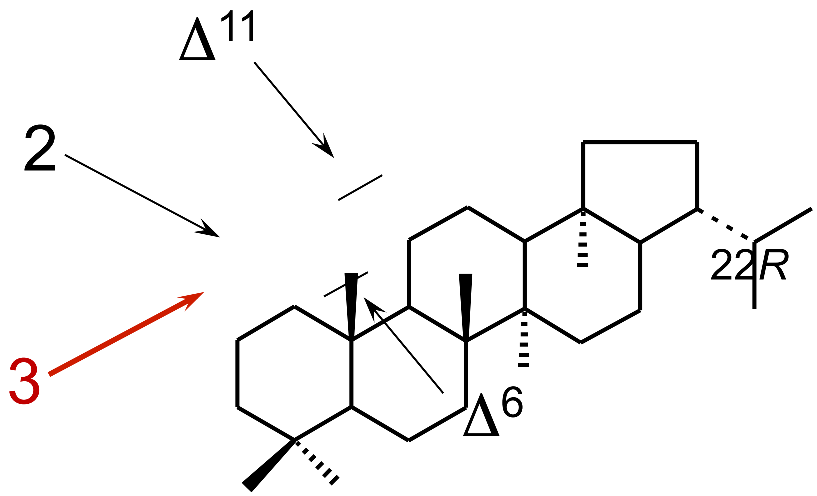
12.158 Lecture 7

- More on cyclic terpenoids
  - Analytical methods for hopanoids
  - LCMS Insights
  - Tricyclic and tetracyclic terpanes
  - Plant sesqui-, di-, triterpenoids

BHP Ring Configurations



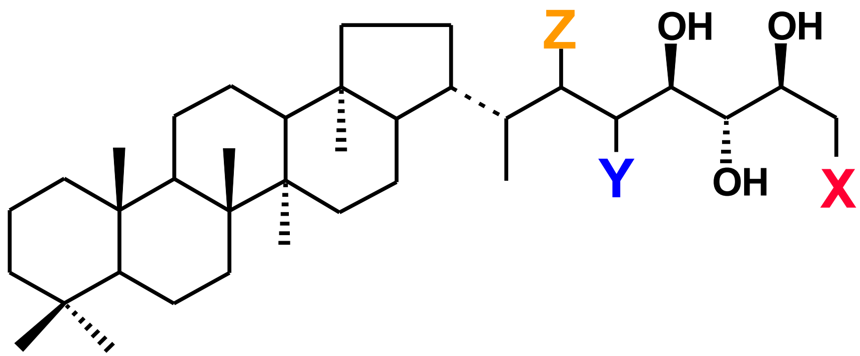
C-2 Me
CYANOBACTERIA
Summons et al., 2000

C-3 Me
METHANOTROPHS
(Acetic Acid bacteria)

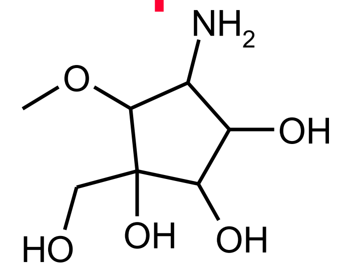
Δ^6 and/or Δ^{11}
ACETIC ACID BACTERIA
(Methanotroph)

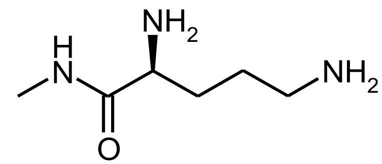
HelenTalbot_nrg

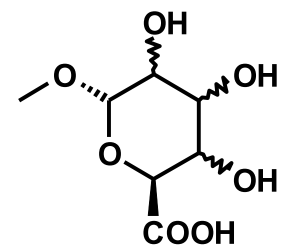
BHP Side-Chain Structures



Composite







TETRA: X=OH, NH_2, composite; Y = Z = H

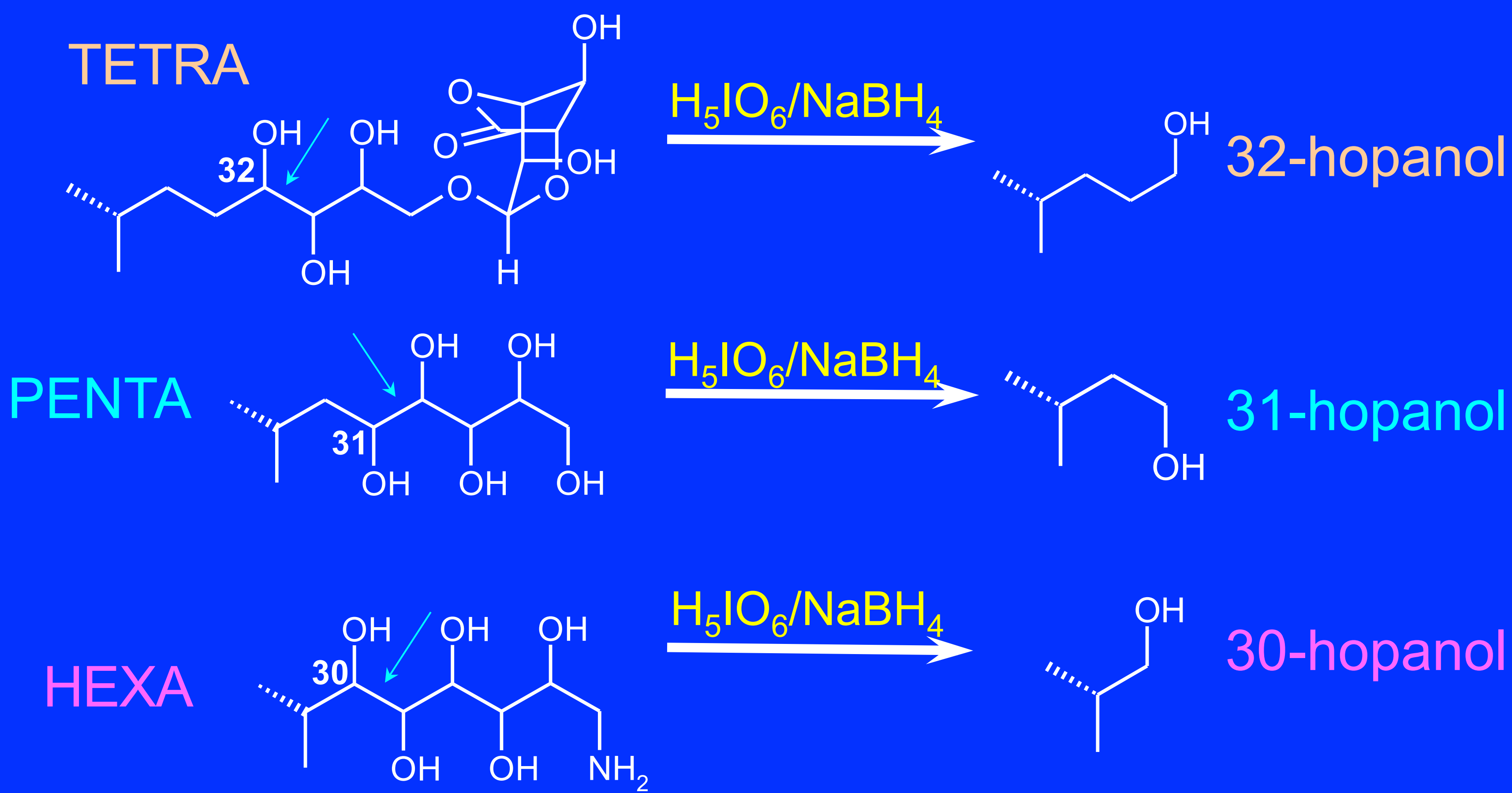
PENTA: X = OH, NH_2, composite; Y = OH, Z = H
X = OH, Y = H, Z = OH

HEXA: X = NH2; Y = Z = OH

Analysis of BHP

- Highly functionalised, amphiphillic
- Not amenable to conventional GC-MS
- Side chain cleavage (Rohmer et al., 1984)
  - Periodic acid/sodium borohydride
  - Product structure directly related to number and position of functional groups in side chain
- Specific nature of functional groups lost

Rohmer periodic acid oxid & red



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Talbot LCMS Methodology

- Extraction:
  - ultrasonication & Soxtherm in 2:1 chloroform/ MeOH
- Acetylation (acetic anhydride/pyridine)
- RP-HPLC (Adapted from Schullenberg-Schell et al., 1989)
  - 15 cm C_{18} column with 1 cm guard column
  - Ternary solvent system

Time (min)	0-5	10	40	70	75
Water (%)	**10**	**5**	**1**	**1**	**10**
Methanol (%)	**90**	**80**	**59**	**59**	**90**
Propan-2-ol (%)	**0**	**15**	**40**	**40**	**0**

- Positive ion APCI (Talbot *et al.*, 2001 J. Chromatogr. A)

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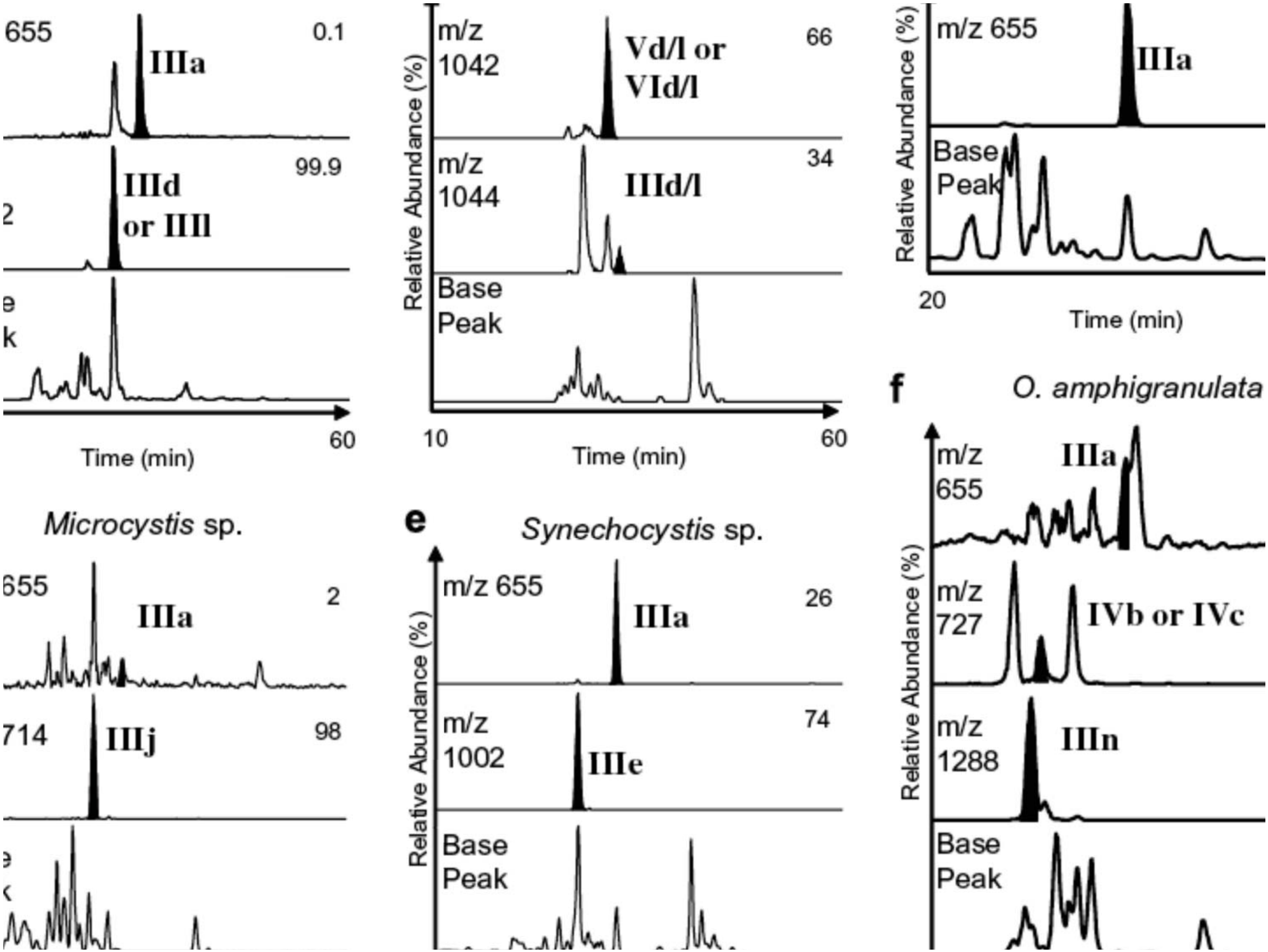
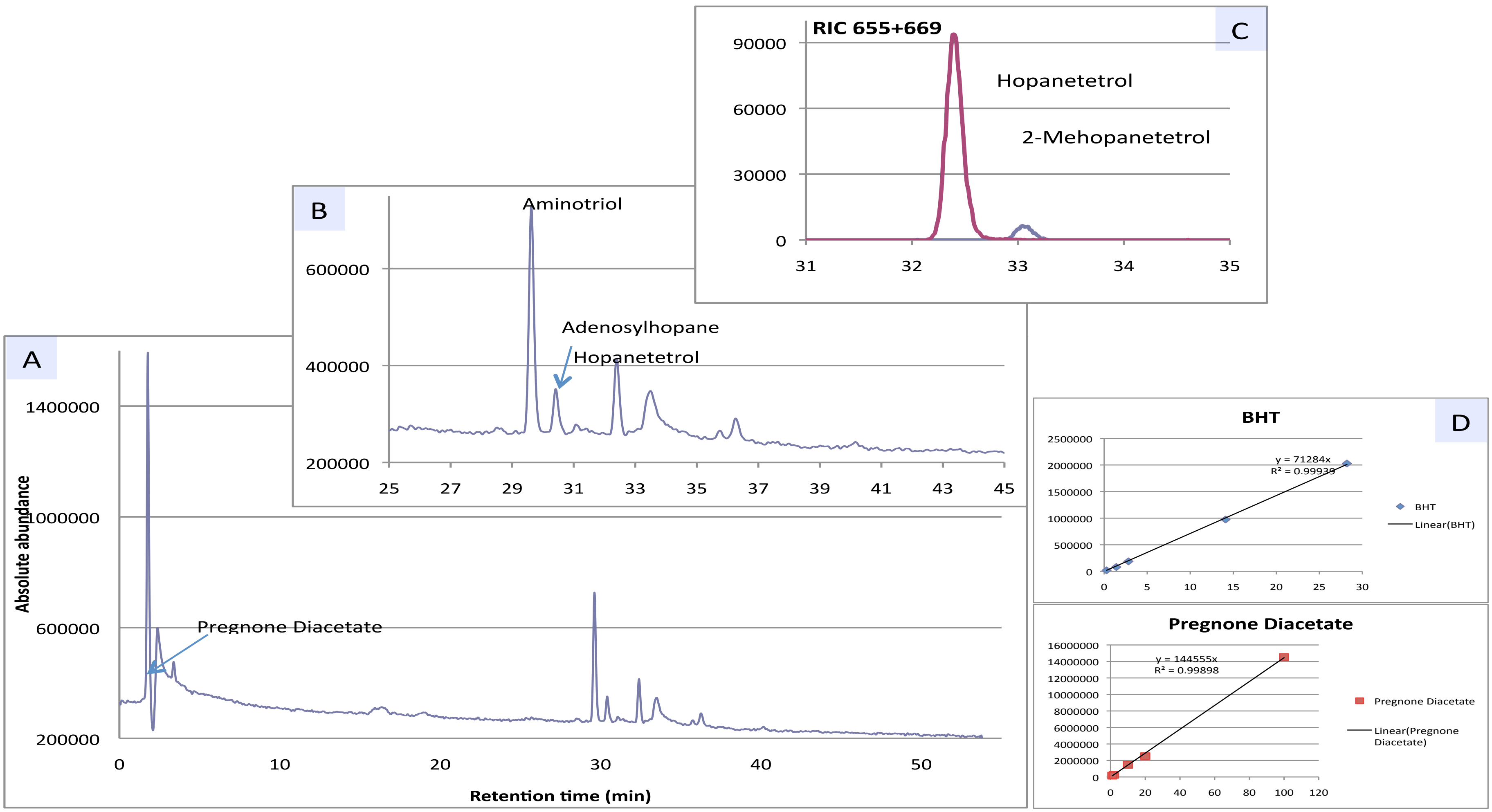
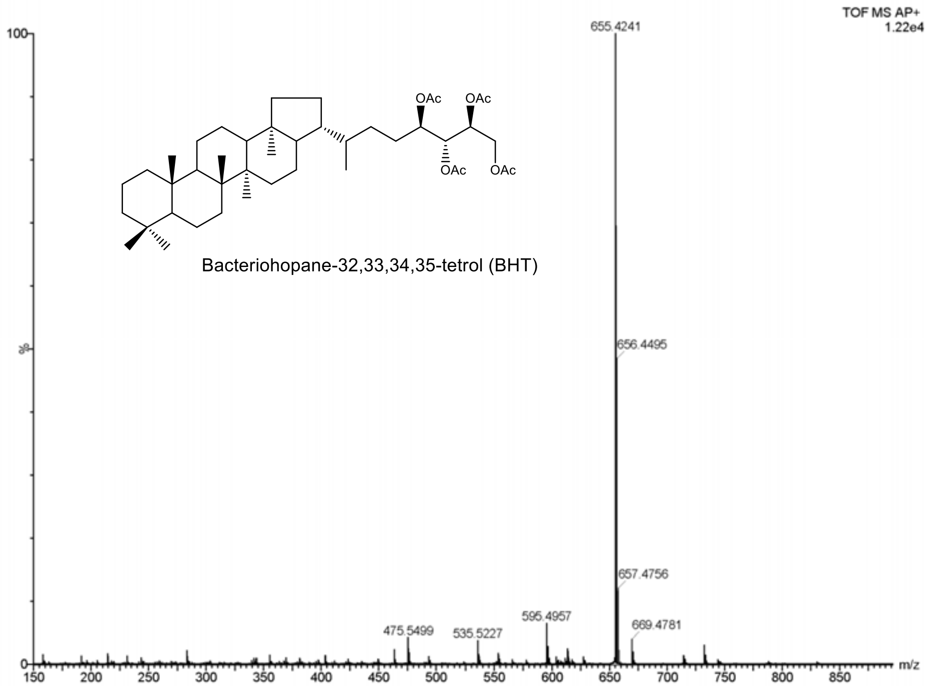
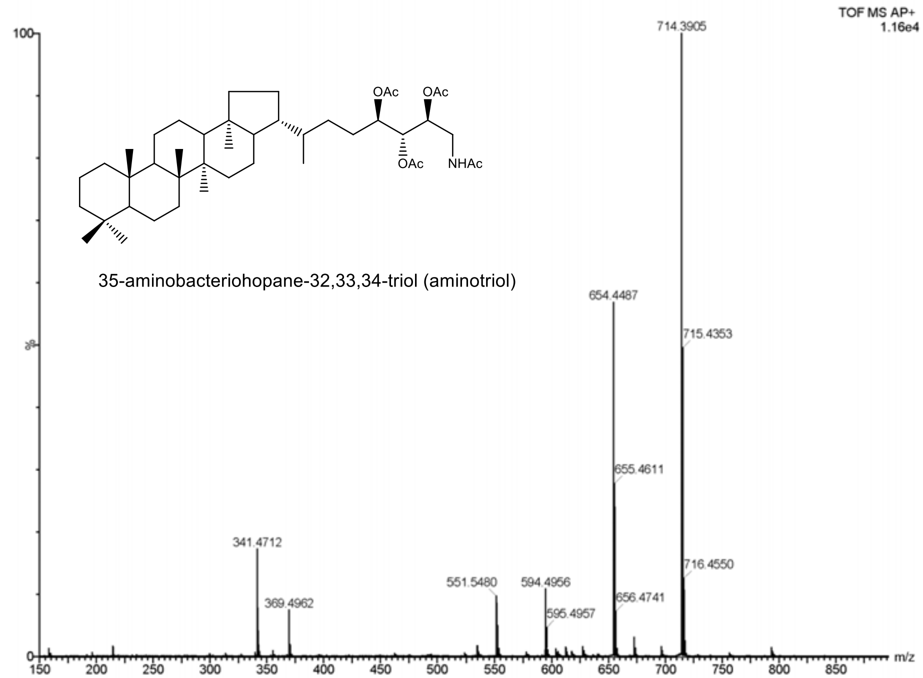
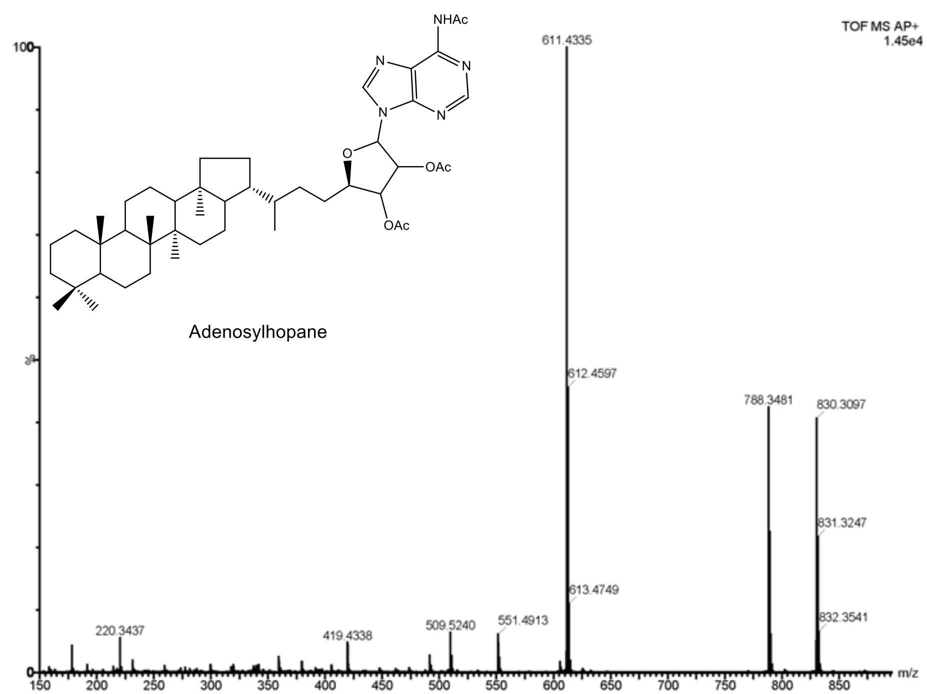

655
0.1
IIIa
99.9
IIId
or IIIl
2
e
k
60
Time (min)
m/z
1042
Vd/l or
VId/l
66
m/z
1044
IIId/l
34
Base
Peak
Relative Abundance (%)
10
Time (min)
60
m/z 655
IIIa
Base
Peak
Relative Abundance (%)
20
Time (min)
Microcystis sp.
655
IIIa
2
714
IIIj
98
e
Synechocystis sp.
m/z 655
IIIa
26
m/z
1002
IIIe
74
Base
Peak
Relative Abundance (%)
f
O. amphigranulata
m/z
655
IIIa
m/z
727
IVb or IVc
m/z
1288
IIIn
Base
Peak
Relative Abundance (%)

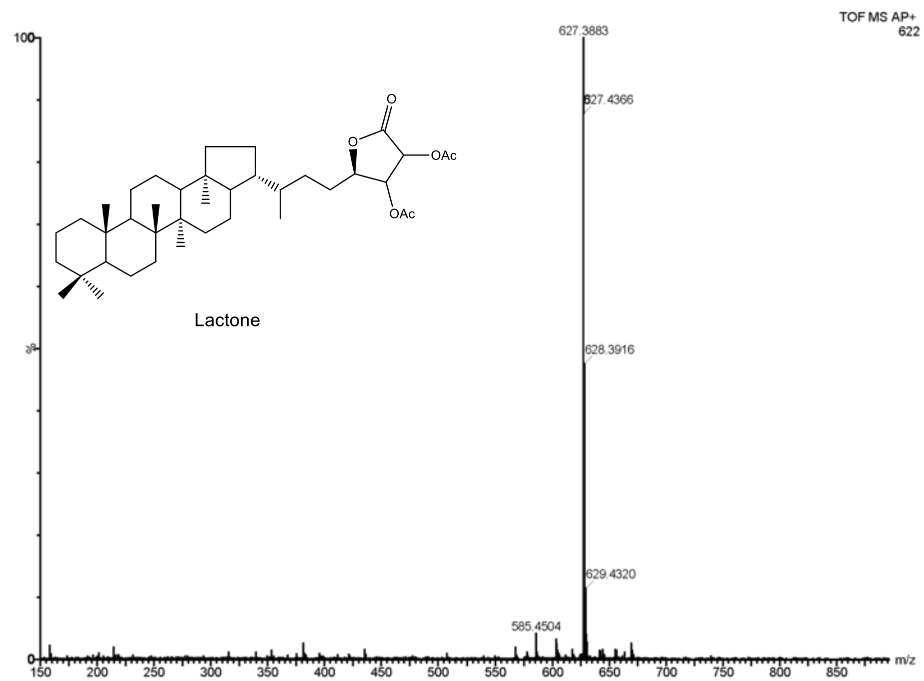
Mehay Modifications to Talbot et al APCI LC-MS




TOF MS AP+
1.22e4
100
%
0
OAc
OAc
OAc
OAc
Bacteriohopane-32,33,34,35-tetrol (BHT)
655.4241
656.4495
657.4756
669.4781
595.4957
535.5227
475.5499
m/z
150 200 250 300 350 400 450 500 550 600 650 700 750 800 850

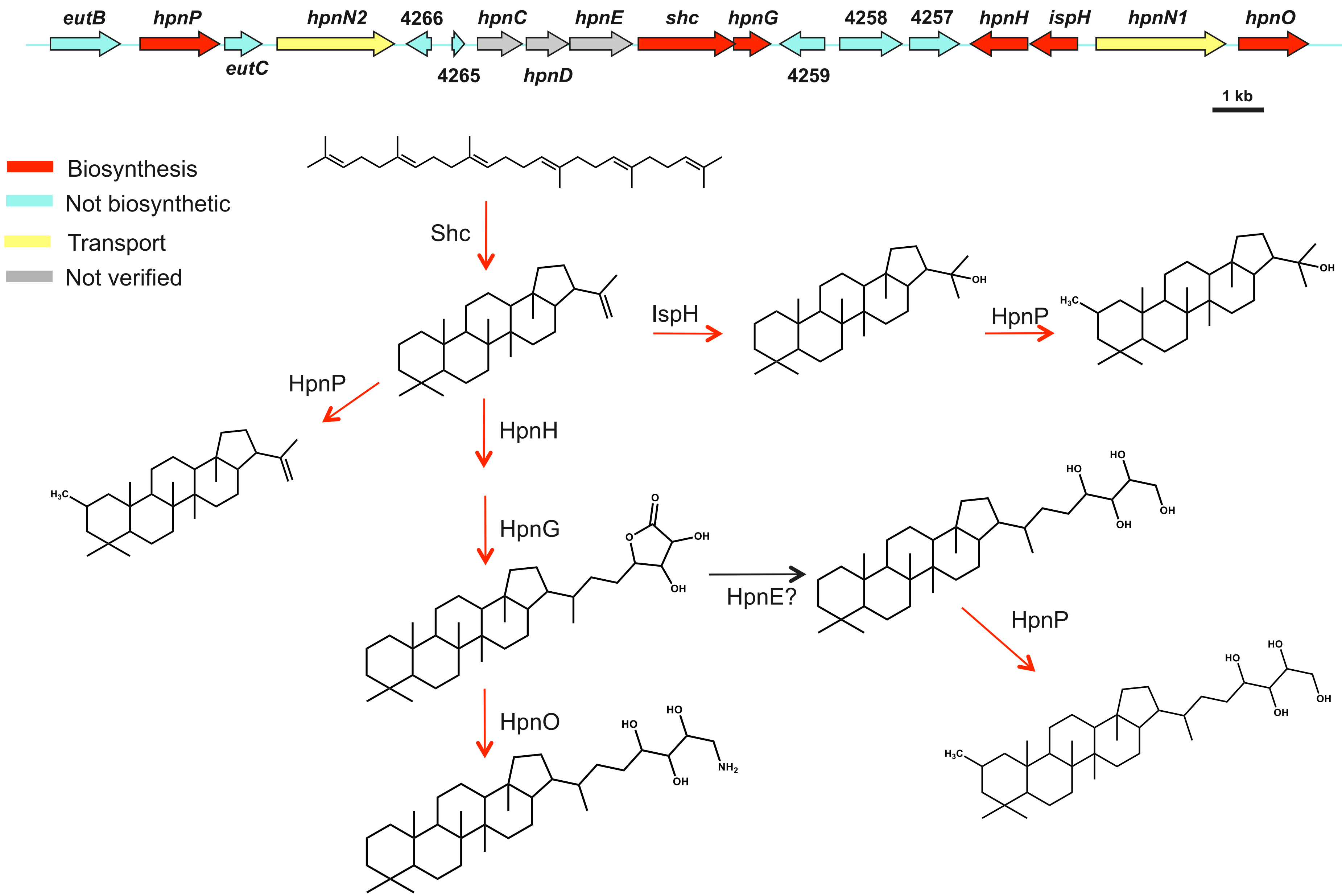

TOF MS AP+
1.16e4
100
%
0
OAc
OAc
OAc
NHAc
35-aminobacteriohopane-32,33,34-triol (aminotriol)
714.3905
654.4487
715.4353
655.4611
716.4550
656.4741
341.4712
369.4962
551.5480
594.4956
595.4957
m/z
150 200 250 300 350 400 450 500 550 600 650 700 750 800 850


TOF MS AP+
1.45e4
100
%
0
NHAc
N
N
N
N
O
OAc
OAc
Adenosylhopane
611.4335
612.4597
613.4749
788.3481
830.3097
831.3247
832.3541
220.3437
419.4338
509.5240
551.4913
m/z
150 200 250 300 350 400 450 500 550 600 650 700 750 800 850

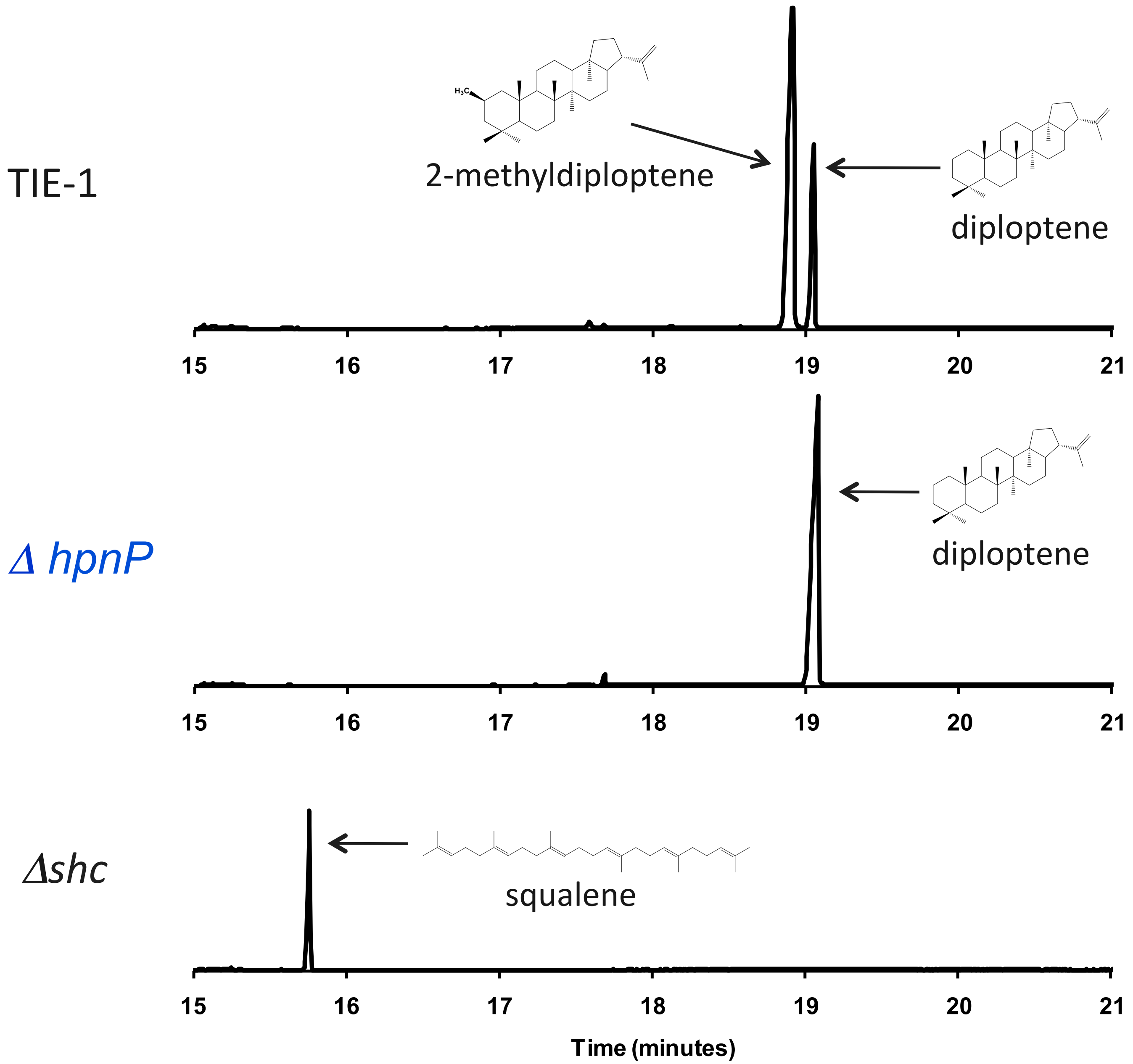

TOF MS AP+
622
100
%
0
O
O
OAc
OAc
Lactone
627.3883
627.4366
628.3916
629.4320
585.4504
m/z
150 200 250 300 350 400 450 500 550 600 650 700 750 800 850

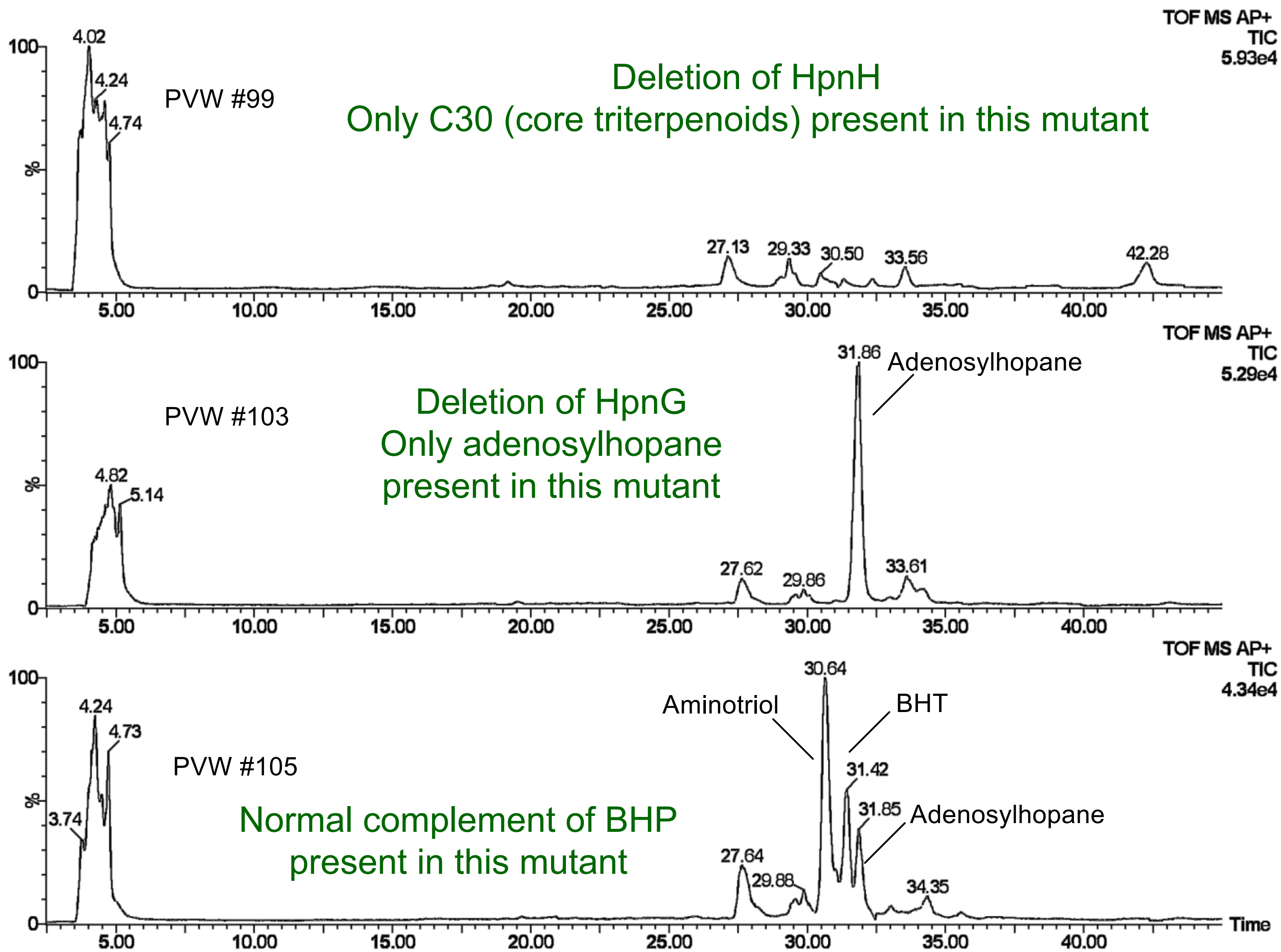
Elucidating Hopanoid Biosynthesis with Mutant Bacteria

Proposed hopanoid biosynthetic pathway

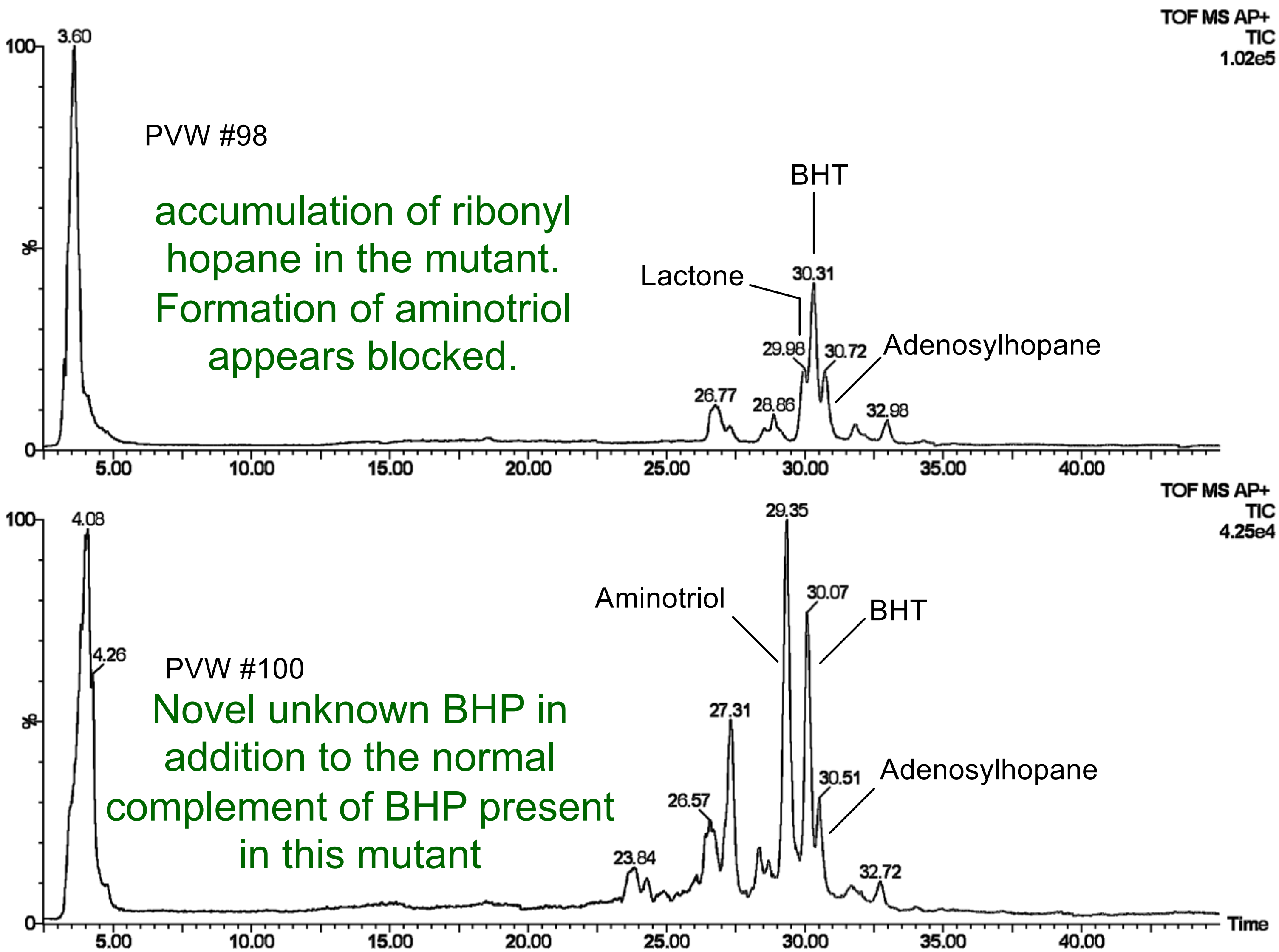


Triterpenes in *shc* and *ΔhpnP* mutants

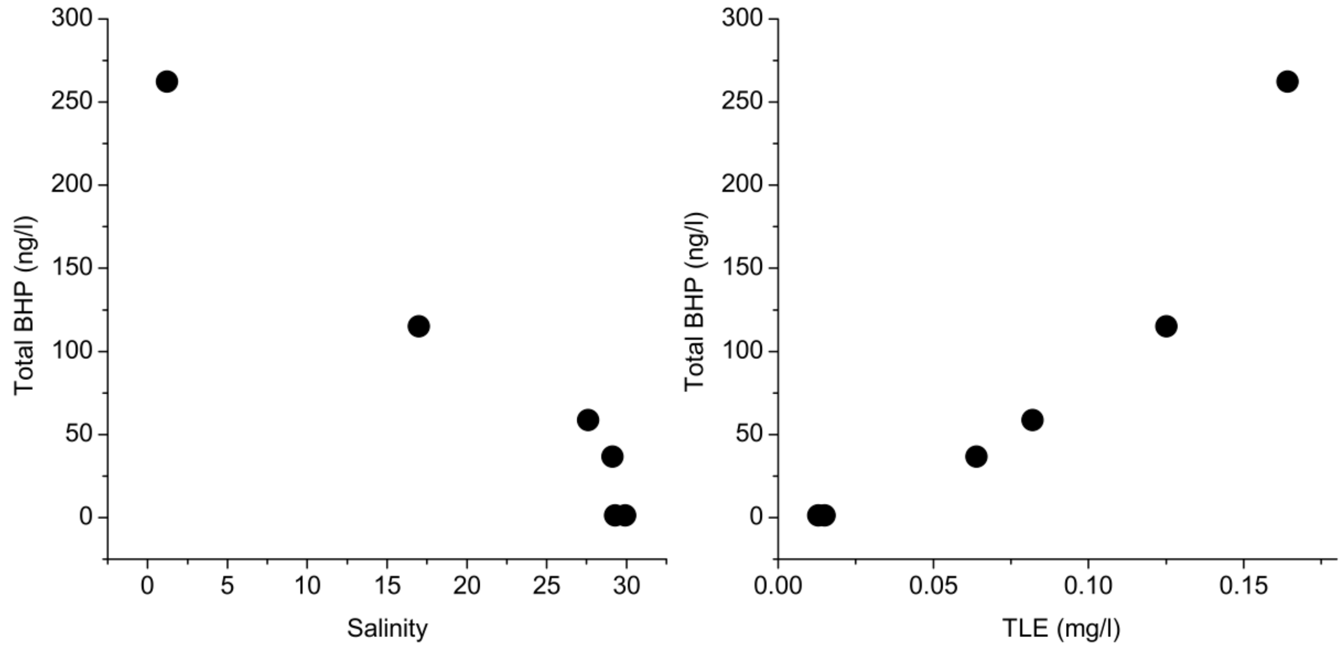




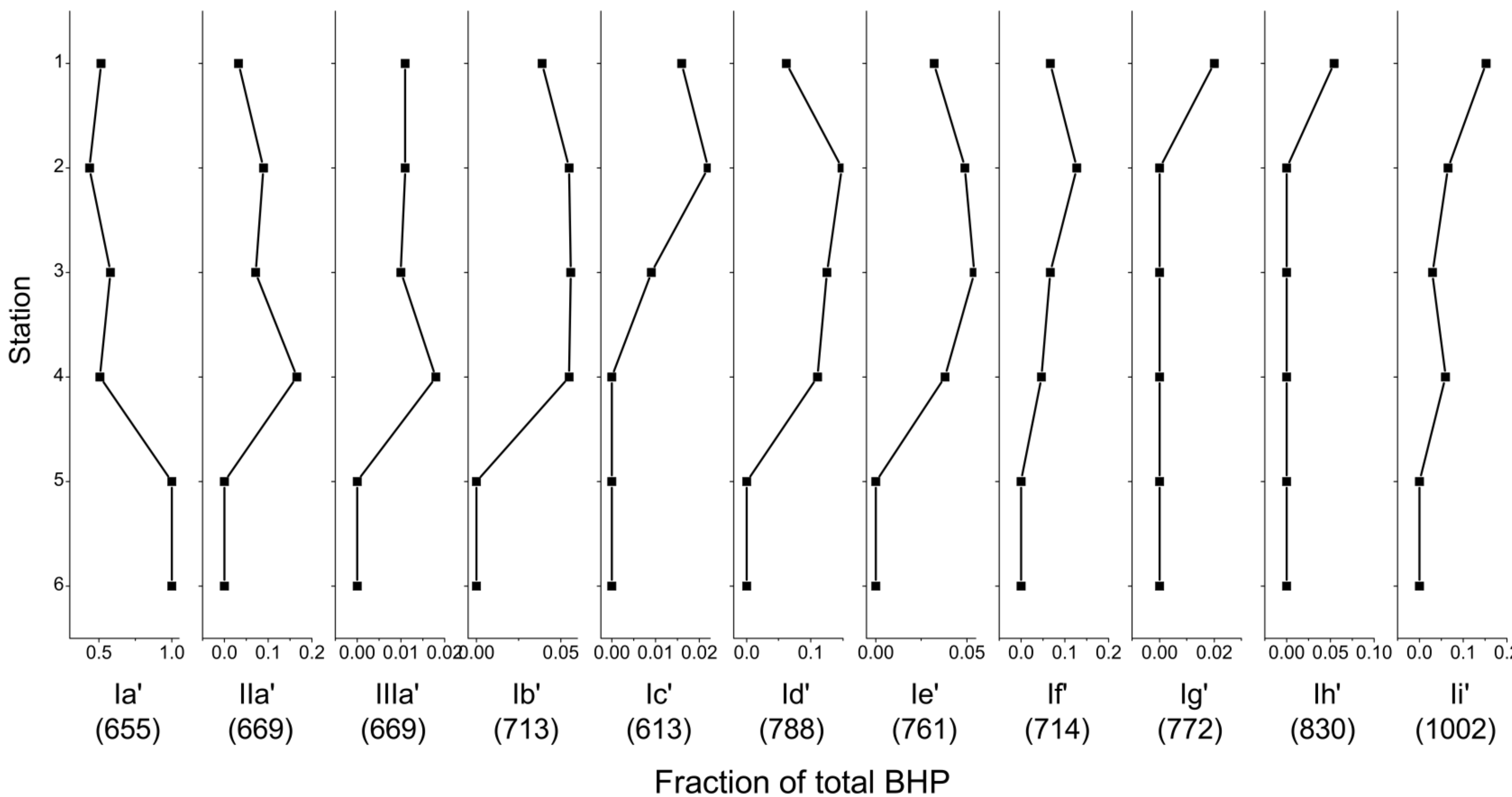
TOF MS AP+
TIC
5.93e4
100
%
0
4.02
4.24
4.74
PVW #99
Deletion of HpnH
Only C30 (core triterpenoids) present in this mutant
27.13
29.33
30.50
33.56
42.28
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
TOF MS AP+
TIC
5.29e4
PVW #103
Deletion of HpnG
Only adenosylhopane
present in this mutant
4.82
5.14
31.86
Adenosylhopane
27.62
29.86
33.61
TOF MS AP+
TIC
4.34e4
4.24
4.73
3.74
PVW #105
Normal complement of BHP
present in this mutant
30.64
Aminotriol
BHT
31.42
31.85
Adenosylhopane
27.64
29.88
34.35
Time

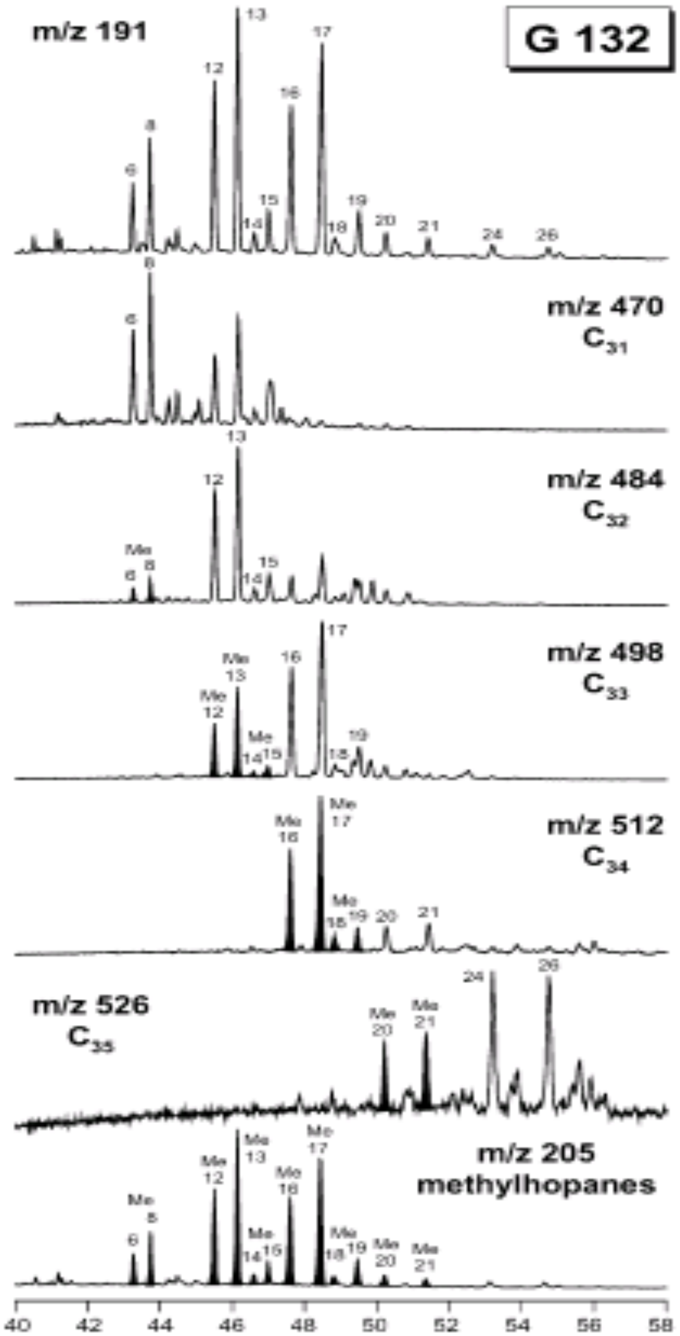

TOF MS AP+
TIC
1.02e5
100
%
0
3.60
PVW #98
accumulation of ribonyl hopane in the mutant. Formation of aminotriol appears blocked.
BHT
Lactone
30.31
29.98
30.72
Adenosylhopane
26.77
28.86
32.98
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
TOF MS AP+
TIC
4.25e4
100
%
0
4.08
4.26
29.35
Aminotriol
30.07
BHT
PVW #100
Novel unknown BHP in addition to the normal complement of BHP present in this mutant
27.31
26.57
30.51
Adenosylhopane
23.84
32.72
5.00
10.00
15.00
20.00
25.00
30.00
35.00
40.00
Time

James Saenz PhD: Panama Liquid Jungle Transect



James Saenz PhD: Panama Liquid Jungle Transect



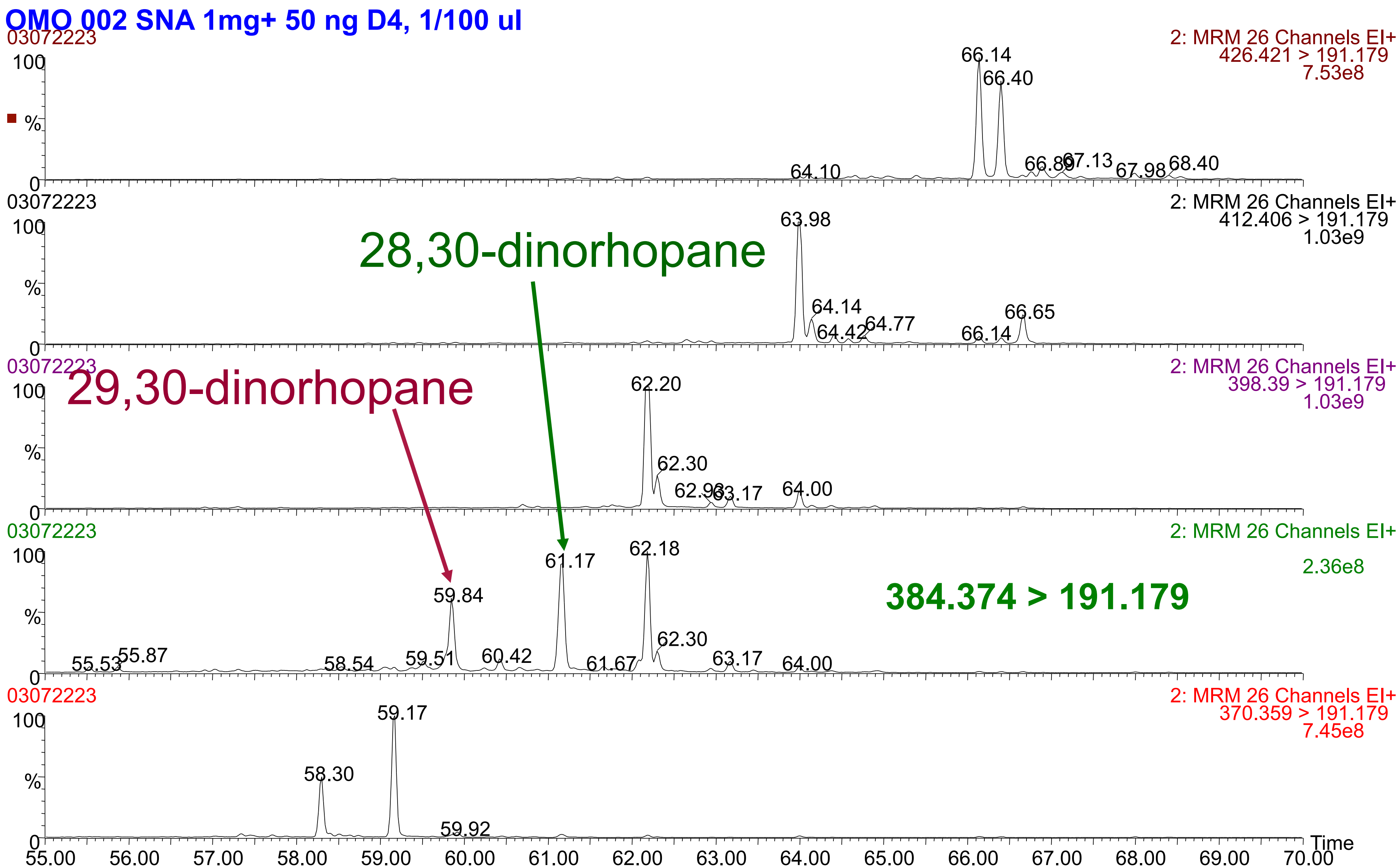


Other sedimentary hopanoids

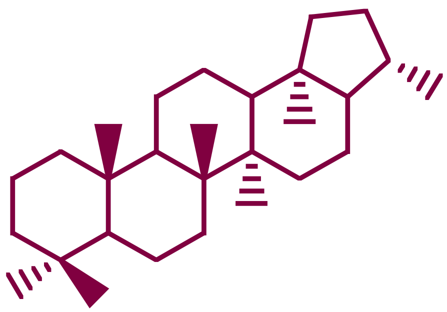
Hopanoic acids in Mesozoic sedimentary rocks: their origin and relationship with hopanes Paul Farrimond, Tony Griffiths, Efthymios Evdokiadis
Organic Geochemistry 33 (2002) 965–977

Fig. 4. An m/z 191 mass chromatogram (top) showing the distribution of hopanoic acids (as methyl esters) in a Triassic sample (Serpiano shale) from Switzerland. The m/z 470, 484, 498, 512 and 526 mass chromatograms represent the molecular ions of C 3135 hopanoic acid methyl esters, including A-ring methylated hopanoic acids (shaded peaks; m/ z 205) which are labelled ‘‘Me’’ and with the numerical code oftheir nonmethylated equivalent hopanoic acid

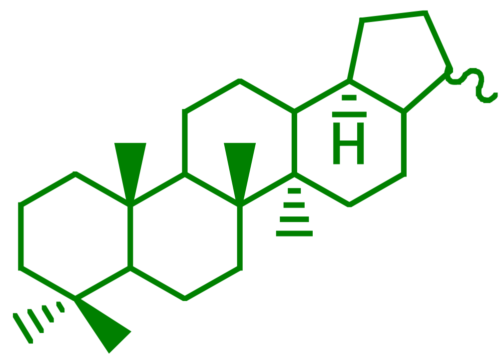
Bisnorhopanes →Dinorhopanes



Bisnorhopanes →Dinorhopanes



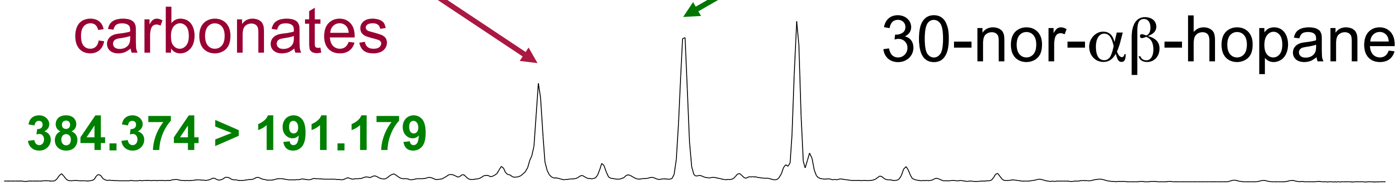
28,30-dinorhopane: free hydrocarbon in highly anoxic sediments; Monterey Fm., Kimmeridge F.m, U-Shale



29,30-dinorhopane; member of homologous series of 3-norhopanes; direct biological precursors? High in carbonates

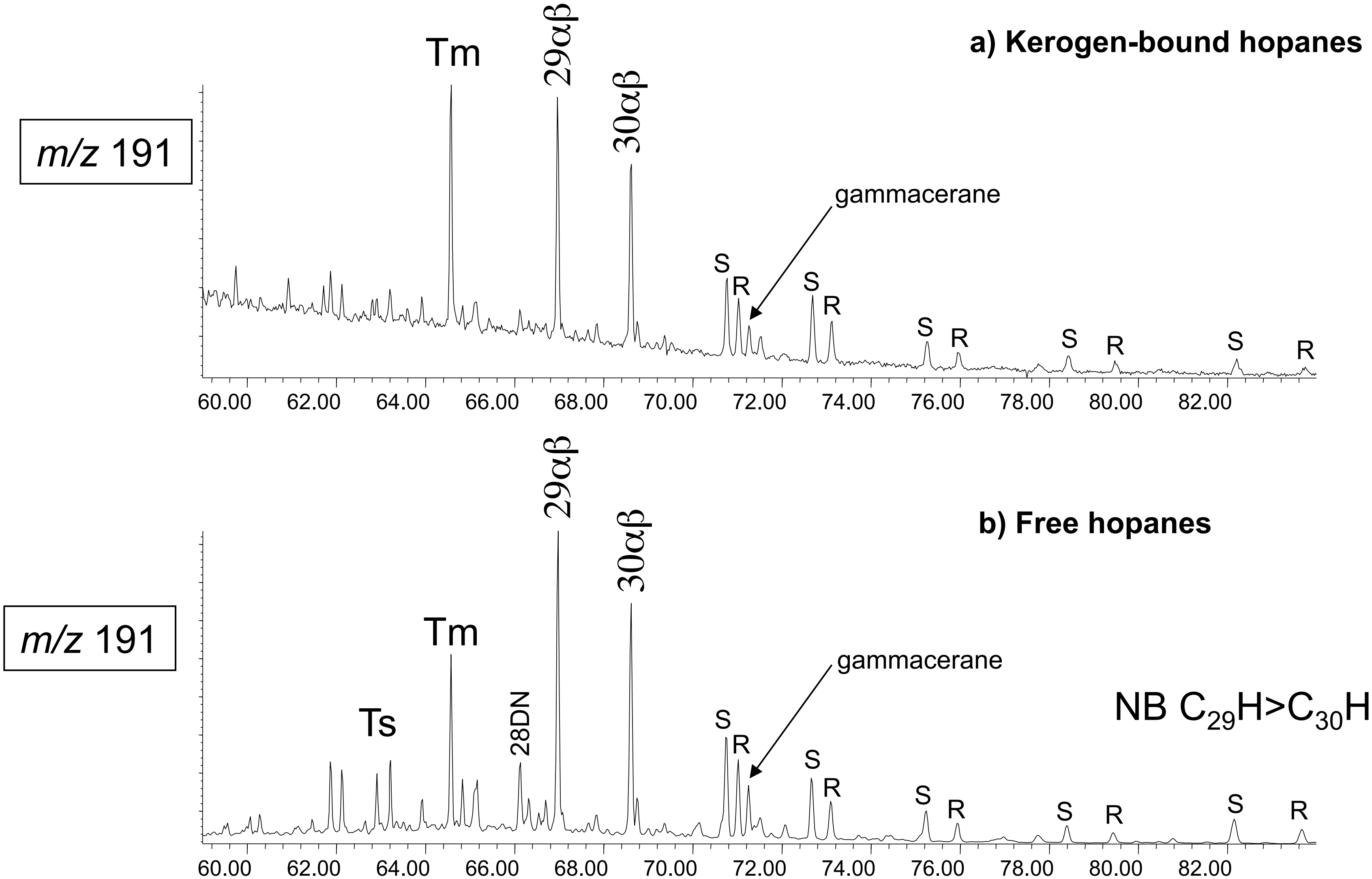
30-nor-αβ-hopane

384.374 > 191.179

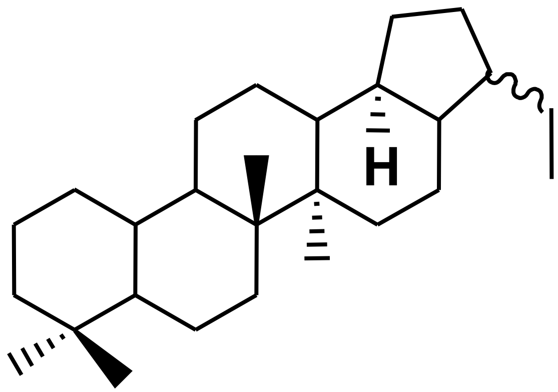


Bisnorhopanes →Dinorhopanes

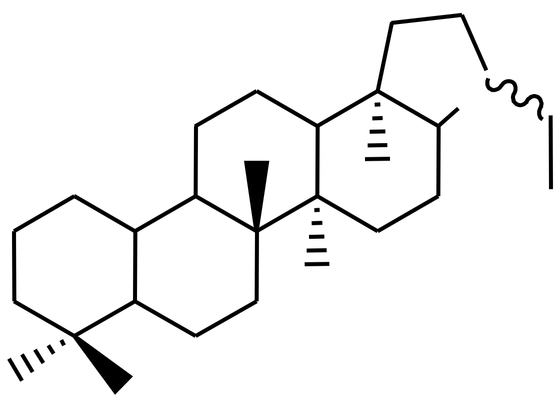
Birba 3 (sapropelic laminite)
Stalvies, Love



Other Dinorhopanes and Trinorhopane

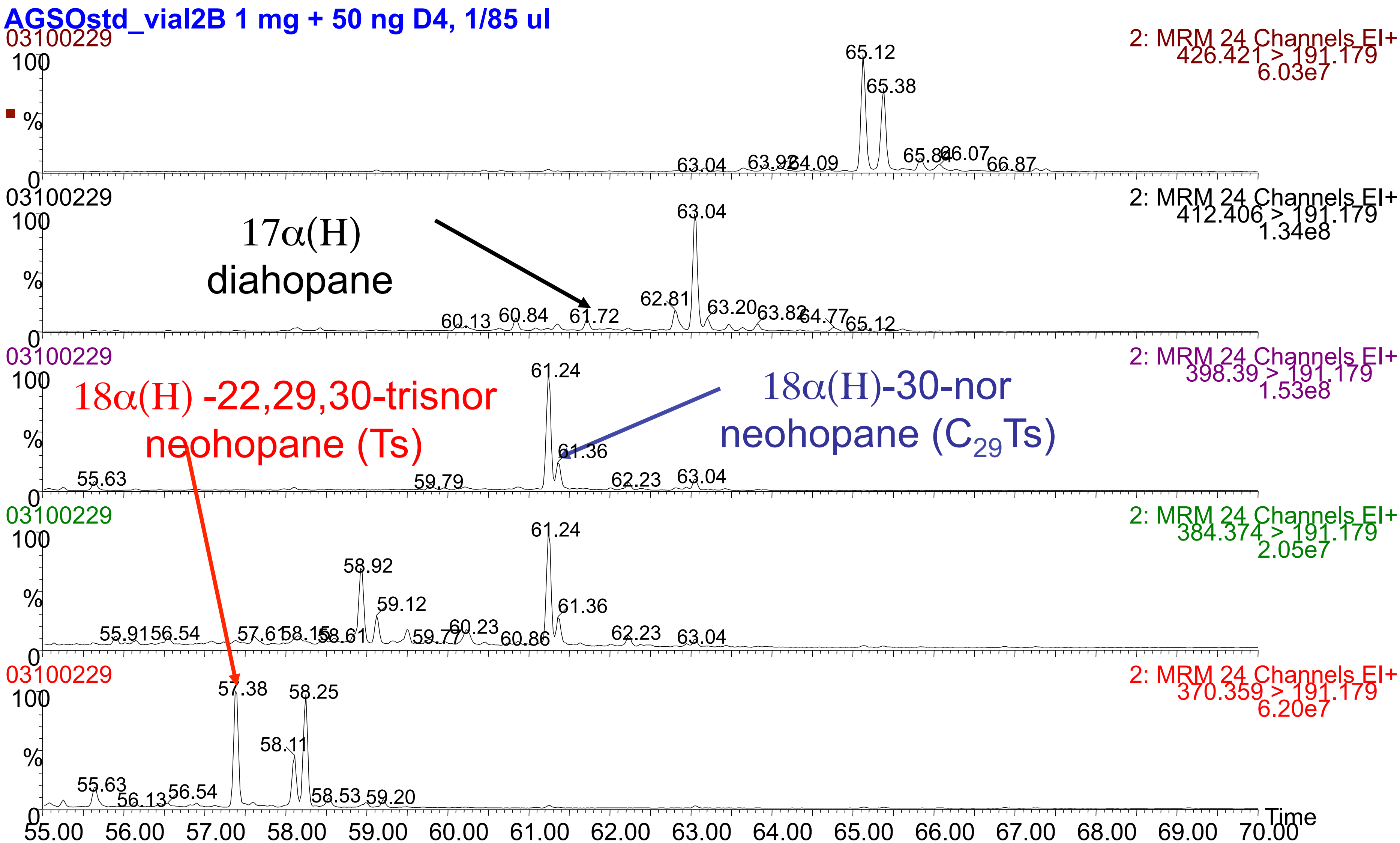


25, 28, 30-trinor-αβ-hopane
often found with 28,30-dinorhopane
sometimes referred to as C27T
major ions 370 M^+, 177 A+B, 163 D+E



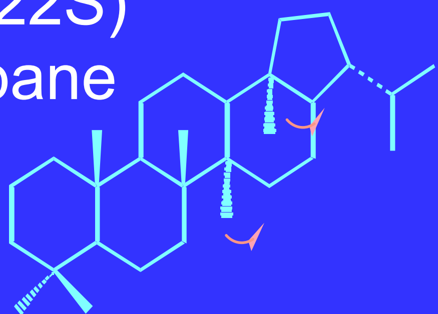
25, 30-dinor-αβ-hopane
biodegradation product of 30-nor-αβ-hopane
major ions 384 M^+, 177 A+B, 163 D+E

Other Triterpenoids- Rearranged Hopanoids

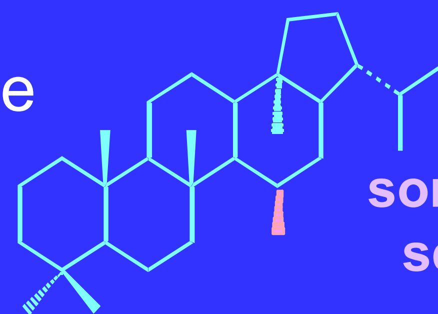


Rearranged Hopanes

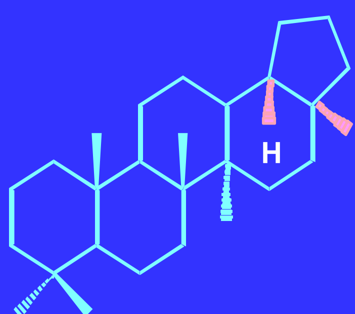
αβ(22S)
hopane



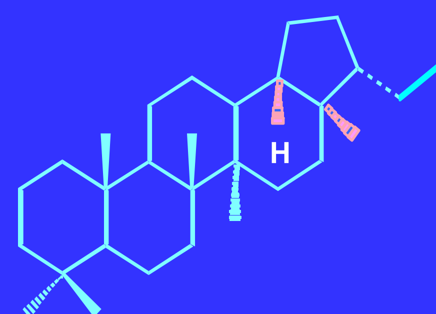
17α(H)
diahopane



some mature
sediments
& oil



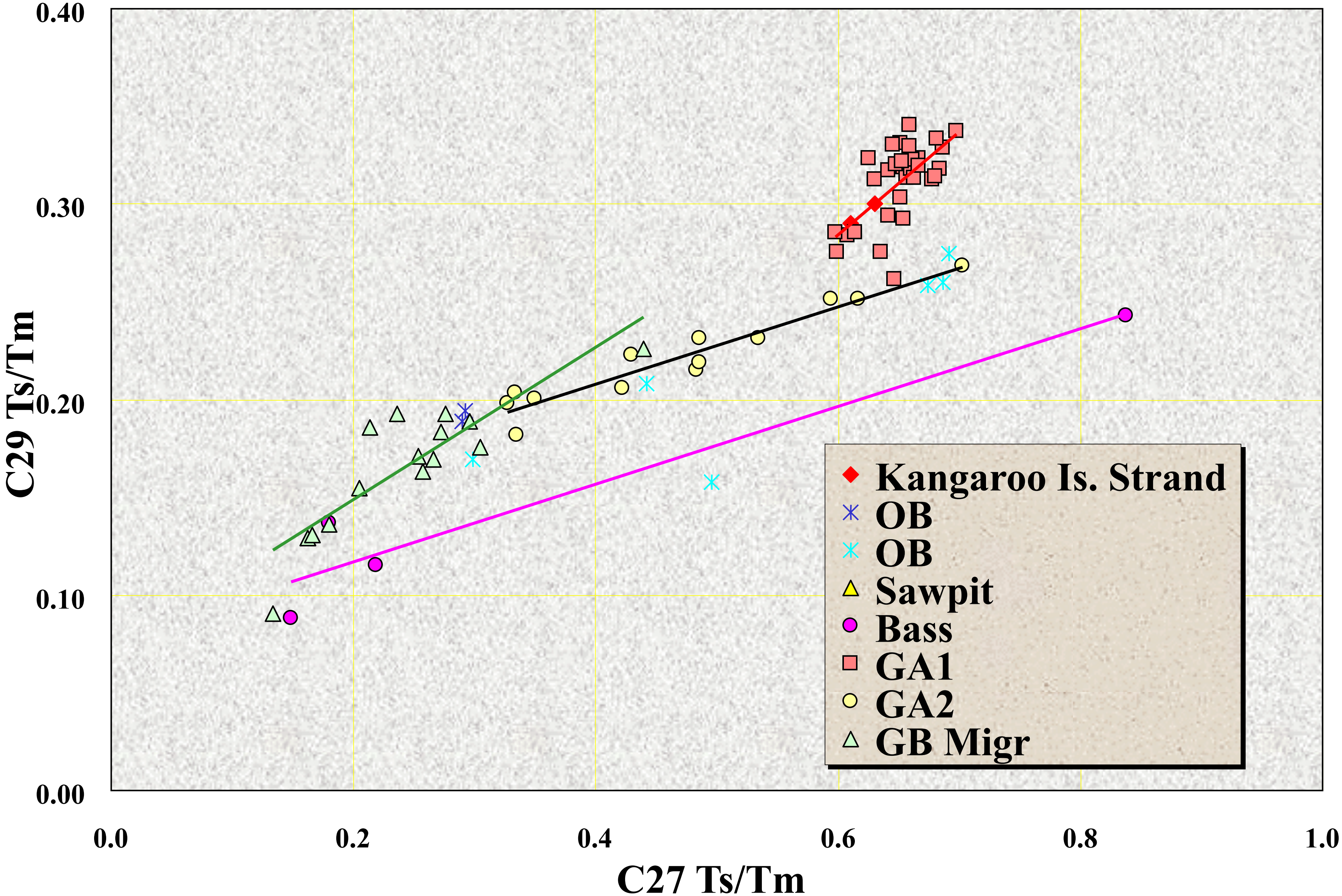
**mature
sediments & oil**

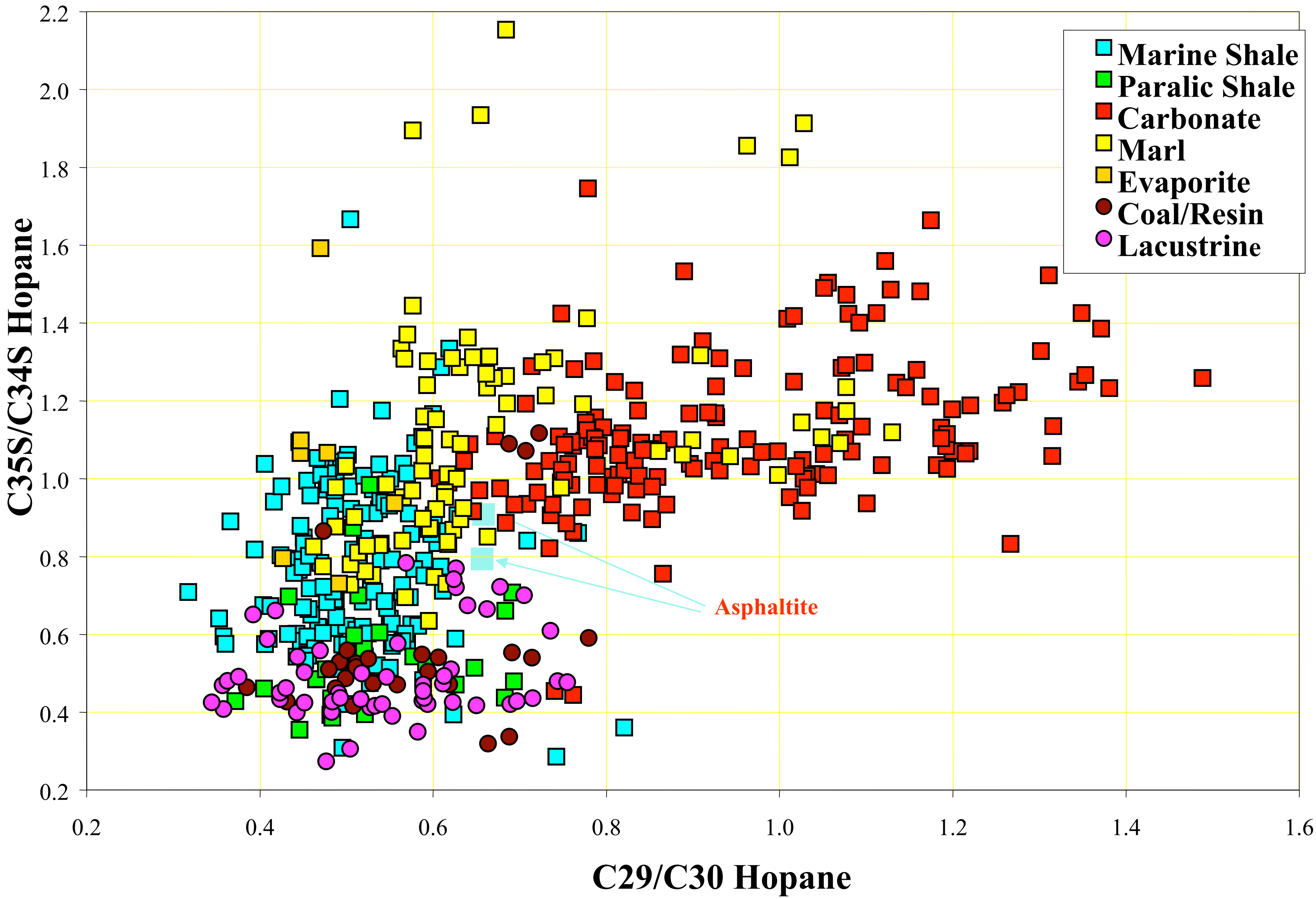


18α(H) -22,29,30-trisnor
neohopane (Ts)

18α(H)-30-nor
neohopane (C_{29}Ts)

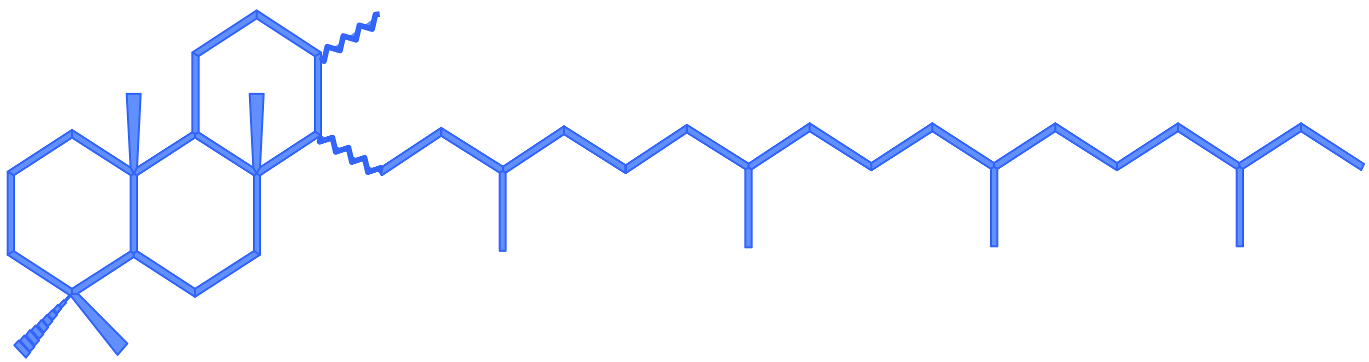
Rearranged Hopanes Source and Maturity Indicators



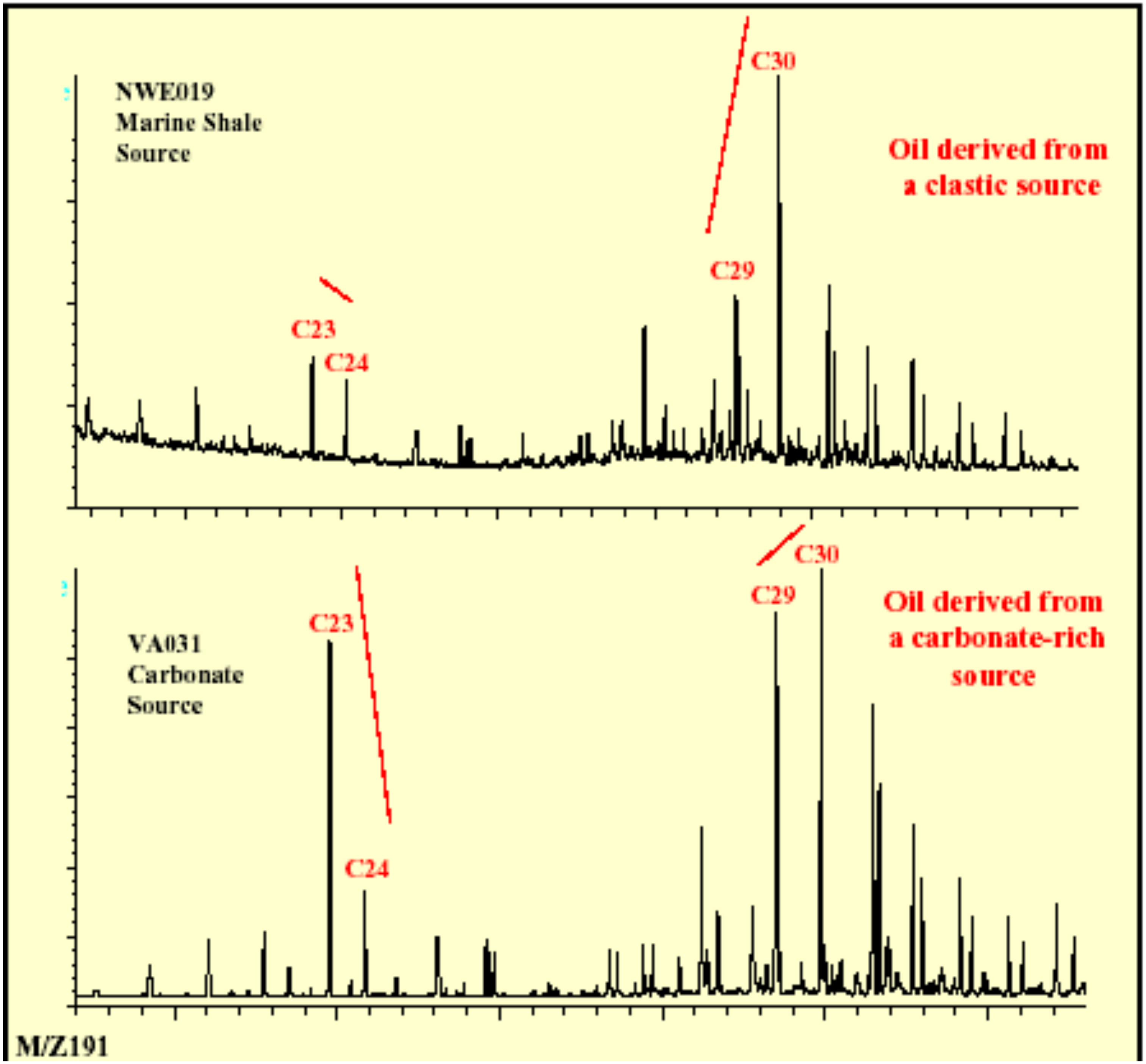


Marine Shale
Paralic Shale
Carbonate
Marl
Evaporite
Coal/Resin
Lacustrine
Asphaltite
C35S/C34S Hopane
C29/C30 Hopane
2.2
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6

Tricyclic Terpanes

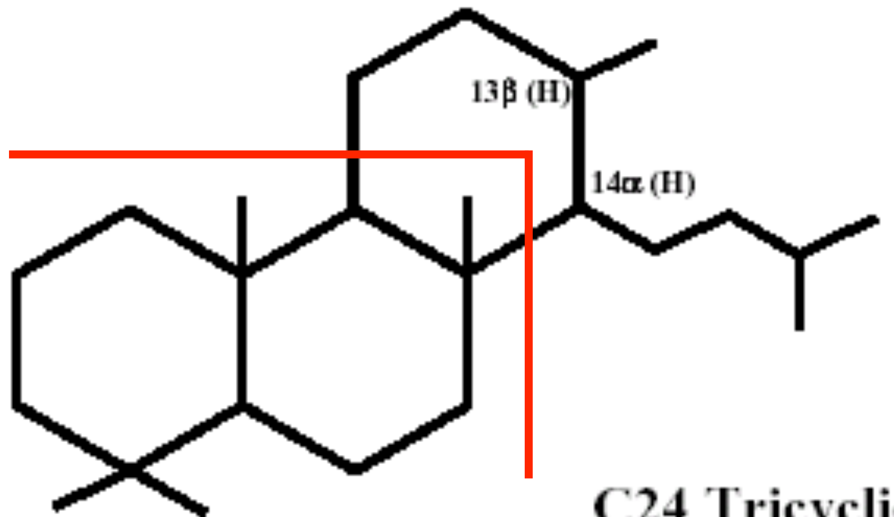


Tricyclics or cheilanthanes extend to C_{40} or C_{45}mostly
marine and mature
regular isoprenoid branching (not like squalene)
unknown source organism


NWE019
Marine Shale
Source
C30
C29
C23
C24
Oil derived from
a clastic source
VA031
Carbonate
Source
C23
C24
C29
C30
Oil derived from
a carbonate-rich
source
M/Z191

Tricyclic Terpanes

m/z 191



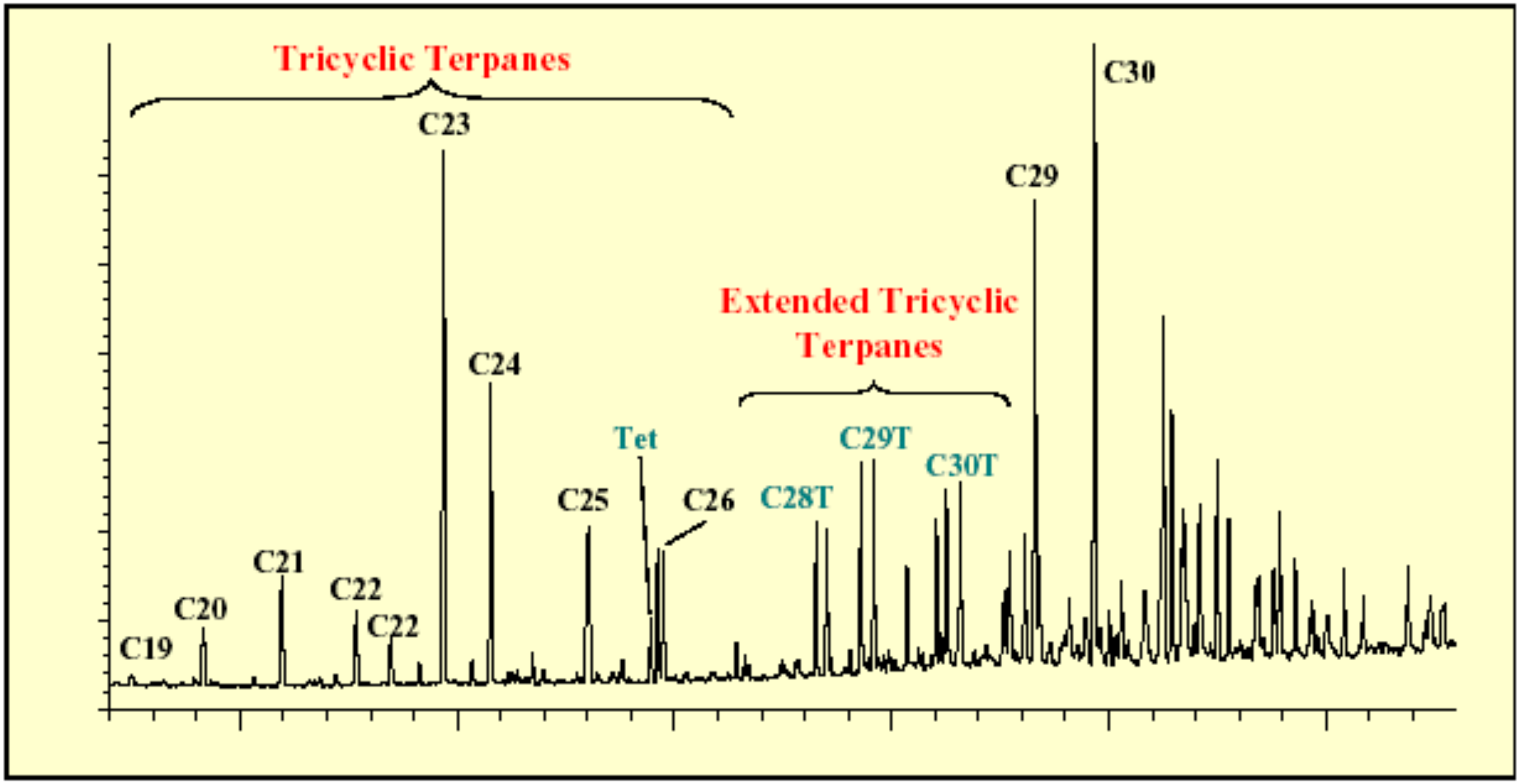
C24 Tricyclic Terpane
All tricyclic terpanes having:

13α, 14β
13α, 14α
13β, 14β
13β, 14α

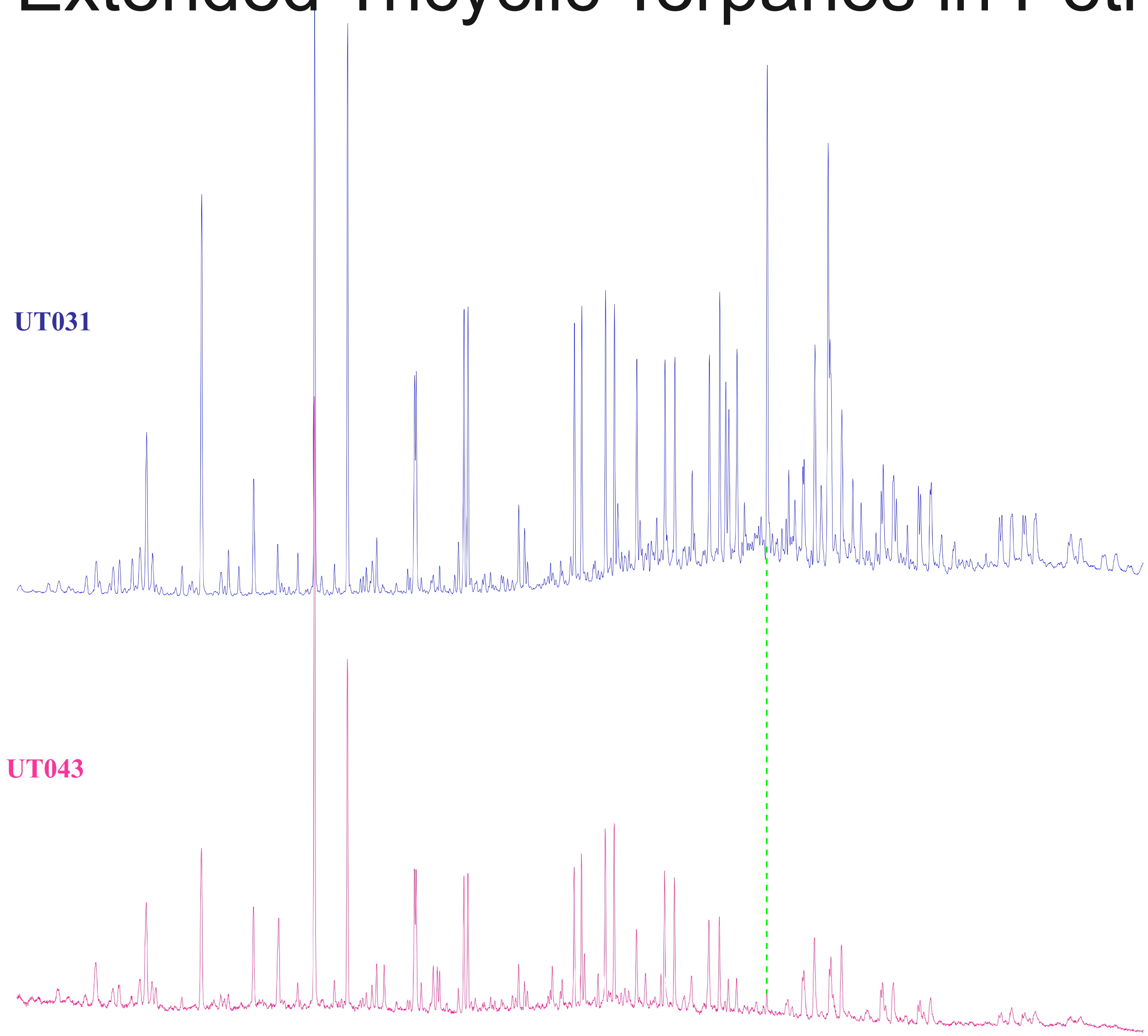
exist in oils.

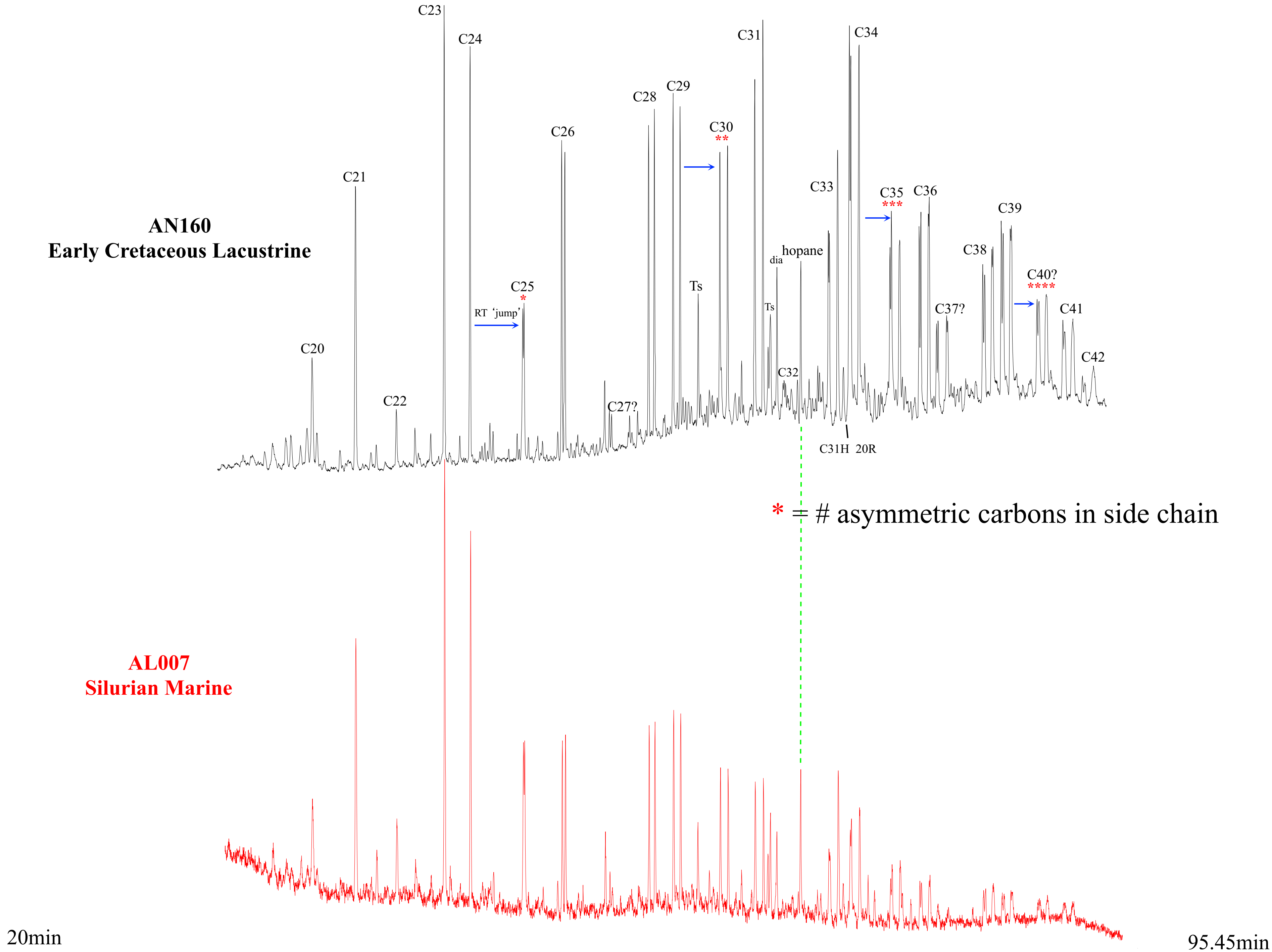
Cheilanthanes = Tricyclic Terpanes

Tricyclic Terpanes

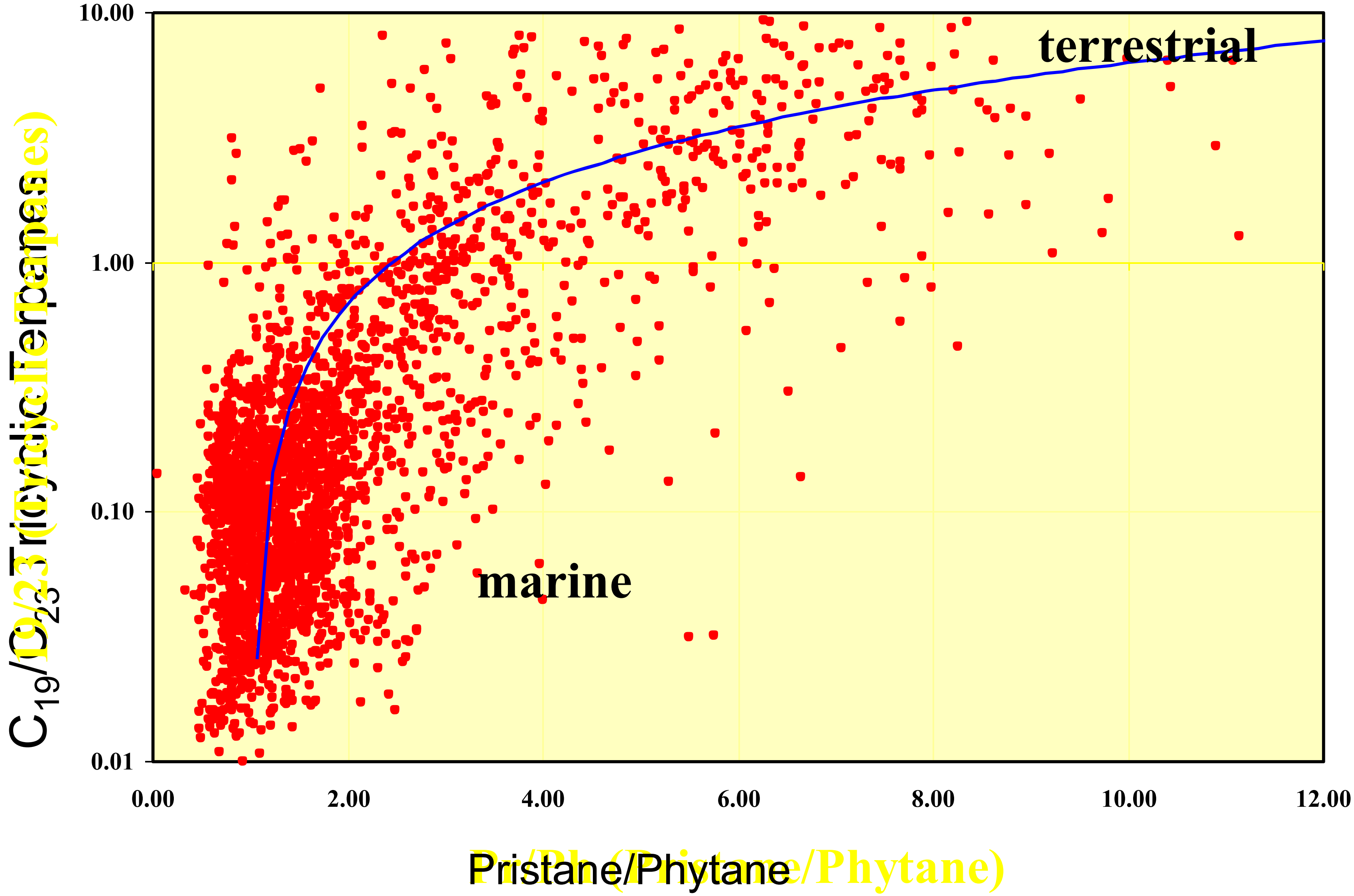


Extended Tricyclic Terpanes in Petroleum

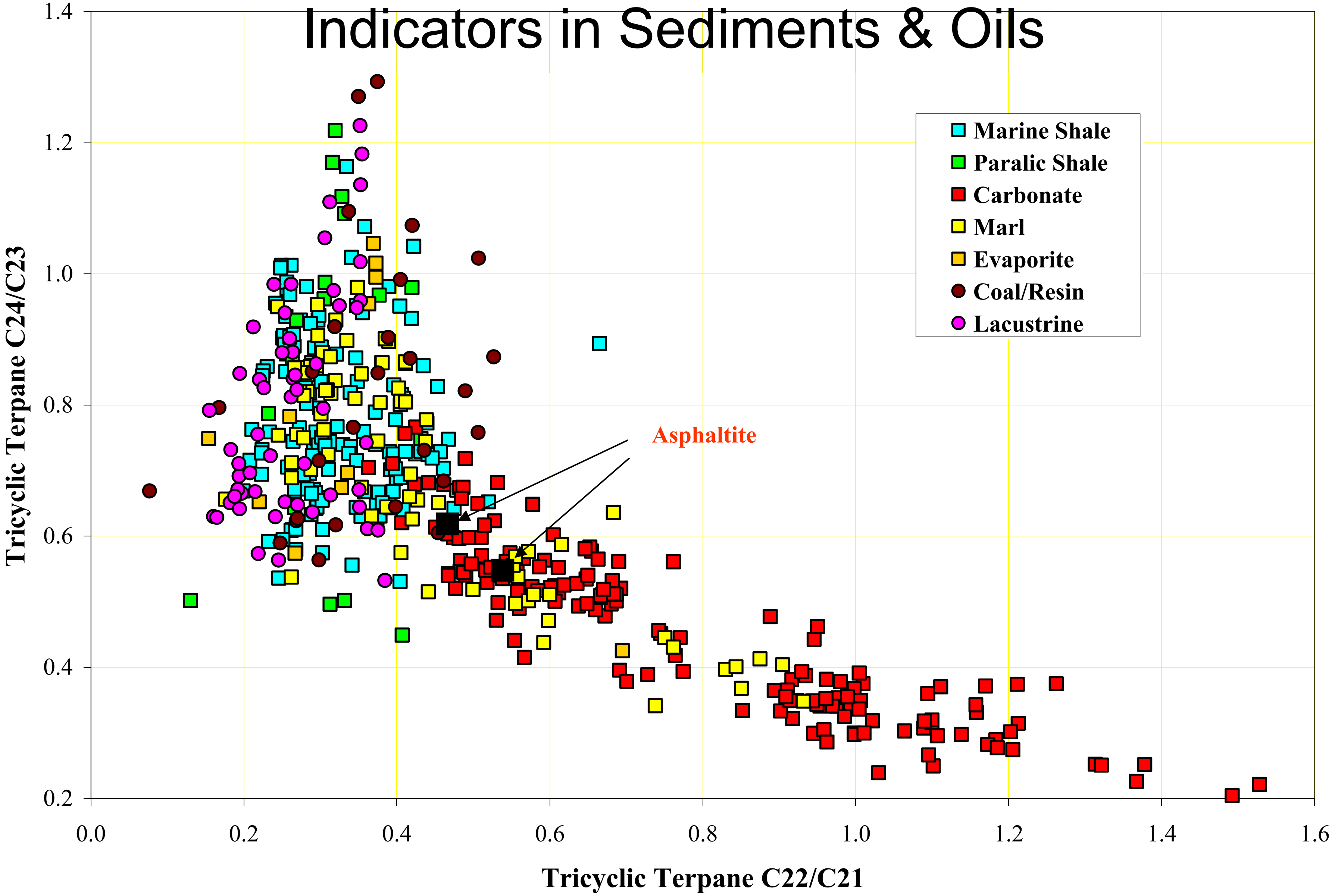



AN160
Early Cretaceous Lacustrine
C20
C21
C22
C23
C24
RT 'jump'
C25
*
C26
C27?
C28
C29
Ts
C30
**
C31
Ts
dia
hopane
C32
C33
C34
C31H 20R
C35
***
C36
C37?
C38
C39
C40?
****
C41
C42
* = # asymmetric carbons in side chain
AL007
Silurian Marine
20min
95.45min

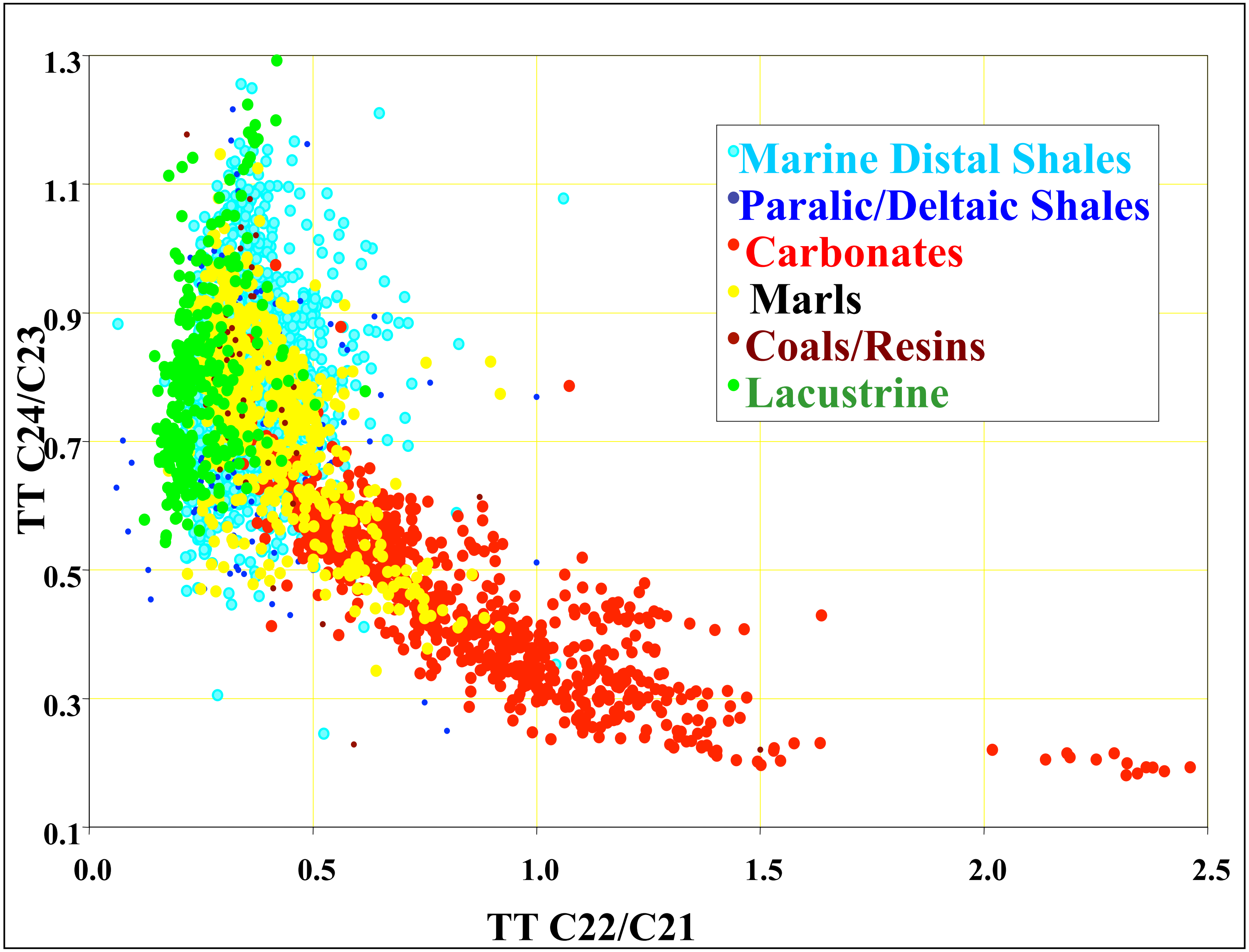
Tricyclic Terpanes Are Powerful Lithology Indicators in Sediments & Oils



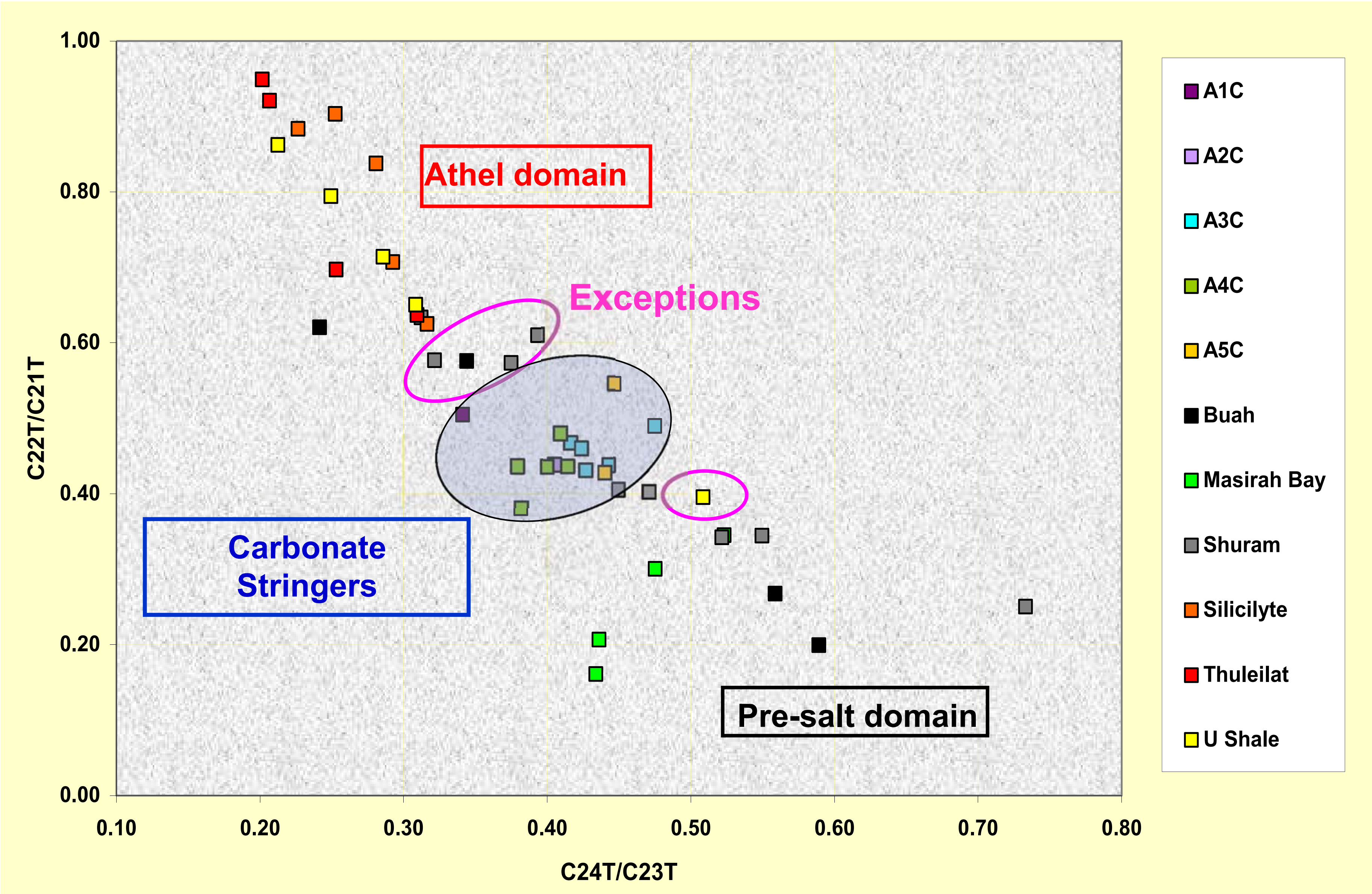
Tricyclic Terpanes Are Powerful Lithology Indicators in Sediments & Oils



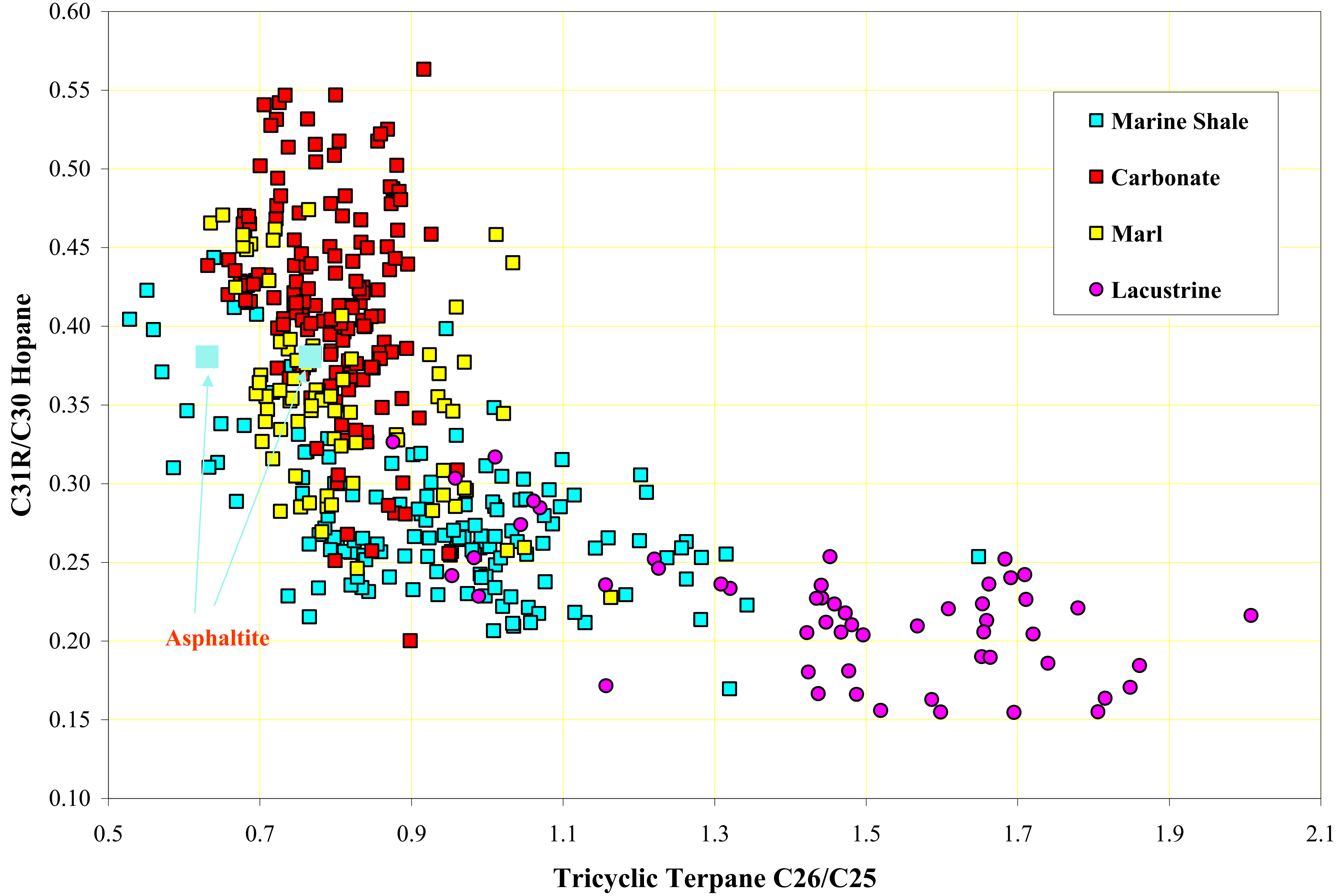
Diagnostic Tricyclic Terpane Ratios



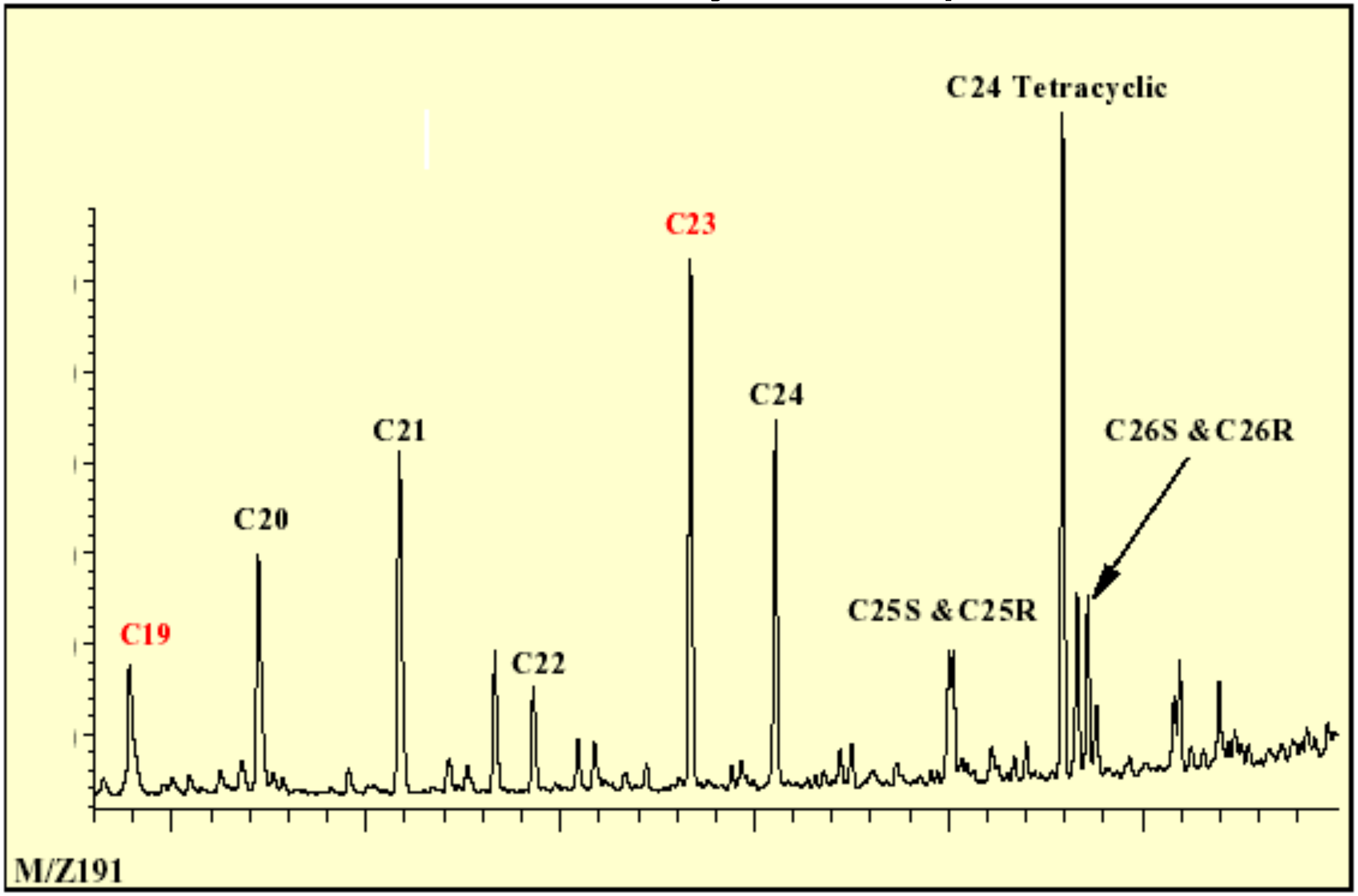
Application to Oman Oils – Nafun (Pre-salt) versus Ara (Intra- salt) biomarker parameters



Diagnostic Hopane vs Tricyclic Terpane Ratios



C24 Tetracyclic Terpane



191 Da mass chromatogram (above) and partial m/z 191 mass chromatogram (below). Peaks in the partial mass chromatogram are normalized to the C24 tetracyclic terpane -- the most abundant terpane in the data. The oil shown is a lacustrine oil from the Cuyo Basin, Argentina.

Principal Component analysis of 800 oils scores & loadings for OilMod variables