Comparison of Terpene Profiles of *Chamaecyparis thyoides* Foliage at Various Levels of Deer Browse from Long-term Study Sites

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Presented at the Ecology & Management of Atlantic White Cedar Symposium
Suffolk, VA June 12-14, 2012

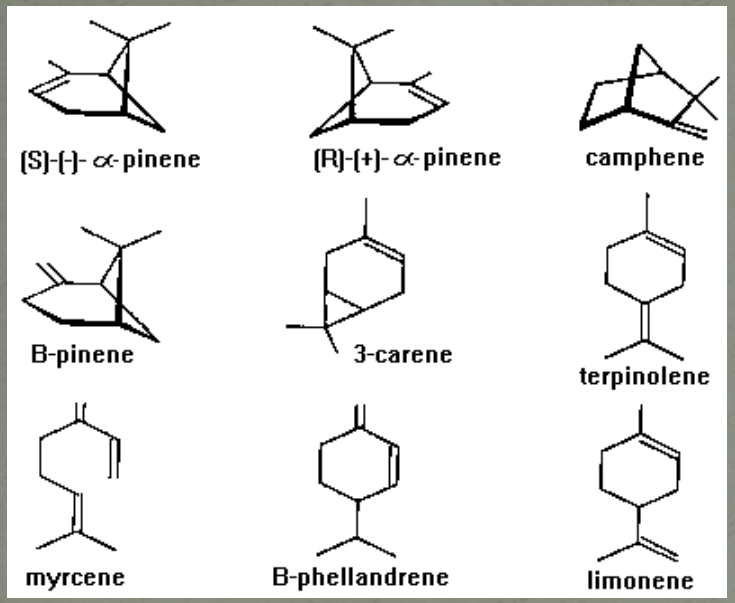
What is a terpene?

Terpenes are carbon based secondary metabolites synthesized by plants, particularly conifers.

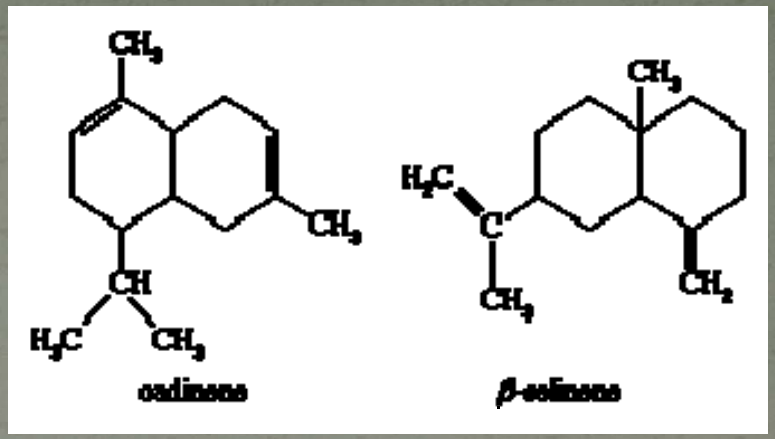
They have many functions including acting as a protective chemical defense against herbivory.

Monoterpenes: $C_{10}H_{16}$
Sesquiterpenes: $C_{15}H_{24}$
Diterpenes: $C_{20}H_{32}$

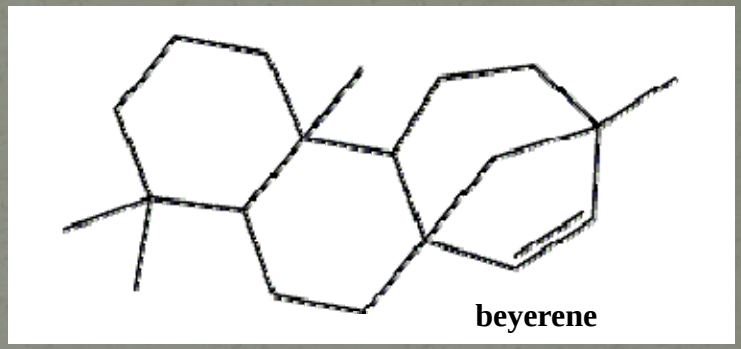
Examples:



monoterpenes



sesquiterpenes



diterpene

Oxidation produces compounds referred to as terpenoids.

Previous Research on Other Species

Based on olfactory and taste senses, herbivory preferentially browse vegetation with lower concentrations of terpenes (Cooper & Owen, 1985; Suomela, et al., 1997).

Oxygenated monoterpenes may inhibit microbial action in ruminants impairing digestion (Oh et al., 1967; Schwartz et al., 1980; Vourc'h et al., 2002).

The production of terpenes in conifers as a chemical defense can be stimulated by herbivory (Bohlmann & Croteau, 1999; Lewinsohn et al., 1991).

Defoliation by herbivory may reduce the carbon pool available to produce secondary metabolites (Bryant et al., 1983; Raffa & Smalley, 1995).

Colletti Site
Brendan T. Byrne State Forest

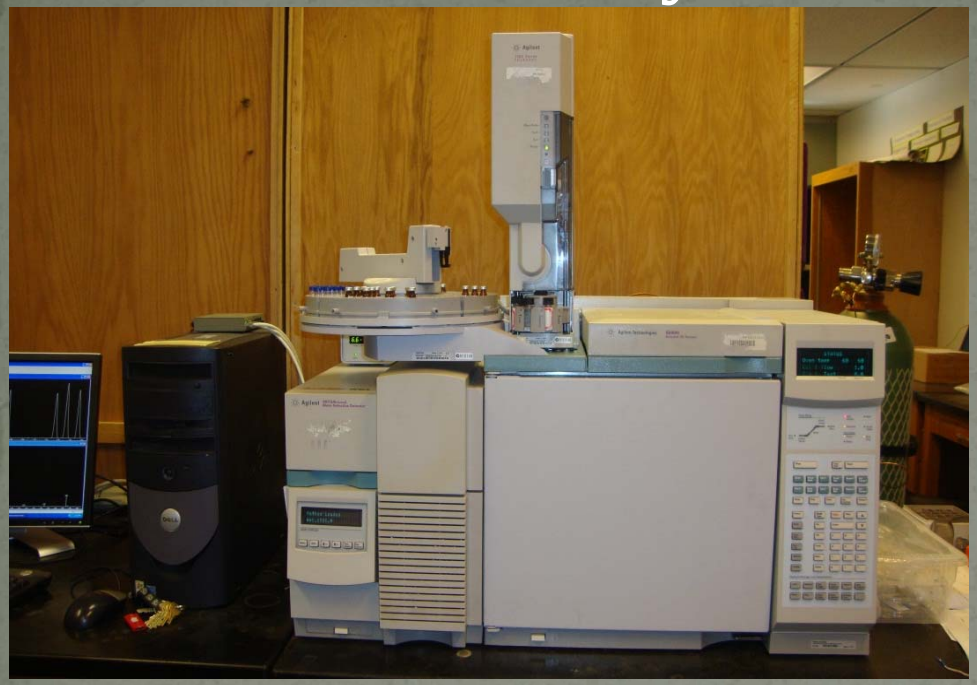
1) Herbicide/ Electric Fence
2) Herbicide/ No Deer Control
3) Herbicide/ Hinder
4) Control/No treatment

Terpene Extraction

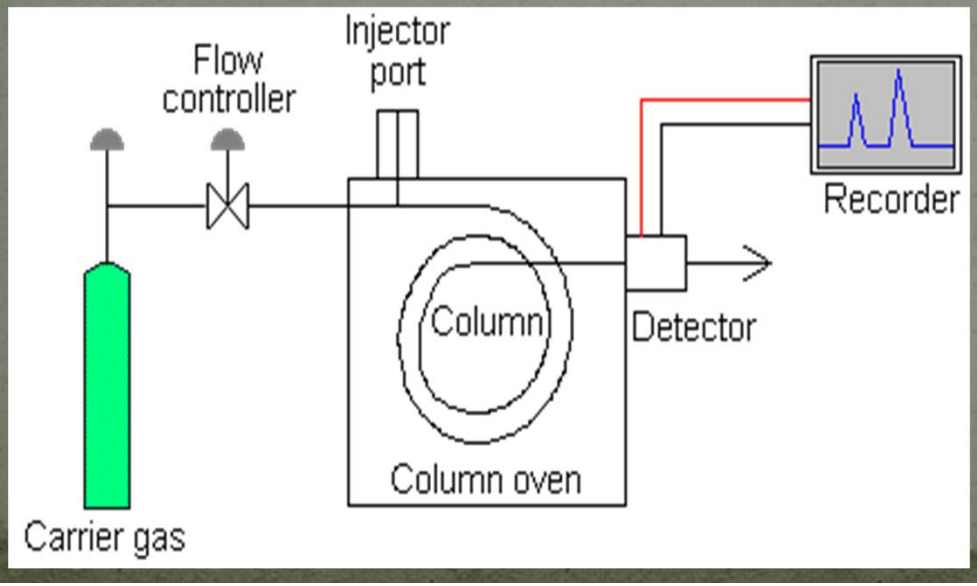
- 1.00 g AWC foliage extracted in 10mL Ultima-grade methanol at room temperature for 48 hrs.
- Foliage from each tree extracted in triplicate.
- Extract decanted & syringe filtered through 0.45 μm PTFE filter.

- Deuterated toluene added to a concentration of 10.0 ppm as an internal standard.
- Extracts stored at -70°C in air tight aluminum crimp seal vials until analysis by gas chromatography/mass spectrometry (GC/MS).

GC/MS Analysis



Agilent 6890N GC/5973 Mass Selective Detector



All extracts analyzed in triplicate using GC/MS.

Carrier Gas: He

Flow Rate: 1.0 mL/min

Injection Volume: 2.0 µL

Injector Temperature: 270 °C

Column Temperature :
60 – 300 °C; ramp 3 °C/min

Column: Agilent HP-5MS;
0.25mm x 30 m x 0.25µm

Split Ratio: 1:20

Ionization source: EI (electron impact)

Source Temperature: 200 °C

The sample molecules eject an electron leaving behind a molecular ion with a positive charge.

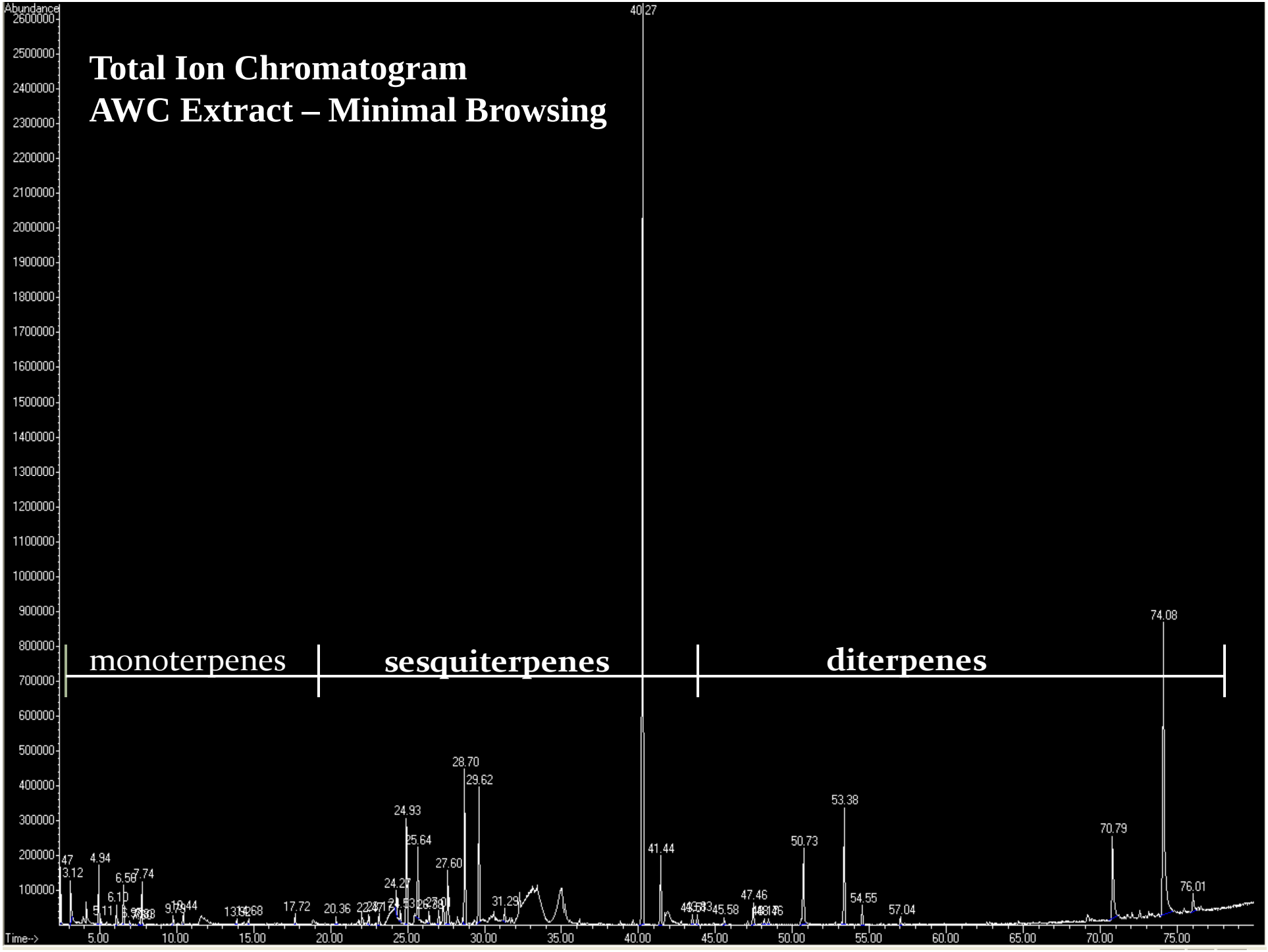
$$M \xrightarrow{e^-} M^{+\bullet} + e^-$$

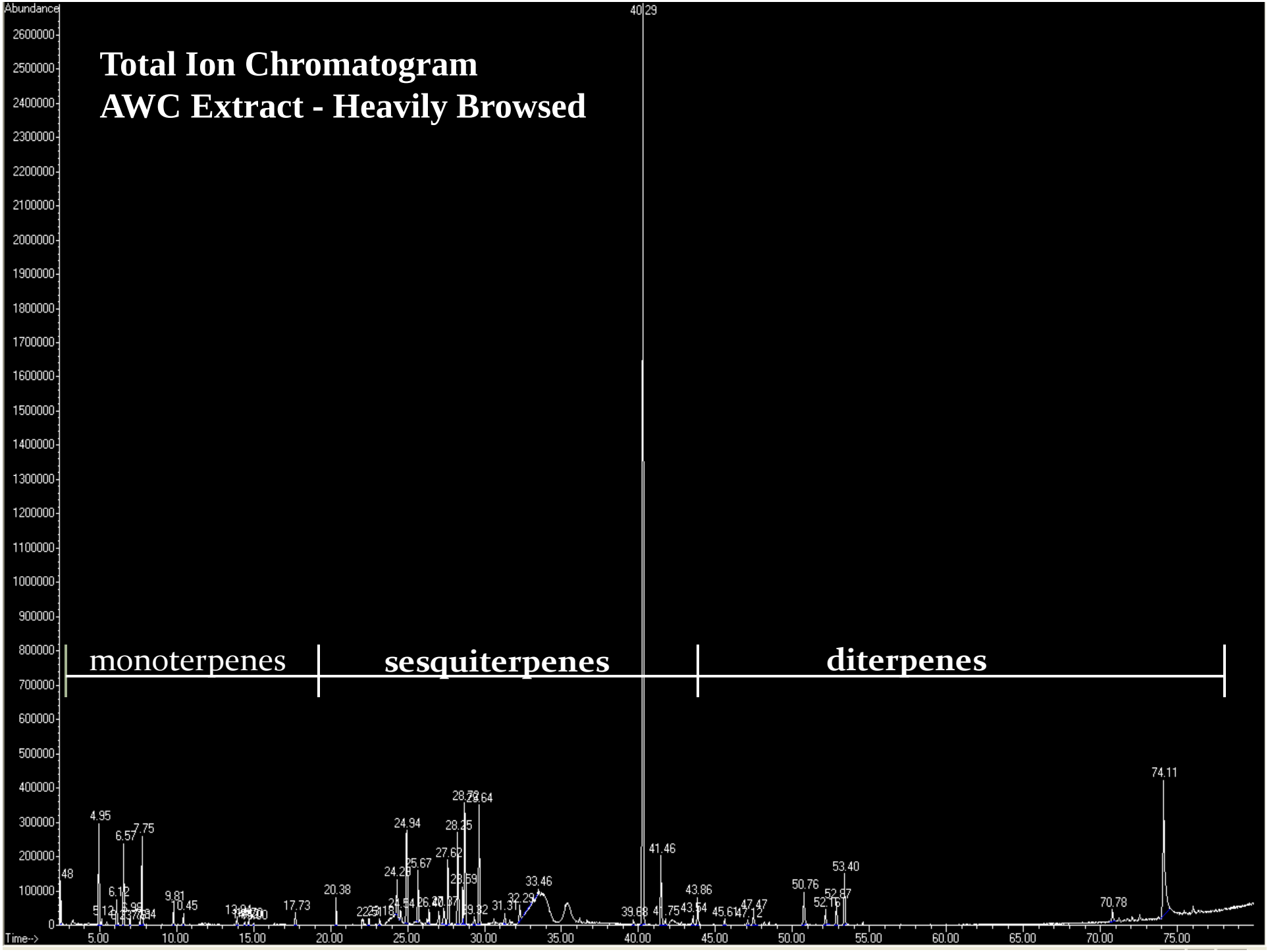
molecule **radical cation**

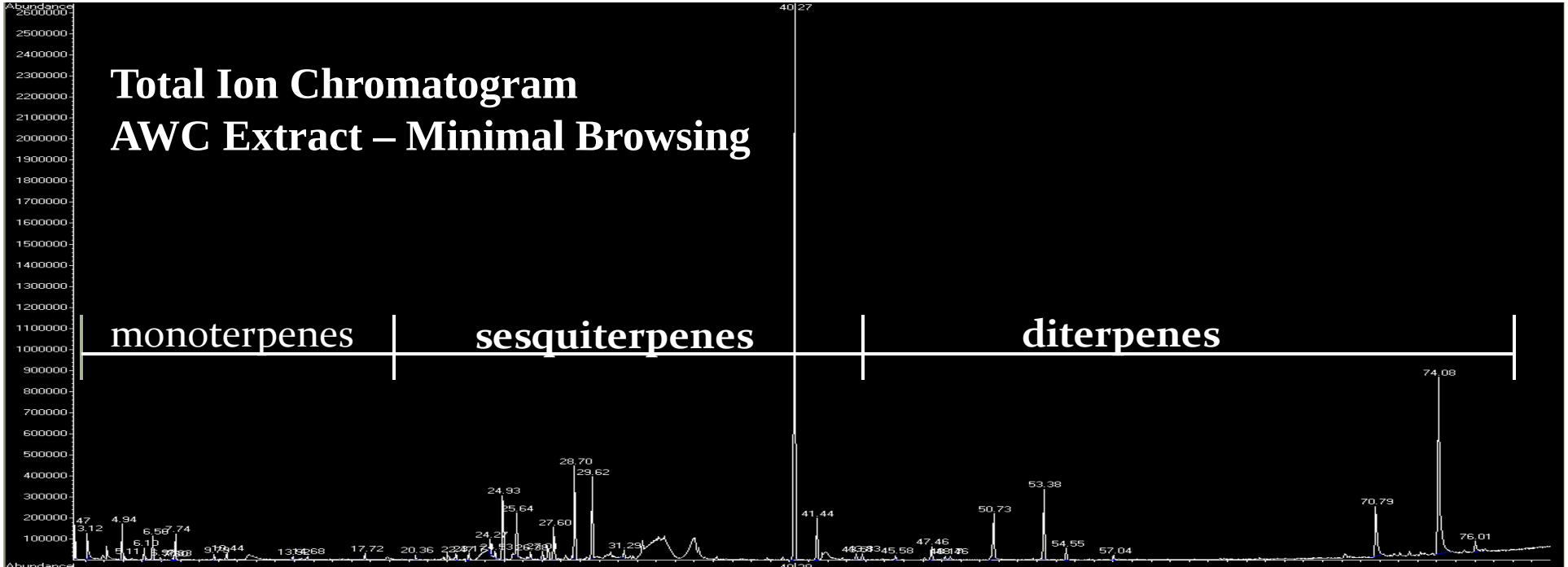
The molecular ion is inherently unstable and fragments. Bonds break resulting in fragments having a lower mass than the molecular ion.

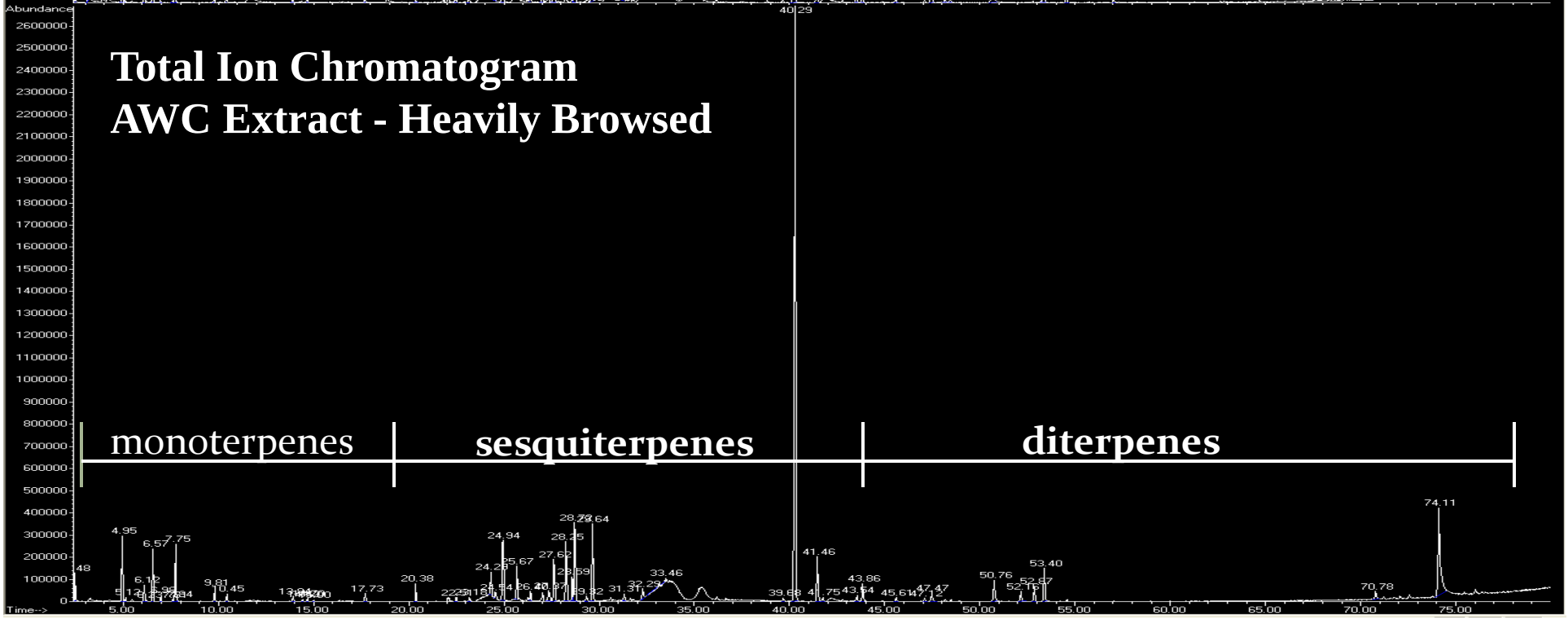
The mass fragmentation pattern of a molecule is a characteristic similar to a fingerprint – it can aid in identification.

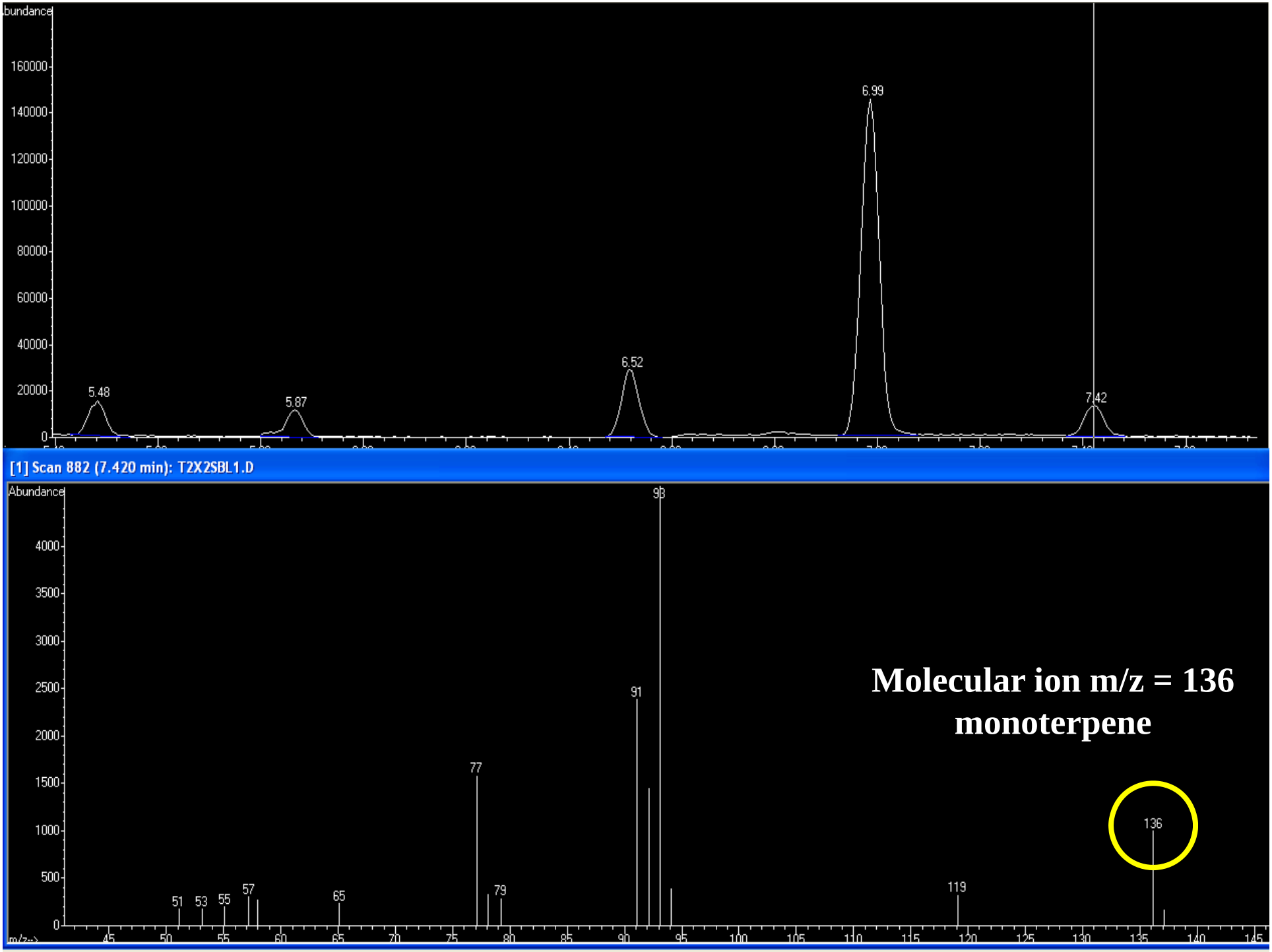
Total Ion Chromatogram
AWC Extract – Minimal Browsing

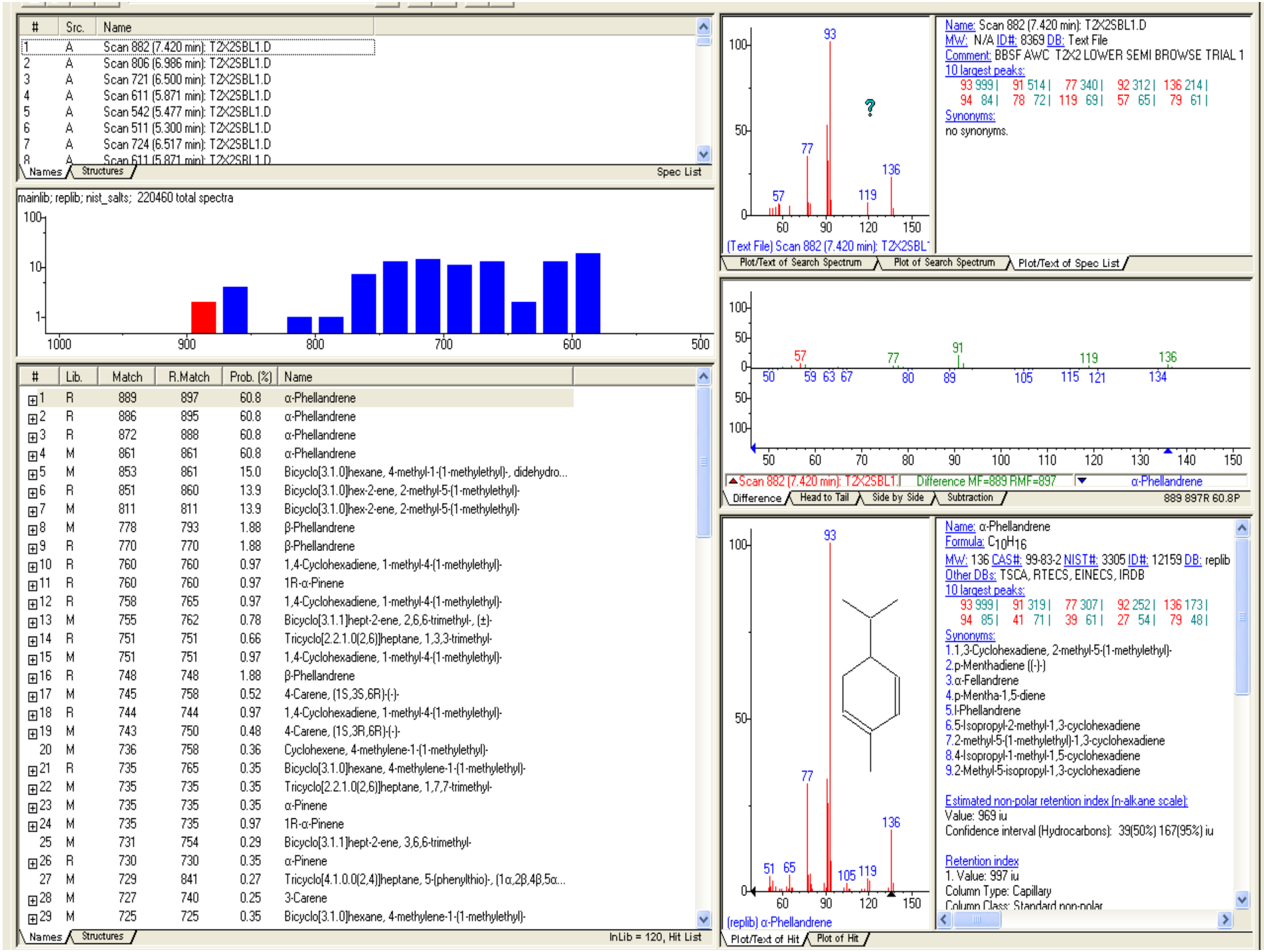


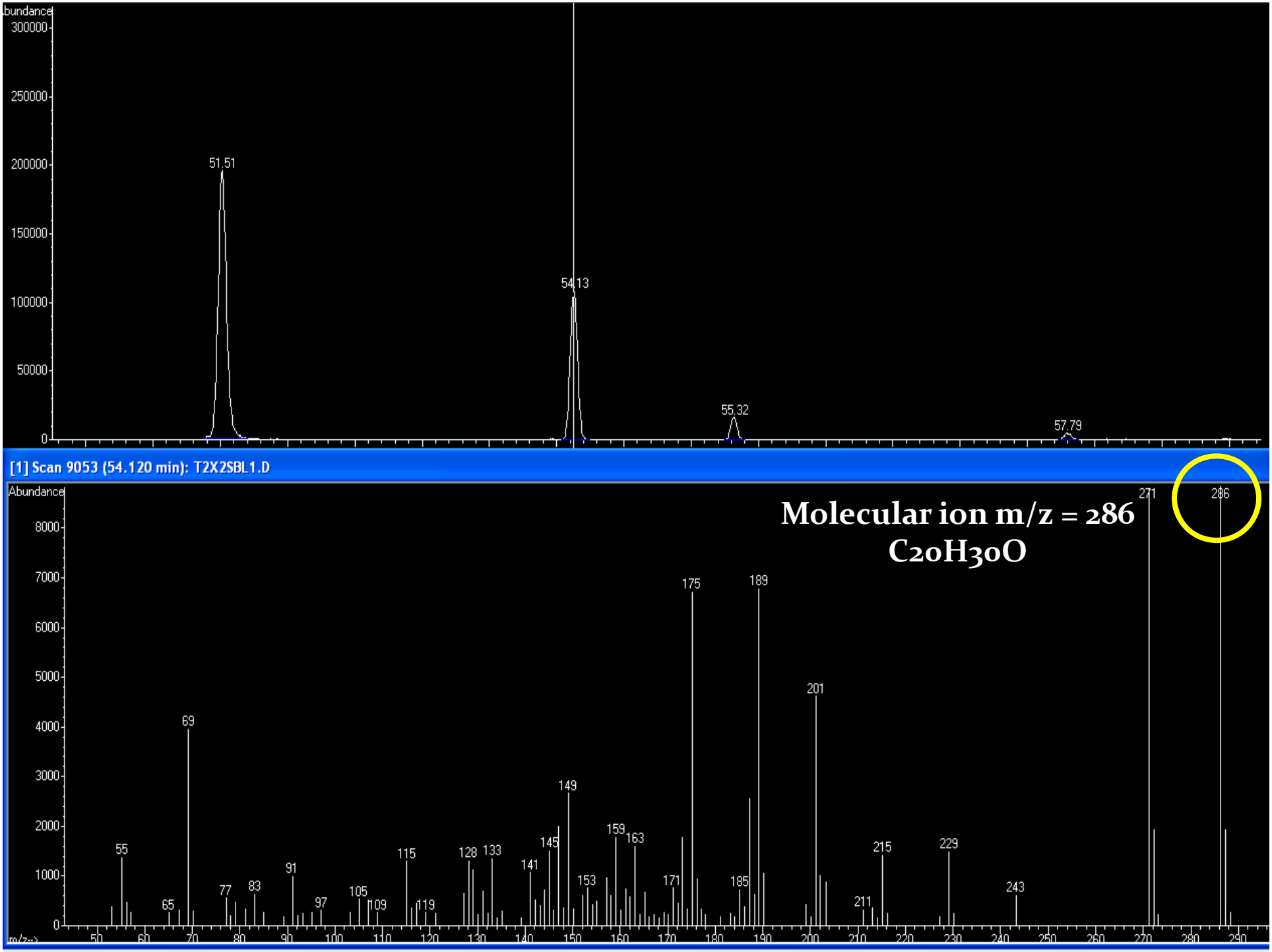

Total Ion Chromatogram
AWC Extract - Heavily Browsed
Abundance
Time-->
monoterpenes
sesquiterpenes
diterpenes
40.29
4.95
6.57
7.75
9.81
10.45
17.73
20.38
24.94
25.67
27.62
28.25
28.64
33.46
41.46
43.86
50.76
53.40
74.11
70.78


Total Ion Chromatogram
AWC Extract – Minimal Browsing
monoterpenes
sesquiterpenes
diterpenes
Abundance
Time-->


Total Ion Chromatogram
AWC Extract - Heavily Browsed
monoterpenes
sesquiterpenes
diterpenes
Abundance
Time-->

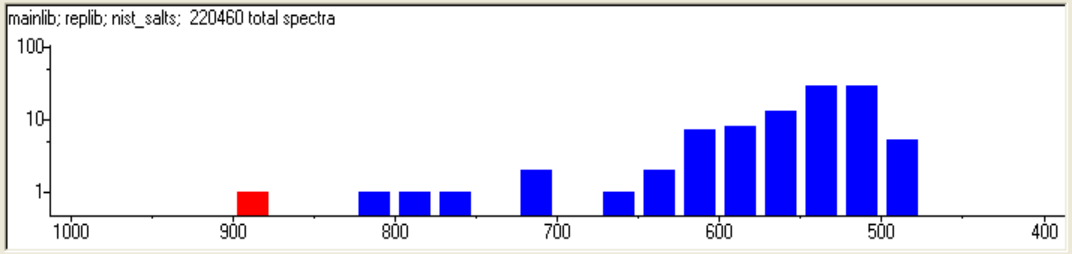

bundance
160000
140000
120000
100000
80000
60000
40000
20000
0
5.48
5.87
6.52
6.99
7.42
[1] Scan 882 (7.420 min): T2X2SBL1.D
Abundance
4000
3500
3000
2500
2000
1500
1000
500
0
93
91
77
79
65
57
55
53
51
119
136
m/z-->
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
120
125
130
135
140
145
Molecular ion m/z = 136
monoterpene


#
Src.
Name
1 A Scan 882 (7.420 min): T2X2SBL1.D
2 A Scan 806 (6.986 min): T2X2SBL1.D
3 A Scan 721 (6.500 min): T2X2SBL1.D
4 A Scan 611 (5.871 min): T2X2SBL1.D
5 A Scan 542 (5.477 min): T2X2SBL1.D
6 A Scan 511 (5.300 min): T2X2SBL1.D
7 A Scan 724 (6.517 min): T2X2SBL1.D
Names
Structures
Spec List
mainlib; replib; nist_salts; 220460 total spectra
100
10
1
1000
900
800
700
600
500
#
Lib.
Match
R.Match
Prob. (%)
Name
1 R 889 897 60.8 α-Phellandrene
2 R 886 895 60.8 α-Phellandrene
3 R 872 888 60.8 α-Phellandrene
4 M 861 861 60.8 α-Phellandrene
5 M 853 861 15.0 Bicyclo[3.1.0]hexane, 4-methyl-1-(1-methylethyl)-, didehydro...
6 R 851 860 13.9 Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-
7 M 811 811 13.9 Bicyclo[3.1.0]hex-2-ene, 2-methyl-5-(1-methylethyl)-
8 M 778 793 1.88 β-Phellandrene
9 R 770 770 1.88 β-Phellandrene
10 R 760 760 0.97 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-
11 R 760 760 0.97 1R-α-Pinene
12 R 758 765 0.97 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-
13 M 755 762 0.78 Bicyclo[3.1.1]hept-2-ene, 2,6,6-trimethyl-, (±)-
14 R 751 751 0.66 Tricyclo[2.2.1.0(2,6)]heptane, 1,3,3-trimethyl-
15 M 751 751 0.97 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-
16 R 748 748 1.88 β-Phellandrene
17 M 745 758 0.52 4-Carene, (1S,3S,6R)-(-)-
18 R 744 744 0.97 1,4-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-
19 M 743 750 0.48 4-Carene, (1S,3R,6R)-(-)-
20 M 736 758 0.36 Cyclohexene, 4-methylene-1-(1-methylethyl)-
21 R 735 765 0.35 Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-
22 M 735 735 0.35 Tricyclo[2.2.1.0(2,6)]heptane, 1,7,7-trimethyl-
23 M 735 735 0.35 α-Pinene
24 M 735 735 0.97 1R-α-Pinene
25 M 731 754 0.29 Bicyclo[3.1.1]hept-2-ene, 3,6,6-trimethyl-
26 R 730 730 0.35 α-Pinene
27 M 729 841 0.27 Tricyclo[4.1.0.0(2,4)]heptane, 5-(phenylthio)-, (1α,2β,4β,5α...
28 M 727 740 0.25 3-Carene
29 M 725 725 0.35 Bicyclo[3.1.0]hexane, 4-methylene-1-(1-methylethyl)-
Names
Structures
InLib = 120, Hit List
93
77
57
119
136
(Text File) Scan 882 (7.420 min): T2X2SBL
Name: Scan 882 (7.420 min): T2X2SBL1.D
MW: N/A ID#: 8369 DB: Text File
Comment: BBSF AWC T2X2 LOWER SEMI BROWSE TRIAL 1
10 largest peaks:
93 999 | 91 514 | 77 340 | 92 312 | 136 214 |
94 84 | 78 72 | 119 69 | 57 65 | 79 61 |
Synonyms:
no synonyms.
Plot/Text of Search Spectrum
Plot of Search Spectrum
Plot/Text of Spec List
57
77
91
119
136
50 59 63 67 80 89 105 115 121 134
Scan 882 (7.420 min): T2X2SBL1.
Difference MF=889 RMF=897
α-Phellandrene
Difference
Head to Tail
Side by Side
Subtraction
889 897R 60.8P
93
77
51 65
105 119
136
(replib) α-Phellandrene
Name: α-Phellandrene
Formula: C10H16
MW: 136 CAS#: 99-83-2 NIST#: 3305 ID#: 12159 DB: replib
Other DBs: TSCA, RTECS, EINECS, IRDB
10 largest peaks:
93 999 | 91 319 | 77 307 | 92 252 | 136 173 |
94 85 | 41 71 | 39 61 | 27 54 | 79 48 |
Synonyms:
1.1,3-Cyclohexadiene, 2-methyl-5-(1-methylethyl)-
2.p-Menthadiene ((-)-)
3.α-Fellandrene
4.p-Mentha-1,5-diene
5.l-Phellandrene
6.5-Isopropyl-2-methyl-1,3-cyclohexadiene
7.2-methyl-5-(1-methylethyl)-1,3-cyclohexadiene
8.4-Isopropyl-1-methyl-1,5-cyclohexadiene
9.2-Methyl-5-isopropyl-1,3-cyclohexadiene
Estimated non-polar retention index (n-alkane scale):
Value: 969 iu
Confidence interval (Hydrocarbons): 39(50%) 167(95%) iu
Retention index
1. Value: 997 iu
Column Type: Capillary
Column Class: Standard non-polar
Plot/Text of Hit
Plot of Hit


bundance
300000
250000
200000
150000
100000
50000
0
51.51
54.13
55.32
57.79
[1] Scan 9053 (54.120 min): T2X2SBL1.D
Abundance
8000
7000
6000
5000
4000
3000
2000
1000
0
m/z-->
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220
230
240
250
260
270
280
290
55
65
69
77
83
91
97
105
109
115
119
128
133
141
145
149
153
159
163
171
175
185
189
201
211
215
229
243
271
286
Molecular ion m/z = 286
C20H30O

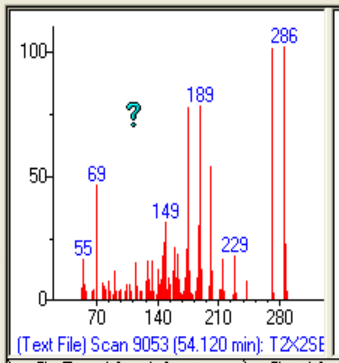
#	Src.	Name
7	A	Scan 8135 (48.873 min): T2X2SBL1.D
8	A	Scan 8136 (48.879 min): T2X2SBL1.D
9	A	Scan 8008 (48.148 min): T2X2SBL1.D
10	A	Scan 7946 (47.793 min): T2X2SBL1.D
11	A	Scan 7945 (47.788 min): T2X2SBL1.D
12	A	Scan 7949 (47.810 min): T2X2SBL1.D
13	A	Scan 7386 (44.593 min): T2X2SBL1.D
14	A	Scan 7332 (44.284 min): T2X2SBL1.D

Names | Structures | Spec List



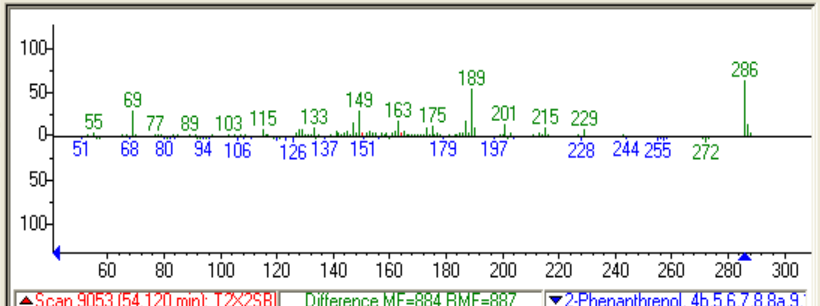
#	Lib.	Match	R.Match	Prob. (%)	Name
1	M	884	887	81.2	2-Phenanthrenol, 4b,5,6,7,8,8a,9,10-octahydro-4b,8,8-trim...
2	M	820	965	13.3	Ferruginol
3	M	782	870	3.19	13-Isopropylpodocarpen-12-ol-20-al
4	M	763	770	1.55	2-Phenanthrenol, 4b,5,6,7,8,8a,9,10-octahydro-4b,8,8-trim...
5	M	722	724	0.35	5-Androstene, 4,4-dimethyl-
6	M	706	727	0.20	3-Phenanthrenol, 4b,5,6,7,8,8a,9,10-octahydro-4b,8,8-trim...
7	M	667	669	0.04	Podocarp-7-en-3-one, 13β-methyl-13-vinyl-
8	M	640	694	0.01	5β-Pregn-11-ene
⊞9	M	629	642	0.00	12β-Hydroxy-5α-pregnane, butyrate (ester)
10	M	615	719	0.00	Podocarpa-8,11,13-trien-17-oic acid, 12-hydroxy-13-isoprop...
11	M	614	614	0.00	Acetic acid, 1-ethyl-9a,11a-dimethylhexadecahydrocyclope...
12	M	613	640	0.00	69-Tetrahydrocannabivarin
13	M	611	612	0.00	9(11)-Dehydrotestosterone
⊞14	R	605	606	0.00	Dromostanolone Propionate
15	M	604	617	0.00	4,14-Retro-retinol
⊞16	R	604	606	0.00	Dromostanolone Propionate
⊞17	M	597	610	0.00	12α-Hydroxy-5α-pregnane, butyrate(ester)
⊞18	M	594	663	0.00	Estra-1,3,5(10)-trien-17-one, 2,3-dihydroxy-
19	M	589	630	0.00	6-Hydroxy-7-isopropyl-1,4a-dimethyl-1,2,3,4,4a,9,10,10a-oc...
⊞20	M	588	588	0.00	Dromostanolone Propionate
⊞21	M	586	648	0.00	12(beta)-Acetoxy-5-(alpha)-pregnane
22	M	583	677	0.00	D:A-Friedoolean-7-ene
⊞23	M	581	581	0.00	Retinol
24	M	577	577	0.00	Pyrano[4,3-a]phenanthren-3-one, 10a,12a-dimethyl-8-hydro...
25	M	575	584	0.00	4a(2H)-Phenanthrenecarboxaldehyde, 1,3,4,9,10,10a-hexa...
26	M	574	575	0.00	1-Phenanthrenecarboxaldehyde, 7-ethenyl-1,2,3,4,4a,4b,5,...
27	M	573	573	0.00	4,6-Androstadien-17β-ol-3-one
⊞28	M	570	619	0.00	1-Phenanthrenemethanol, 1,2,3,4,4a,9,10,10a-octahydro-1...
⊞29	M	566	576	0.00	12β-Hydroxy-5α-pregnane, trimethylsilyl ether

Names | Structures | InLib = 112, Hit List

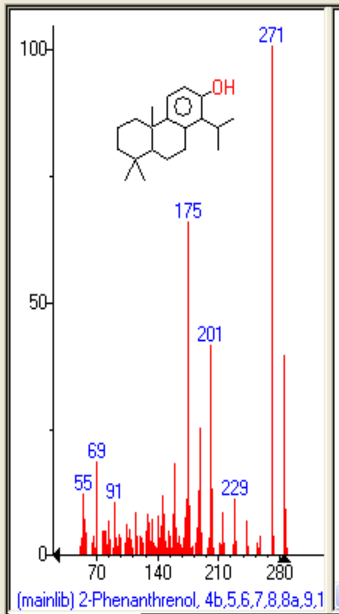


Name: Scan 9053 (54.120 min): T2X2SBL1.D
MW: N/A ID#: 8458 DB: Text File
Comment: BBSF AWC T2X2 LOWER SEMI BROWSE TRIAL 1
10 largest peaks:
286 999 | 271 991 | 189 766 | 175 759 | 201 521 |
69 447 | 149 301 | 187 289 | 147 226 | 272 217 |
Synonyms:
no synonyms.

Plot/Text of Search Spectrum | Plot of Search Spectrum | Plot/Text of Spec List



Difference | Head to Tail | Side by Side | Subtraction
884 887R 81.2P



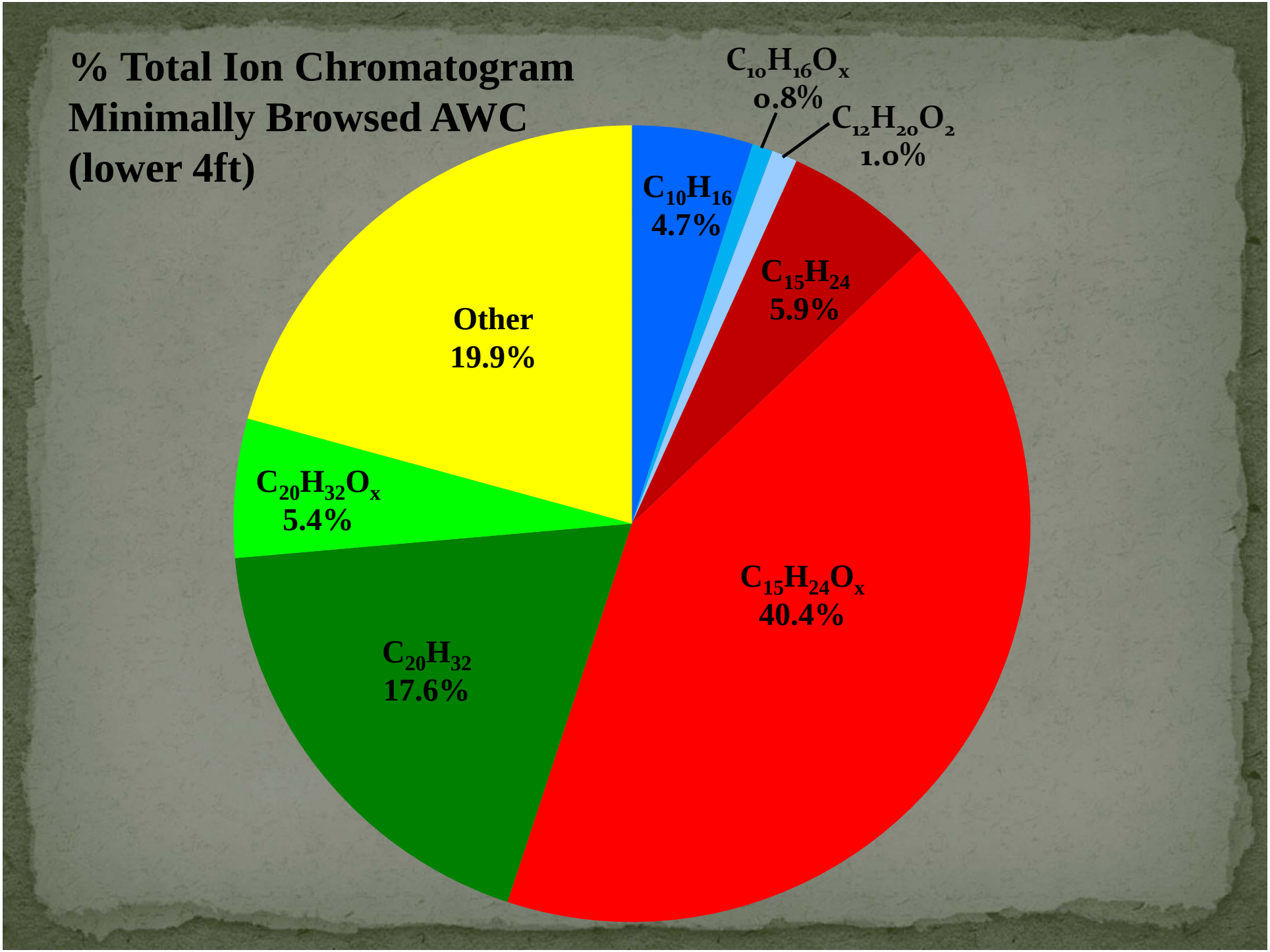
Name: 2-Phenanthrenol, 4b,5,6,7,8,8a,9,10-octahydro-4b,8,8-
Formula: $C_{20}H_{30}O$
MW: 286 CAS#: 511-15-9 NIST#: 42592 ID#: 171589 DB: m
Other DBs: NIH
Contributor: R RYHAGE MS-LAB KAROLINSKA INSTITUTET
10 largest peaks:
271 999 | 175 653 | 201 409 | 286 391 | 272 258 |
189 249 | 69 182 | 41 178 | 159 177 | 43 140 |
Synonyms:
1.Podocarpa-8,11,13-trien-13-ol, 14-isopropyl-
2.Totarol
3.Totarol (b637832k176)
4.(+)-Totarol
5.14-Isopropylpodocarpa-8,11,13-trien-13-ol #

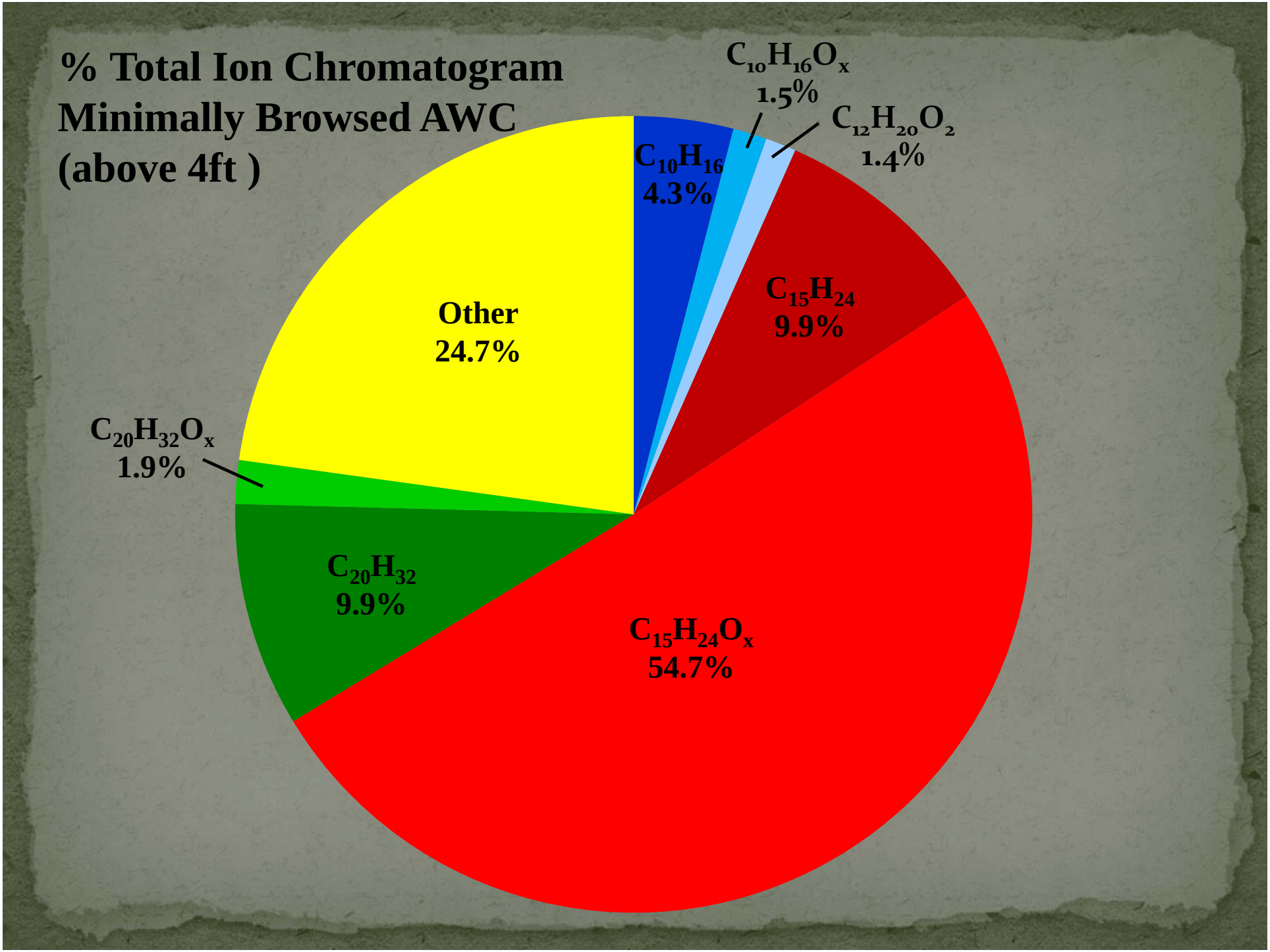
Estimated non-polar retention index (n-alkane scale):
Value: 2225 iu
Confidence interval (Phenols): 70(50%) 301(95%) iu

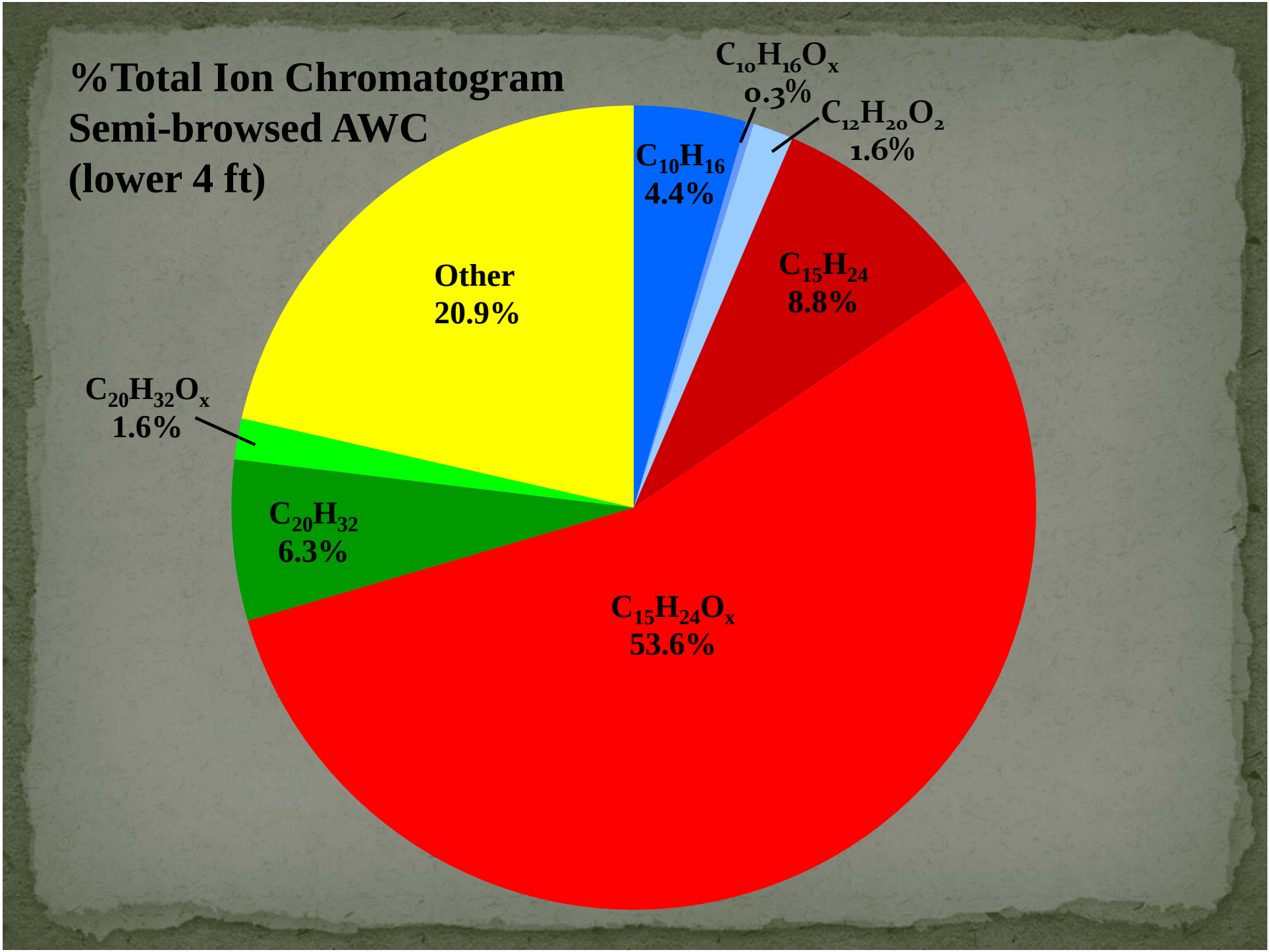
Retention index
1. Value: 2268 iu
Column Type: Capillary
Column Class: Standard non-polar
Active Phase: BP-1
Column Length: 50 m
Carrier Gas: He

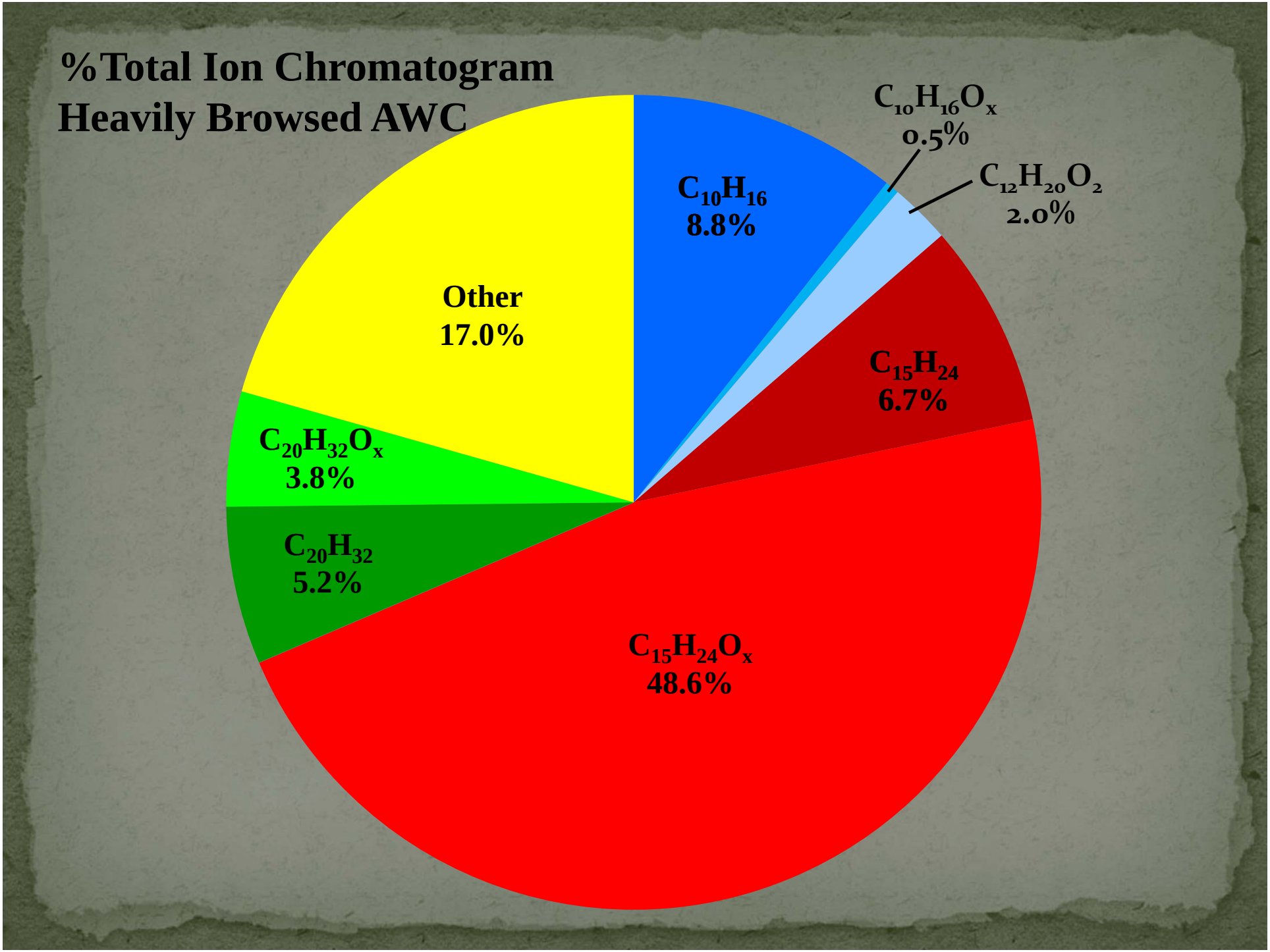
Plot/Text of Hit | Plot of Hit

Lib. Search | Other Search | Names | Compare | Librarian | MSMS


% Total Ion Chromatogram
Minimally Browsed AWC
(lower 4ft)
$C_{10}H_{16}O_x$
0.8%
$C_{12}H_{20}O_2$
1.0%
$C_{10}H_{16}$
4.7%
$C_{15}H_{24}$
5.9%
Other
19.9%
$C_{20}H_{32}O_x$
5.4%
$C_{15}H_{24}O_x$
40.4%
$C_{20}H_{32}$
17.6%


% Total Ion Chromatogram
Minimally Browsed AWC
(above 4ft)
$C_{10}H_{16}O_x$
1.5%
$C_{12}H_{20}O_2$
1.4%
$C_{10}H_{16}$
4.3%
$C_{15}H_{24}$
9.9%
Other
24.7%
$C_{20}H_{32}O_x$
1.9%
$C_{20}H_{32}$
9.9%
$C_{15}H_{24}O_x$
54.7%


%Total Ion Chromatogram
Semi-browsed AWC
(lower 4 ft)
$C_{10}H_{16}O_x$
0.3%
$C_{12}H_{20}O_2$
1.6%
$C_{10}H_{16}$
4.4%
$C_{15}H_{24}$
8.8%
Other
20.9%
$C_{20}H_{32}O_x$
1.6%
$C_{20}H_{32}$
6.3%
$C_{15}H_{24}O_x$
53.6%


%Total Ion Chromatogram
Heavily Browsed AWC
$C_{10}H_{16}$
8.8%
$C_{10}H_{16}O_x$
0.5%
$C_{12}H_{20}O_2$
2.0%
$C_{15}H_{24}$
6.7%
$C_{15}H_{24}O_x$
48.6%
$C_{20}H_{32}$
5.2%
$C_{20}H_{32}O_x$
3.8%
Other
17.0%

Conclusions

Monoterpene contribution to the total ion chromatogram in heavily browsed AWC foliage was nearly twice that observed in minimally browsed and semi-browsed trees. This may be result of a stress response to herbivory.

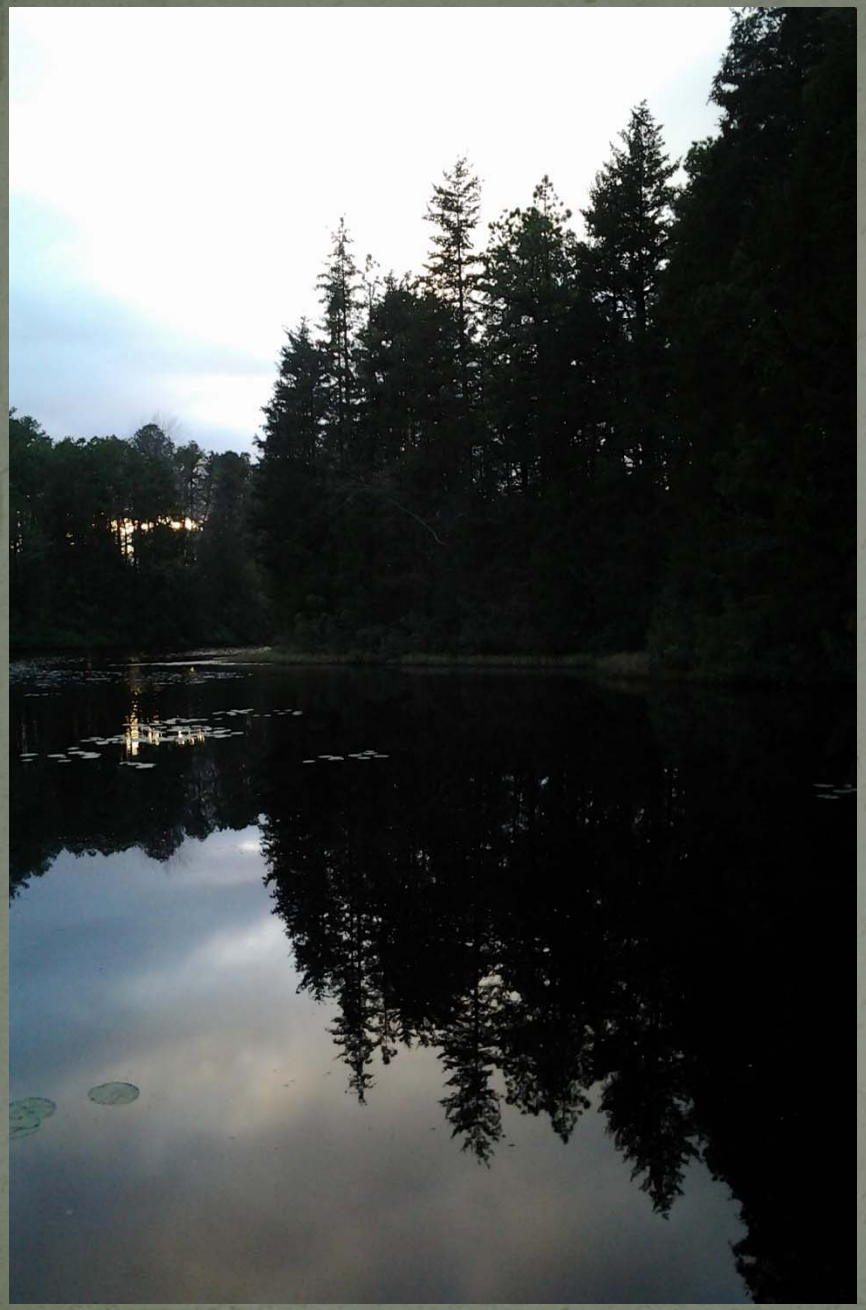
OR

Measuring remaining AWC that survived and perhaps these trees survived because a higher abundance of monoterpenes made them less palatable.

Conclusions

Oxygenated monoterpenoids which inhibit microbial action in ruminants making digestion difficult represented only 2-3% of the total ion chromatogram regardless of browse level.

The diterpene fraction represented 23% of the total ion chromatogram for minimally browsed AWC foliage, but only 8-9% for browsed foliage suggesting browsed AWC lack the carbon pool to synthesize the larger terpenes.



Future Directions

Increase sample population!!!

Investigate terpene profile:

- **geographically**
- **seasonally**
- **intra-tree**
- **greenhouse experiments & browsing studies**

Acknowledgements

- **Past undergraduate students:**
  Victoria Johnson
  Dana Bezaire
  Alyssa Schlueter
  Ryan Roff

- **Support: The Richard Stockton College of New Jersey, Career Development Award**