Bioactive Anti-Cancer Compound(s) in Devil’s Club: a Medicinal Plant of the Pacific Northwest

Discussion

- Use/Collection/Processing
- Bioassay 3D Cell suspensions
- LC/MS, MS/MS, and accurate mass (+) ESI
- Overview LC/MS ESI, APCI, and APPI

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Devil’s Club: “Oplopanax horridus”

Araliacea family which contains Ginsengs. DC was formerly called Alaskan Ginseng
First Nations Use of Devil’s Club

Cultural Linguistic Groups (Over 38):
- Over 34 medicinal uses known
- Spiritual uses 8

Mode of Use Inner Bark/Berries:
- Chew inner bark
- Infusion or Decoction (i.e. as a tea)
- Mixed with berries as a paste (crush both together) and taken orally or applied externally to wounds

Stem Processing for Inner Bark

- Cut Stems
- Remove outer Bark
- Score longitudinal
- Peal Inner Bark Off

Dry @ 30°C 8 hrs

Grind - Extract 70% EtOH/H₂O
Grind - Extract 95°C H₂O

Bioassay
LC/MS Profile

LC Fractionation
Activity

MS and MS MS accurate mass
Human Panc-1 Treated with Different Agents: 3D – 3 days

- **Control**
  - DC extract
  - ~ 1.5 µM - cells still viable
  - ~ 6 µM - cells dead

- **Different Panc Cell Line**
  - Gemcitabine 1st line drug
  - 10 µM - cells still viable
  - 40 µM – mostly cells dead

- **Paclitaxel 2nd line drug**
  - 10 nM – cells still viable
  - 80 nM - cells dead
Chromatographic Profiles: 70 % EtOH Extract Inner Bark

- **ESI Positive Ion TIC**
- **ESI Negative Ion TIC**

[A: H₂O  B: AcN; A and B 0.05% formic acid]
B: 10% 2 min, 40% 22 min, 60% 26 min, 100% 40 min
Initial Mass Spectra Found
ESI (+) Ion Spectra of Bioactive Compound

- **A:** H$_2$O; 0.05% formic acid
- **B:** AcN; 0.05% formic acid
- Gradient 10 -100% B in 40 min
- 4.6 x 150 mm Zorbax C18

**Agilent G1946D ESI(+)**
- 50-1500 m/z
- Vcap: 4.0 kV
- Fragmentation voltage: 90
- Nebulizer gas: 10 l/min
- N$_2$ temperature: 350 °C
Devil's Club Polyynes with Antibacterial and Antimycobacterial Activity

G.H.N. Towers et. al. J. Natural Products 1997, 60(11), 1210-1213

Compound (4)
C20 H28 O4: 332.4

Oplopanoik acetate (5)
C20 H30 O4: 334.4

Falcarinol (1)
C17 H24 O : 244.4

Falcarindiol (2)
C17 H24 O2: 260.4

Oplopanoik (3)
C17 H26 O2 : 262.4
LC/MS Polyacetylene Positive Ion Spectral Characteristics

ESI, APCI positive ions reported in literature:

- $[\text{M} + \text{Na}]^+$; $[\text{M} + \text{K}]^+$; $[\text{M} + \text{H}]^+$; $[2\text{M} + \text{H}]^+$, $[\text{M} + \text{NH}_4]^+$

- $[\text{M} - (n \text{H}_2\text{O}) + \text{H}]^+$: $n = 0,1,2...$

- $[\text{M} - (n \text{H}_2\text{O}) + m (\text{CH}_3\text{OH or AcN}) + \text{H}]^+$ : $n = 0,1,2..$; $m = 0,1,2..$
<table>
<thead>
<tr>
<th>Nominal Mass</th>
<th>m/z Ion Observed</th>
<th>Proposed Correlation</th>
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</thead>
<tbody>
<tr>
<td>“Tower’s Cmpds”</td>
<td></td>
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<tr>
<td>1. 244</td>
<td>297</td>
<td>[(M – 2H₂O) + H]⁺</td>
</tr>
<tr>
<td>2. 260</td>
<td>315</td>
<td>[(M – H₂O) + H]⁺</td>
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<tr>
<td></td>
<td>332</td>
<td>[M – H₂O + NH₄]⁺, M⁺</td>
</tr>
<tr>
<td>3. 262</td>
<td>350</td>
<td>[M + NH₄]⁺</td>
</tr>
<tr>
<td></td>
<td>355</td>
<td>[M + Na]⁺</td>
</tr>
<tr>
<td>4. 332</td>
<td>411</td>
<td>[?]⁺</td>
</tr>
<tr>
<td></td>
<td>429</td>
<td>[?]⁺</td>
</tr>
<tr>
<td></td>
<td>555</td>
<td>[(M – 2H₂O) + (M – C₃H₄O - H₂O) + H]⁺</td>
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<tr>
<td>5. 334</td>
<td>593</td>
<td>[2(M – 2H₂O) + H]⁺</td>
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<td>611</td>
<td>[(M – 2H₂O) + (M - H₂O) + H]⁺</td>
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<tr>
<td></td>
<td>629</td>
<td>[2(M – H₂O) + H]⁺</td>
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<tr>
<td></td>
<td>664</td>
<td>[M + (M – H₂O + NH₄)]⁺, ......?</td>
</tr>
</tbody>
</table>
+ve ESI mass spectra of active peak – Waters QTof

350.2329 (-0.7ppm $[C_{20}H_{28}O_{4}+NH_4]^+$)

355.1882 (-0.9ppm $[C_{20}H_{28}O_{4}+Na]^+$)

MS/MS of m/z 350 Ar@5eV

- 332.2214 (theoretical = 332.2226)
  loss of $H_2O$ from m/z 350 – 3.5 ppm

- 315.1971 (theoretical = 315.1960)
  loss of $NH_3$ from m/z 332 +3.4 ppm

- 297.1868 (theoretical = 297.1854)
  loss of $H_2O$ from m/z 315 +4.5 ppm
Positive Ion ESI; APCI; APPI [MeOH H₂O]

- ESI: [(M-2H₂O)+H]⁺, [M+Na]⁺
- APCI: [(M-2H₂O)+CH₃OH+H]⁺
- APPI:toluene: [(M-2H₂O)+2CH₃OH+H]⁺
Negative Ion ESI; APCI; APPI [MeOH H₂O]
Initial Identification of Compounds

Positive Ion Thoughts

- Where does NH$_4^+$ come from? NH$_4^+$ addition did not increase 350 m/z but did suppressed dimers.

- Addition of metal ions (Na$^+$) to indicate [M + Na]$^+$ and M; search of – H$_2$O.

- Positive ion may predict number of OH groups and dimers can be useful for initial interpretation.

- Different MS Sources do not necessarily give the same results.

- LC and MS should be clean and mobile phase fresh.