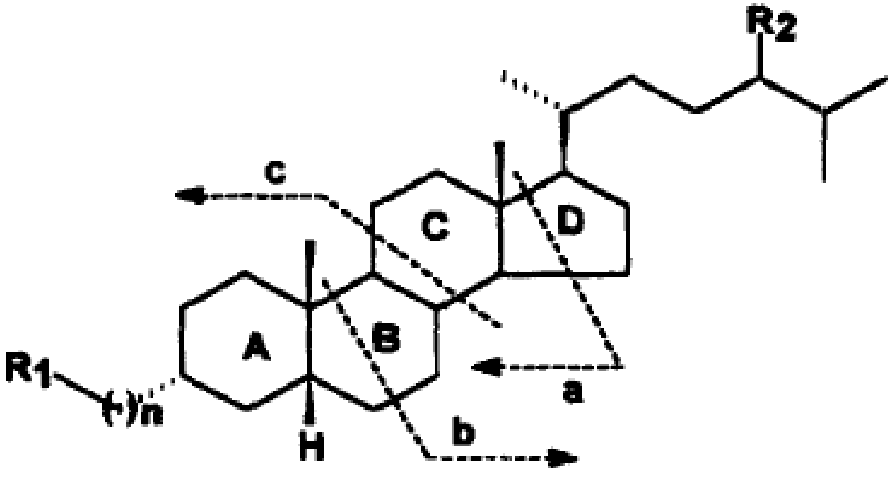
Since the first reports of 3-methyl (Summons and Capon, 1988; Summons et al. 1988), and 3-carboxyl-steranes (Dany et al., 1990), other sterane bio-markers substituted at the C-3 positions have been reported (Summons and Capon, 1991; Dahl et al., 1992; Lopes et al., 1992, 1994; Schaeffer et al., 1993, 1994) belonging to the 5α(H) series of cholestane (R2 = H), ergostane (R2 = Me) and stigmastane (R2 = Et). These compounds are of geological interest because they have unusual substitution patterns and because logical precursors have not been identified in any living orghanism.

Org. Geochem. Vol. 26, Nos. 11–12, pp. 787–790, 1997

2- & 3-alkylsteranes



1 R_1=H, R_2=H, n=1 to 4
2 R_1=H R_2=CH_3, n=1 to 5
3 R_1=H R_2=CH_2CH_3, n=1 to 3
4 R_1=CO_2H, R_2=H, n=0 to 5
5 R_1=CO_2H, R_2=CH_3, n=0 to 5
6 R_1=CO_2H, R_2=CH_2CH_3, n=0 to 5



7 R_1=H, R_2=H, n=1 to 2
8 R_1=CO_2H, R_2=H, n=0 to 4
9 R_1=CO_2H, R_2=CH_3, n=3
10 R_1=CO_2H, R_2=CH_2CH_3, n=3 to 4

he alkyl or carboxyalkyl moiety at the C-3 position. Fragment *b* includes R_2 and allows the differentiation of the degree of alkylation at C-24.

Fragment *c* confirms the nature of the R_1 substituent and also establishes the configuration at C-5. The ratio between the ions *m/z* 149 and 151 in cholestane ($R_1 = R_2 = H$, n = 0) depends on the A/B ring junction configuration (Gallegos, 1971). Compounds with 5β(H) configuration show a higher *m/z* 151 peak than that of *m/z* 149 while those with 5α(H) have an increased *m/z* 149.

Org. Geochem. Vol. 26, Nos. 11–12, pp. 787–790, 1997

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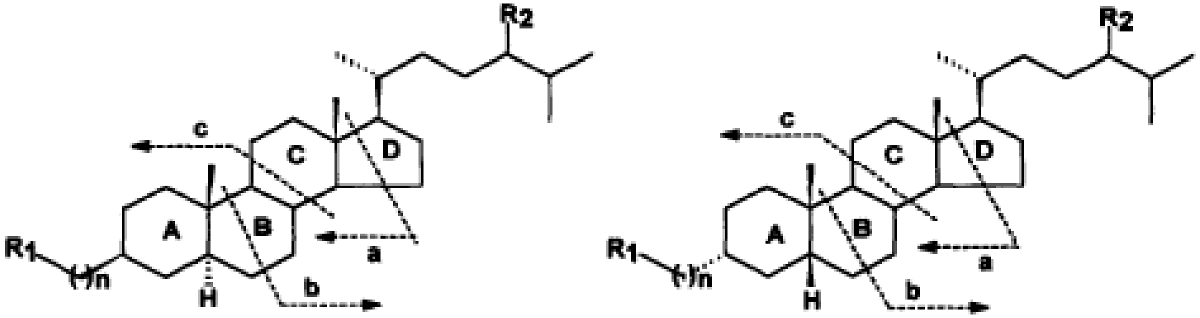
Fossil steranes no longer have the original oxygen at C3

Fossil steranes with unprecedented methylation in ring-A, *1988*.
Roger E. Summons and Robert J. Capon
Geochimica et Cosmochimica Acta 52, 2733-2736

Identification and significance of 3β-ethyl steranes in sediments and petroleum, *1991.*
Roger E. Summons and Robert J. Capon
GCA, 55, 2391-2395

Extended 3β-alkyl steranes and 3-alkyl triaromatic steroids in crude oils and rock extracts *1995*.
Jeremy Dahl, J. Michael Moldowan, Roger E. Summons, Mark A. McCaffrey, Paul Lipton, D. S. Watt and Janet M. Hope
GCA, 59,, 3717-3729

Geosteranes: Identification and synthesis of a novel series of 3-substituted steranes. *1997.*
JoséA. D. Lopes, Eugênio V. Santos Neto, Márcio R. Mello and Francisco De A.M. Reis
Org. Geochem., 26, 787-790



1 R_1=H, R_2=H, n=1 to 4
2 R_1=H R_2=CH_3, n=1 to 5
3 R_1=H R_2=CH_2CH_3, n=1 to 3
4 R_1=CO_2H, R_2=H, n=0 to 5
5 R_1=CO_2H, R_2=CH_3, n=0 to 5
6 R_1=CO_2H, R_2=CH_2CH_3, n=0 to 5

7 R_1=H, R_2=H, n=1 to 2
8 R_1=CO_2H, R_2=H, n=0 to 4
9 R_1=CO_2H, R_2=CH_3, n=3
10 R_1=CO_2H, R_2=CH_2CH_3, n=3 to 4

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Diagenetic Pathways of Sterols

Δ2-sterenes are primary sterol dehydration products as shown in lab expts. and data from immature sediments.

Nature 269, 978, 1977

Diverse 3-alkylated steranes direct evidence of 3β-OH

Steroid incorporated in kerogen bound through C-3 as shown by chemolysis with deuterated reagents

Sterol Methyltransferases -SMT

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- Responsible for alkylation at C24; stereochemically precise with strict substrate selectivity

-Use the 3β-OH group for recognition and anchoring

12.158 Molecular Biogeochemistry
Fall 2011