

Sesquiterpene Lactones from *Helenium flexuosum*

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Introduction

- Biodiversity is the diversity of life including plants, animals, and microorganisms.
- Chemotaxonomy is the biological classification of organisms based on their similarities in chemical composition.
- Primary metabolites are chemical compounds in living organisms include DNA, RNA, proteins, lipids, and carbohydrates.
- Secondary metabolites are smaller chemical compounds produced by all living things often for purposes of survival, defense, and communication.¹

- 1. Glaxo Wellcome Research & Development: Stevenage, Herts, U.K. *Natural Products Isolation*. Ed. Cannell, R.J.P. et. al. Humana Press: Totowa, New Jersey. 1998. Electronic Book.



Natural Products

- Natural Products are secondary metabolites - chemical compounds discovered from living organisms.
- Approximately one-half of the U.S. Food and Drug Administration approved drugs are derived from natural products.
- Bio-prospecting is the search for novel natural products found in nature by researchers around the world to analyze and evaluate for potential medicinal drug discoveries.¹

- 1. Glaxo Wellcome Research & Development: Stevenage, Herts, U.K. *Natural Products Isolation*. Ed. Cannell, R.J.P. et. al. Humana Press: Totowa, New Jersey. 1998. Electronic Book.



Synthesis of Natural Products

- Once a natural product has been discovered and passed multiple clinical trials it is synthesized in a laboratory
- Many polymers (plastics) and pharmaceuticals are synthesized.
- Organic synthesis involves the use of common reactants under controlled pH and temperature, using organic solvents, to synthesize the desired product.
- Bioactivity of the product is reliant on chirality. Racemic mixtures are separated using chiral resolution.²



Natural Products

- Penicillin was the first antibiotic discovered in 1928, a natural product produced from the fungus *Penicillium chrysogenum*.³
- Vancomycin is the first line treatment for methicillin resistant *Staphylococcus aureus* (MRSA) infections. It was discovered in 1953, from soil samples in the country of Borneo, from the bacterium *Amycolatopsis orientalis*.⁴
- 3. American Chemical Society International Historic Chemical Landmarks. Discovery and Development of Penicillin.
<http://www.acs.org/content/acs/en/education/whatischemistry/landmarks/flemingpenicillin.html>
(Accessed March 28, 2016).
- 4. Shenoy, E. S., Paras, M. L., Noubary, F., Walensky, R. P., & Hooper, D. C. (2014). Natural history of colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE): a systematic review. *BMC Infectious Diseases*, 14, 177.
<http://doi.org/10.1186/1471-2334-14-177>



Natural Products

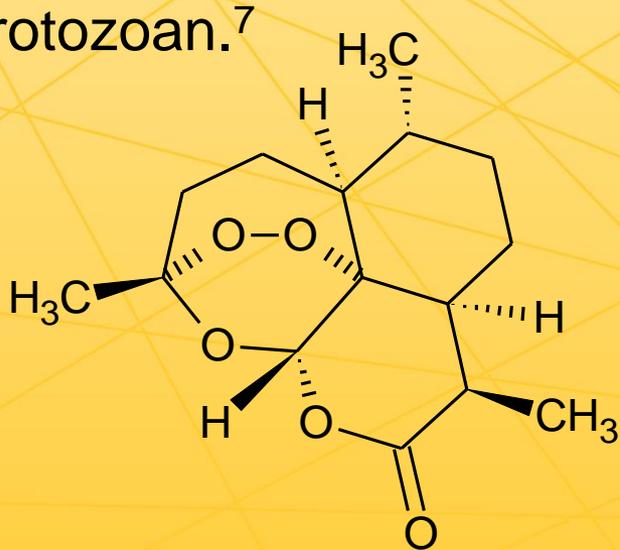
- Epinephrine (adrenaline) is a compound produced by the adrenal glands of mammals and a hormone that works as a vasopressor in the sympathetic nervous system. It was discovered in 1895. It can be extracted from animal serum or synthesized.⁵
- Acetylsalicylic acid ASA (aspirin) is a natural product produced by willow tree bark sap and used since antiquity as an analgesic (pain reliever).⁶

- 5. Sneader, W. The Discovery and Synthesis of Epinephrine. *Drug News Prospect*. Vol 14.8 (2001). pp.491-494
- 6. Mahdi, J., Mahdi, A., Bowen, I. The Historical Analysis of Aspirin Discovery, its relation to the willow tree and antiproliferative and anticancer potential. *Cell Proliferation*. Vol. 39.2 (2006). pp.147-155



Natural Products

- Artemisia annua contains a large concentration of artemisinin. Artemisinin is a chemical compound in the sesquiterpene lactone class. Youyou Tu shared the 2015 Nobel Prize in Physiology or Medicine for the discovery of artemisinin. Artemisinin-based Combination Therapies (ACT) are being used to treat malaria caused by several species of the parasitic Plasmodium protozoan.⁷



Artemisia annua
“Sweet Wormwood”

- 7. "The Nobel Prize in Physiology or Medicine 2015". *Nobelprize.org*. Nobel Media AB 2014. Web. 28 Mar 2016. <http://www.nobelprize.org/nobel_prizes/medicine/laureates/2015/>

Natural Product Research on Helenium Flexuosum

- Helenium Flexuosum is a Native American daisy species, from the Asteraceae family. The Asteraceae family is known for the production of sesquiterpene lactones, a class of compounds with anti-cancer and anti-inflammatory properties.
- A sesquiterpene lactone compound is a 15-carbon compound, formed from three isoprene units, with a lactone ring. Their biological effects such as tumor treatment is due to the α -methylene- γ -lactone (α MyL) group.⁸

- 8. Chadwick, M., Trewin, H., Gawthrop, F., & Wagstaff, C. (2013). Sesquiterpenoids Lactones: Benefits to Plants and People. *International Journal of Molecular Sciences*, 14(6), 12780–12805. 
<http://doi.org/10.3390/ijms140612780>

Sesquiterpene Lactones

- 3 Isoprene Units (2-methyl-1,3-butadiene)
- Lactone – 5, 6, or 7 member ring ester
- α MyL group is reactive by the process of the Michael Reaction – the addition of a nucleophile to an α - β unsaturated carbonyl group.
- Sesquiterpene lactones are lipophilic, enabling movement across cell membranes, altering transcription of genes in the nucleus and signal transduction pathways.⁸

- 8. Chadwick, M., Trewin, H., Gawthrop, F., & Wagstaff, C. (2013). Sesquiterpenoids Lactones: Benefits to Plants and People. *International Journal of Molecular Sciences*, 14(6), 12780–12805. <http://doi.org/10.3390/ijms140612780>



Gathering Daisies

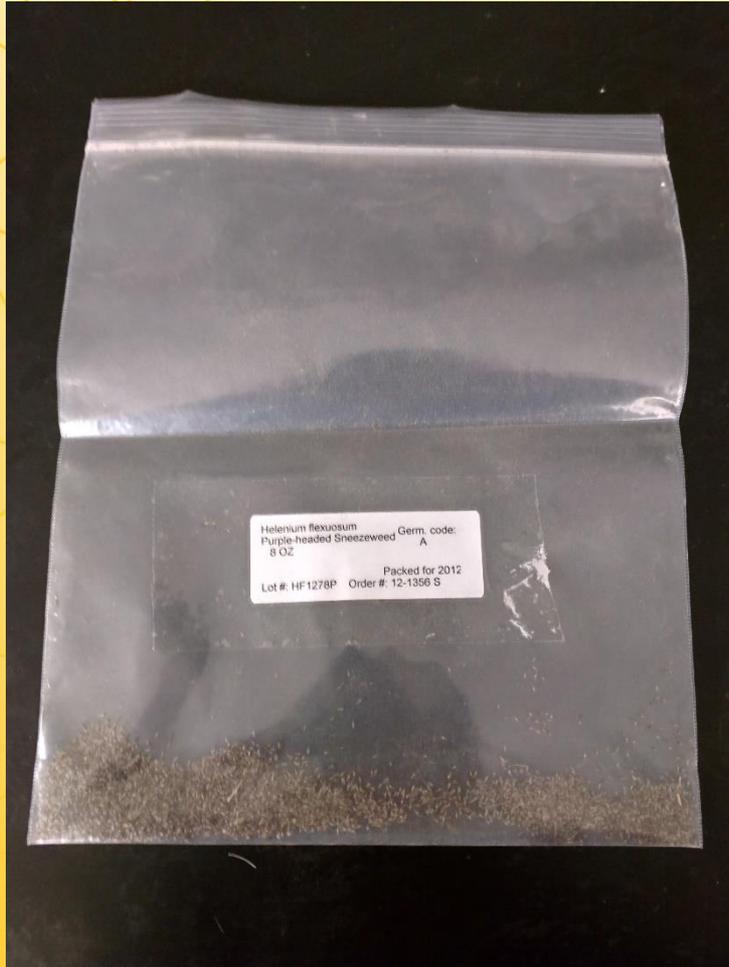
- *Helenium flexuosum* (Purple Sneezeweed) is a species in the daisy family; an herbaceous perennial wildflower, native to North America. It grows up to 3 feet in height.
- Each globoid flowerhead is 1 inch across and has 8-14 florets. At the apex of the flowerhead there are numerous achenes (small, dry, one-seeded fruits). The achenes are the source of the medicinal sesquiterpene lactones.⁹



- 9.Hilty, J. *Helenium flexuosum*. Asteraceae Family.. Prairie Wildflowers of Illinois. UNIVERSITY OF MINNESOTA CROOKSTON
http://www.illinoiswildflowers.info/prairie/plant_index.htm#ph_sneezeweed



Crude Extract from Seeds



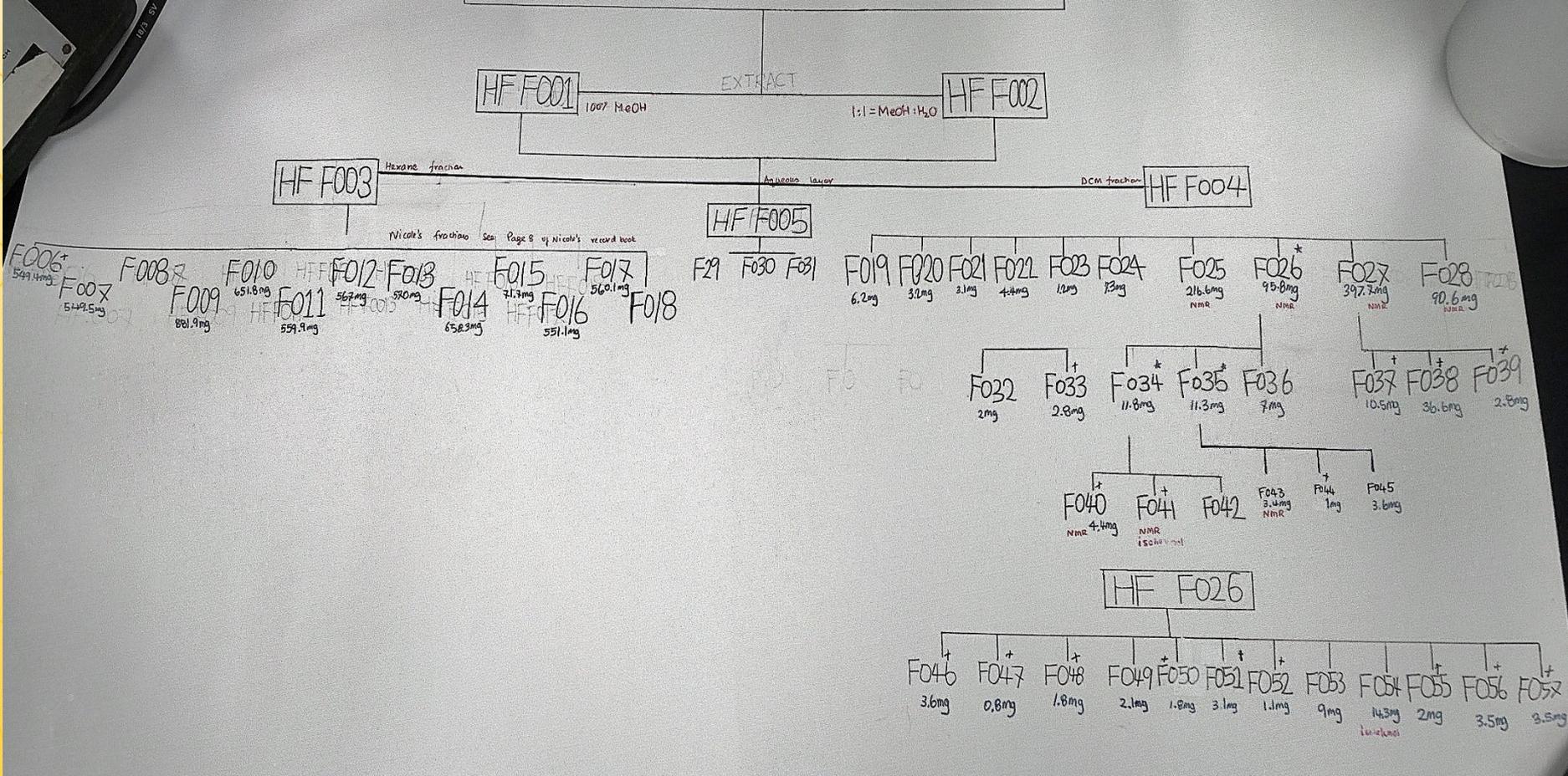
Fractionation

- The seeds were ground using a seed grinder.
- Next the powder was extracted with methanol. The methanolic extract was Kupchan partitioned into hexane, dichloromethane, and aqueous fractions.
- Categorization of fractions were named as such:
 - Helenium flexuosum – HF
 - Fraction F003 is hexane
 - Fraction F004 is dichloromethane
 - Fraction F005 is aqueous
- The fractions under research are HFF004.
- The fractions that showed potential from Thin Layer Chromatography (TLC) included: HFF051, HFF036, HFF037, HFF041, and HFF027.



Helenium Flexuosum Seed

* Used up
 † HPLC analysed (analytical)



- 10. Baek, G, Mukku, V. (2013). Fractionation of Helenium flexuosum seeds. University of Minnesota, Crookston.



What is HPLC?

- High Pressure Liquid Chromatography is a type of Chromatography technique which separates compounds based on adsorption.
- HPLC involves two phases – a stationary phase and a mobile phase. The stationary phase is a nonpolar silica-based column. The mobile phase is a mixture of water and methanol or acetonitrile.
- With such stationary phases, retention time is longer for molecules which are less polar, while polar molecules elute more readily.¹¹

- 11. Lindsay, S., & Kealey, D. (1987). *High performance liquid chromatography*. United States: John Wiley and Sons, New York, NY.



HPLC Equipment



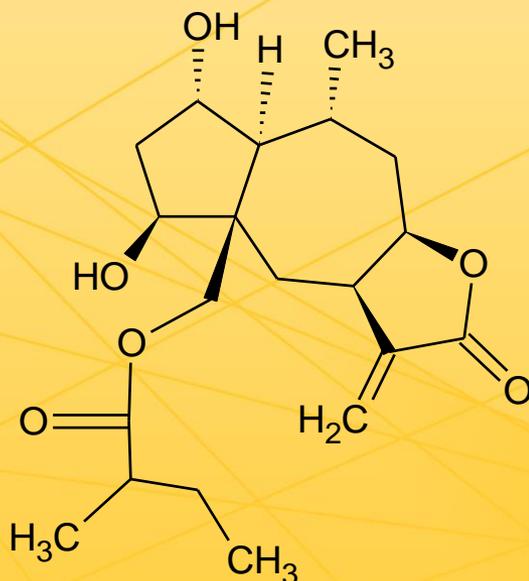
Separation Method by HPLC

- HFF051, HFF036, HFF037, HFF041, and HFF027 were injected into the analytical column (C18).
- Next the chromatograms were analyzed for potential compounds for isolation.
- Many compounds were isolated using the preparatory column (C18).
- Isolates were collected into sample vials. Of the many compounds, two known compounds were found using ^1H NMR spectral analysis.



Spectral Data

- The compound discovered from *Helenium flexuosum* is an un-named sesquiterpene lactone; a helenanolide, previously reported from the aerial parts of the plant *Gaillardia powelii*.¹²
- ¹H NMR
- ¹³C NMR
- ESI MS
- HMBC
- HSQC

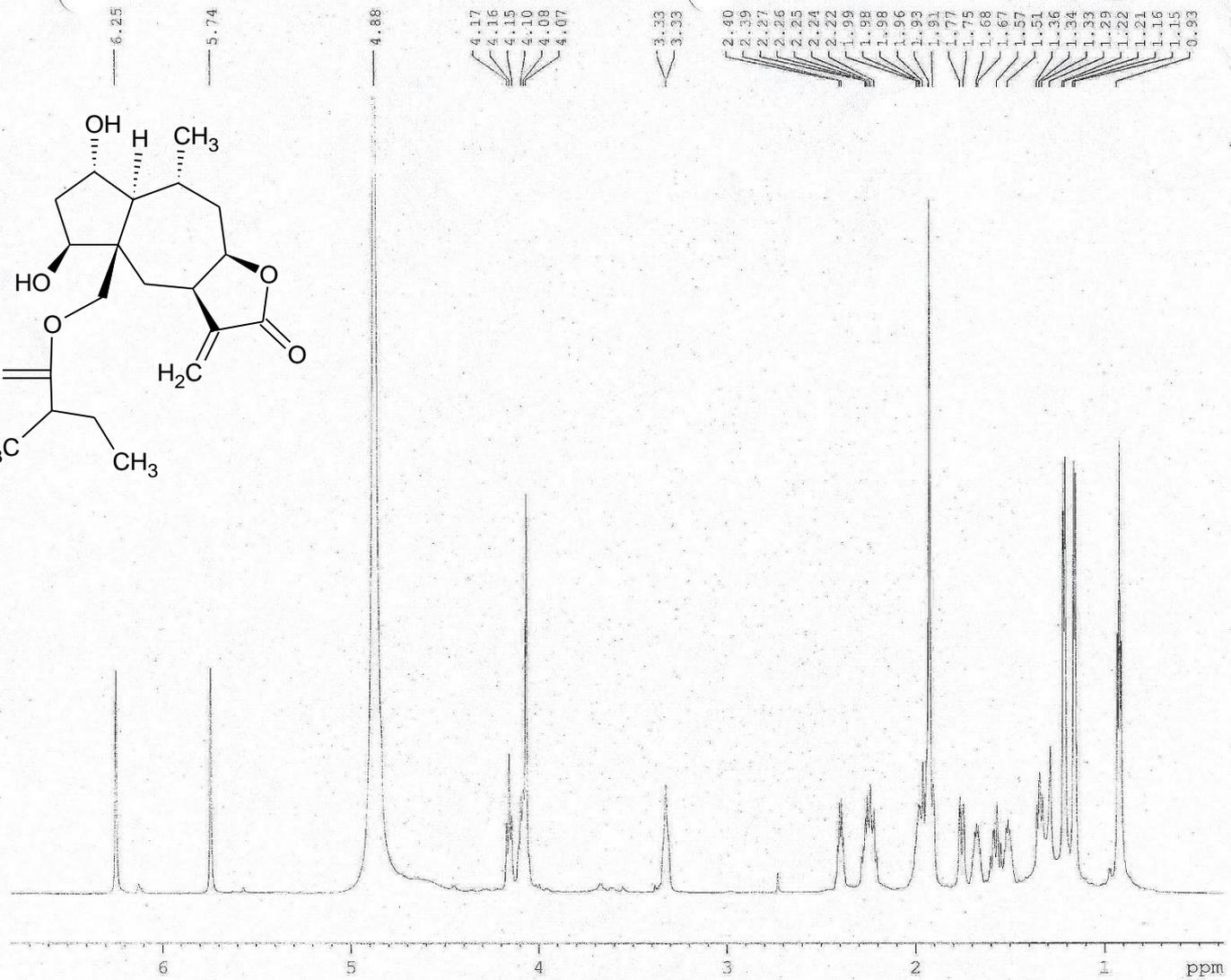
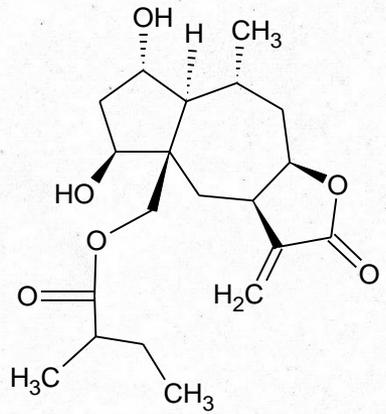


- 12. Herz, W., Bruno, M. Helenanolides from *Gaillardia Powellii*. *Phytochemistry*. Vol 26, No 1. (1987). pp 201-204.



HFF27B

27B H



4.17
4.16
4.15
4.10
4.08
4.07

3.33
3.33

2.90
2.39
2.27
2.26
2.25
2.21
2.22
1.99
1.98
1.98
1.96
1.93
1.91
1.77
1.75
1.68
1.67
1.57
1.51
1.36
1.34
1.33
1.23
1.22
1.21
1.18
1.15
0.93

6.25
5.74

1.000

1.000

1.177
3.166

1.565

1.144
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6.512

1.197

1.225

1.299

1.175

1.782

1.491

6.935

3.961

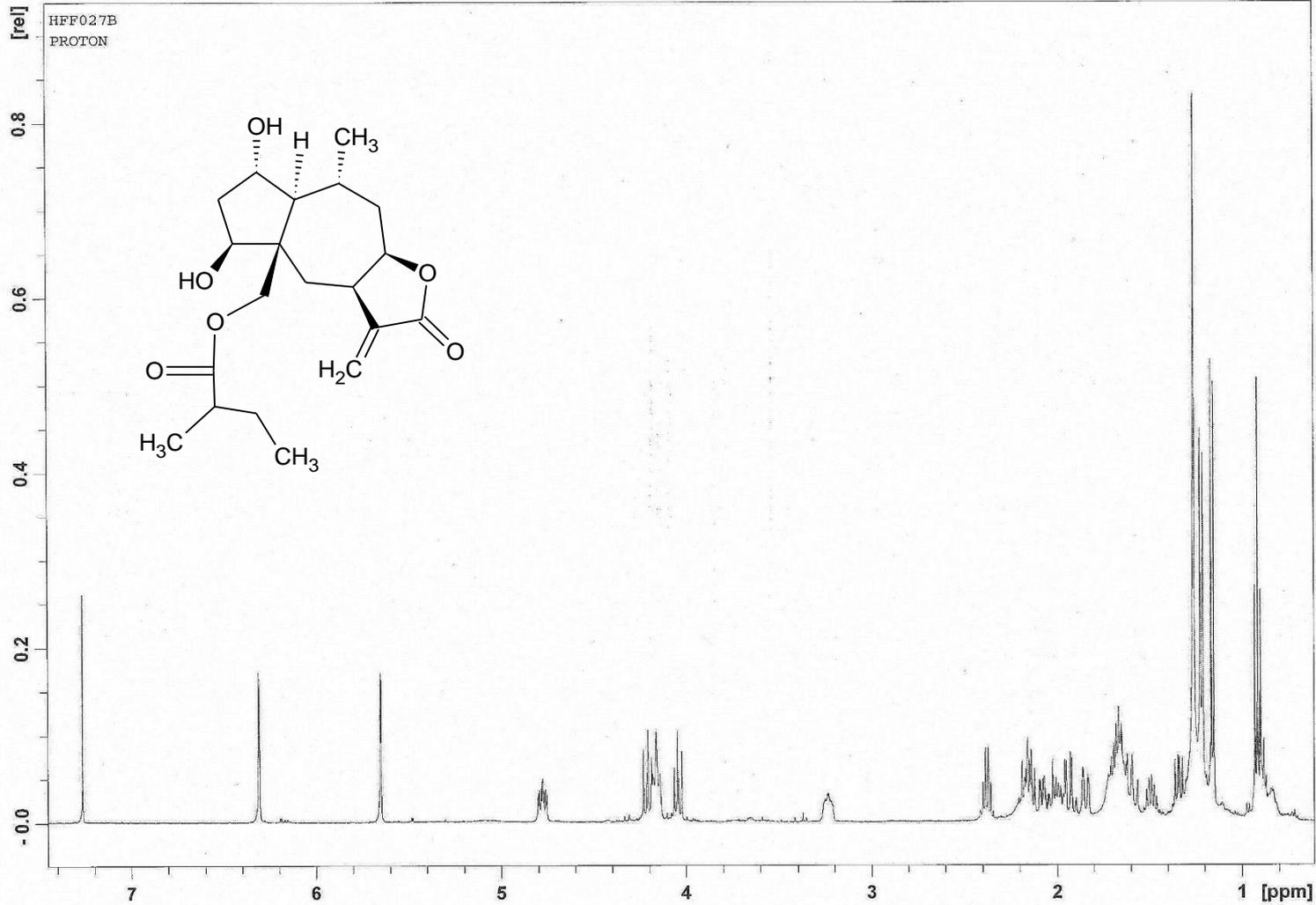
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FIDRES 0.127724 Hz
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TE 298.2 K
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TDO 1

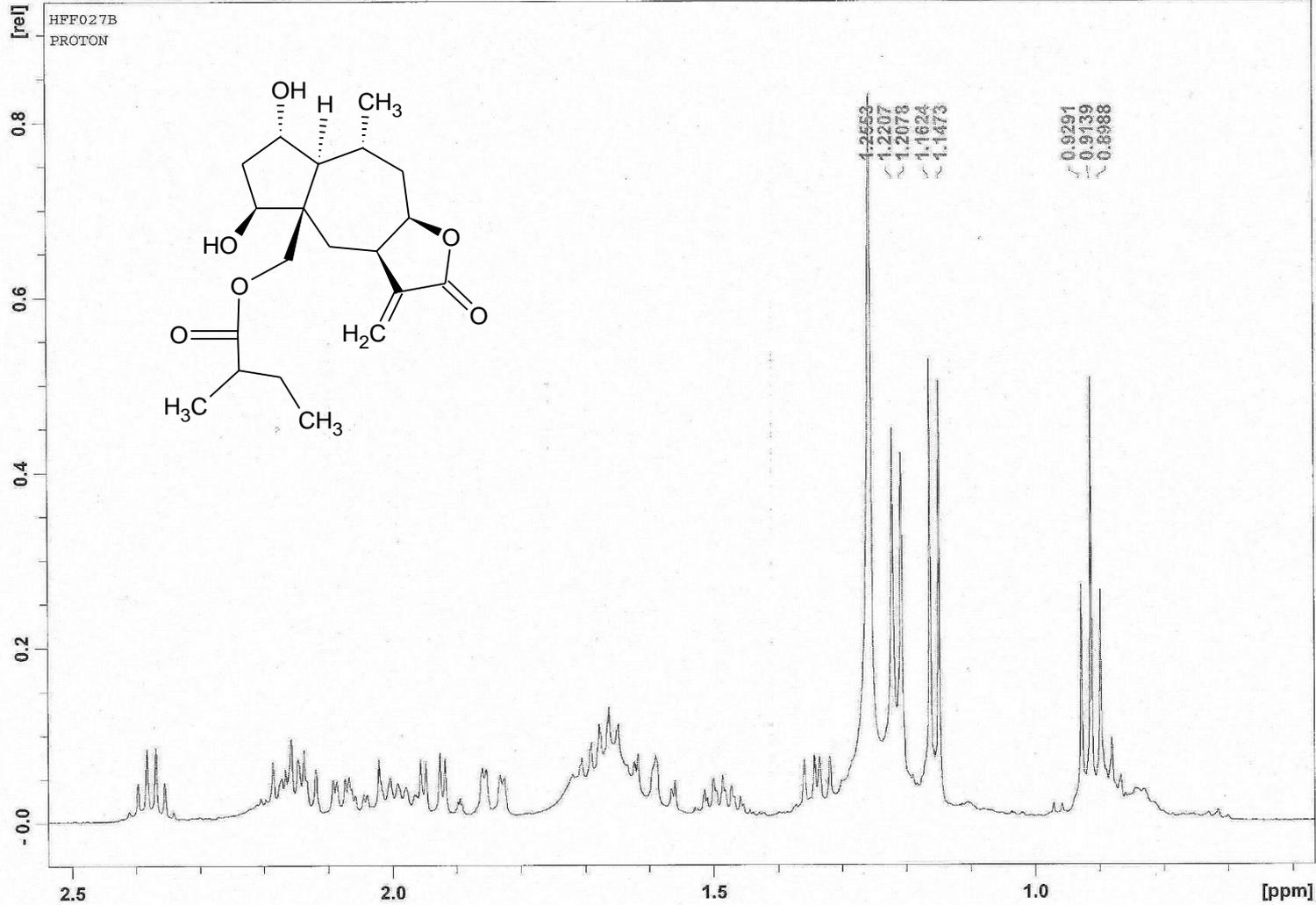
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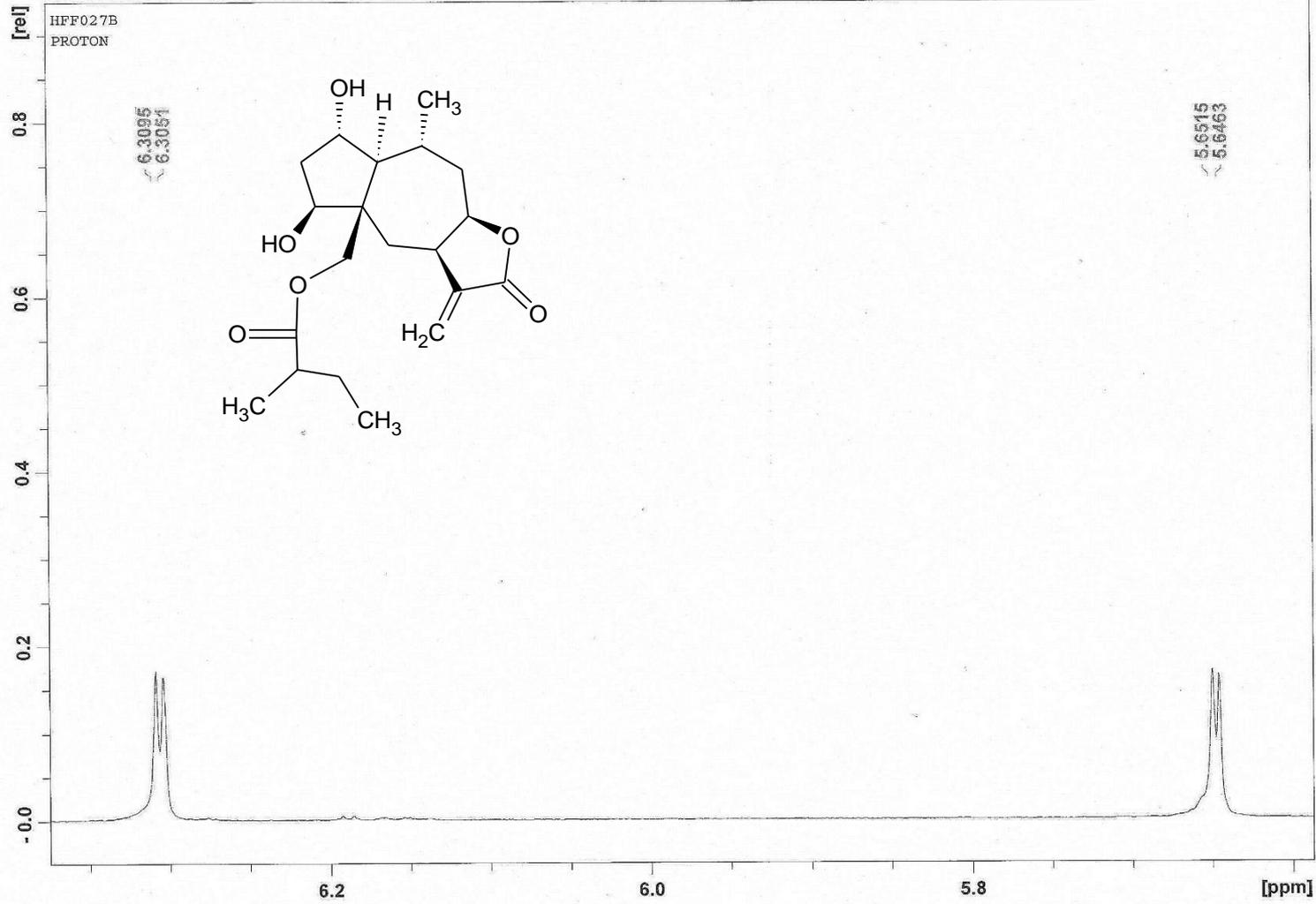
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Nov25-2015 7 1 c: umc

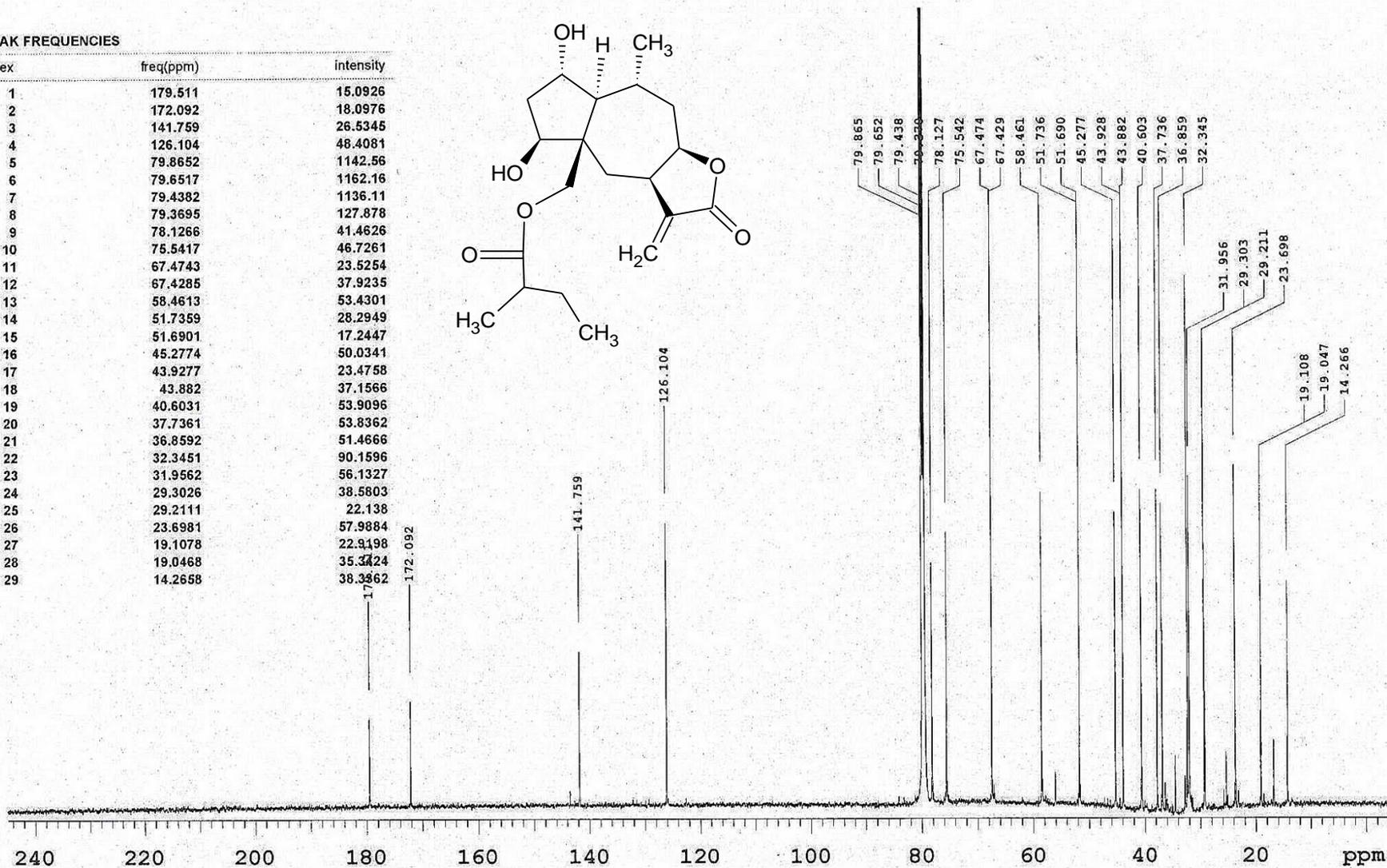
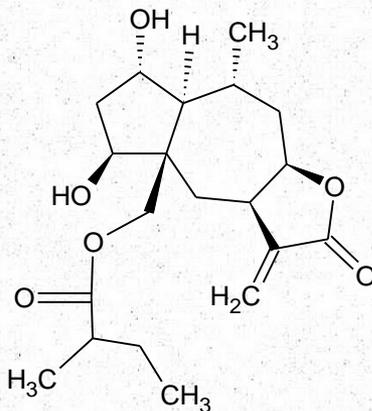


Nov25-2015 7 1 c: umc



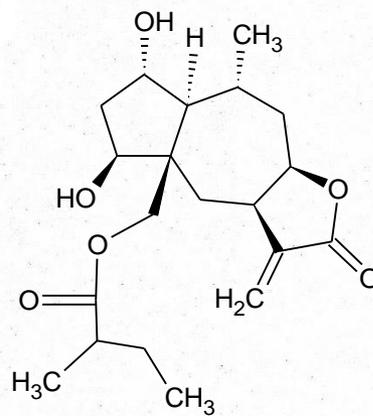
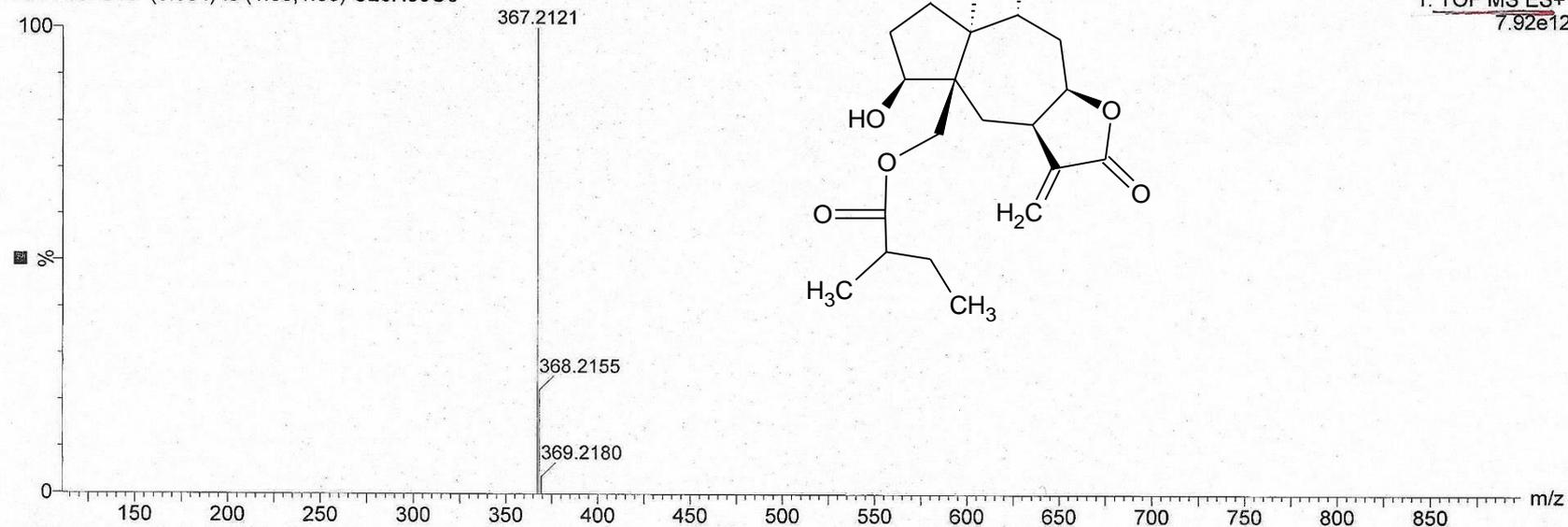
PEAK FREQUENCIES

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3	141.759	26.5345
4	126.104	48.4081
5	79.8652	1142.56
6	79.6517	1162.16
7	79.4382	1136.11
8	79.3695	127.878
9	78.1266	41.4626
10	75.5417	46.7261
11	67.4743	23.5254
12	67.4285	37.9235
13	58.4613	53.4301
14	51.7359	28.2949
15	51.6901	17.2447
16	45.2774	50.0341
17	43.9277	23.4758
18	43.882	37.1566
19	40.6031	53.9096
20	37.7361	53.8362
21	36.8592	51.4666
22	32.3451	90.1596
23	31.9562	56.1327
24	29.3026	38.5803
25	29.2111	22.138
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28	19.0468	35.3424
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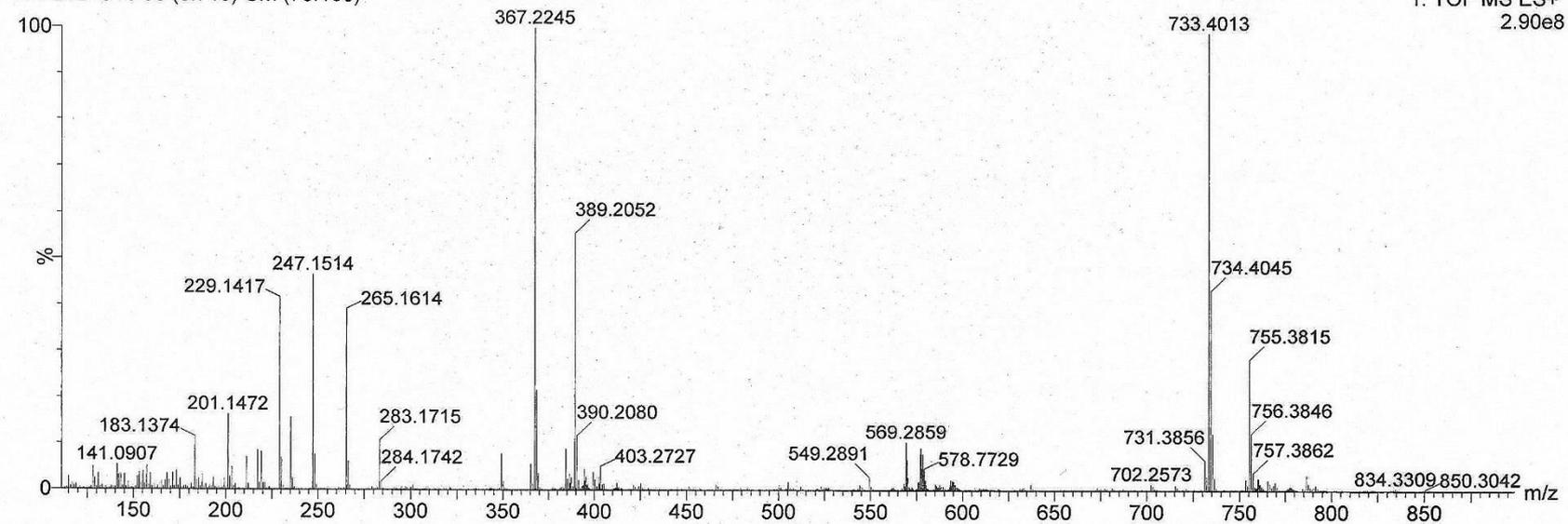
HPLC purified

HFF28B-C18 (0.054) Is (1.00,1.00) C₂₀H₃₀O₆

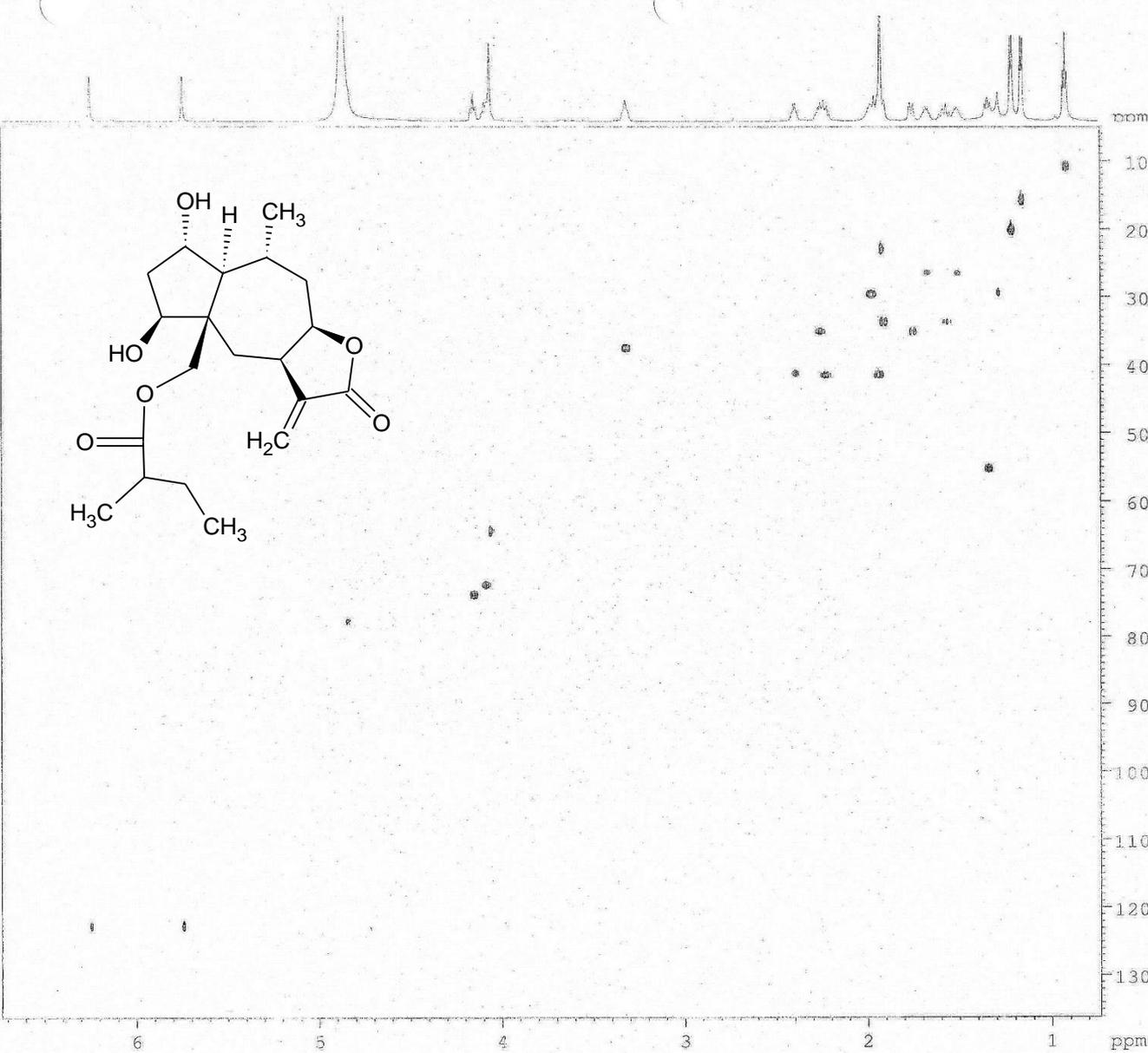


1: TOF MS ES+
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HFF28B-C18 98 (3.710) Cm (78:100)



1: TOF MS ES+
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TE        298.2 K
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CNS17    -0.500000
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P24      2000.00 usec
PE3      1500.00 usec
PLWC     0 W
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PLM12    4.66510010 W
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SFOFFS31 0 Hz
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GPNAM[2] SMSQ10.100
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CP22     20.10 %
CP23     11.00 %
CP24     -5.00 %
P18      1000.00 usec
P19      600.00 usec

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F2MODE    Echo-Antiecho

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WDW       QSINE
SSB       2
GB        0 Hz
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2710 HMBC

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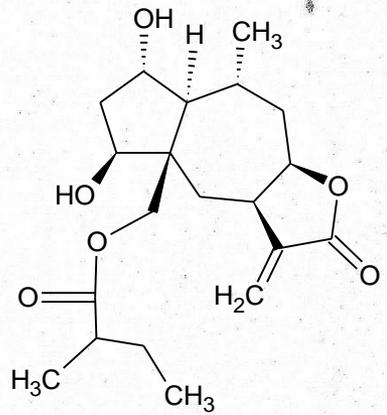
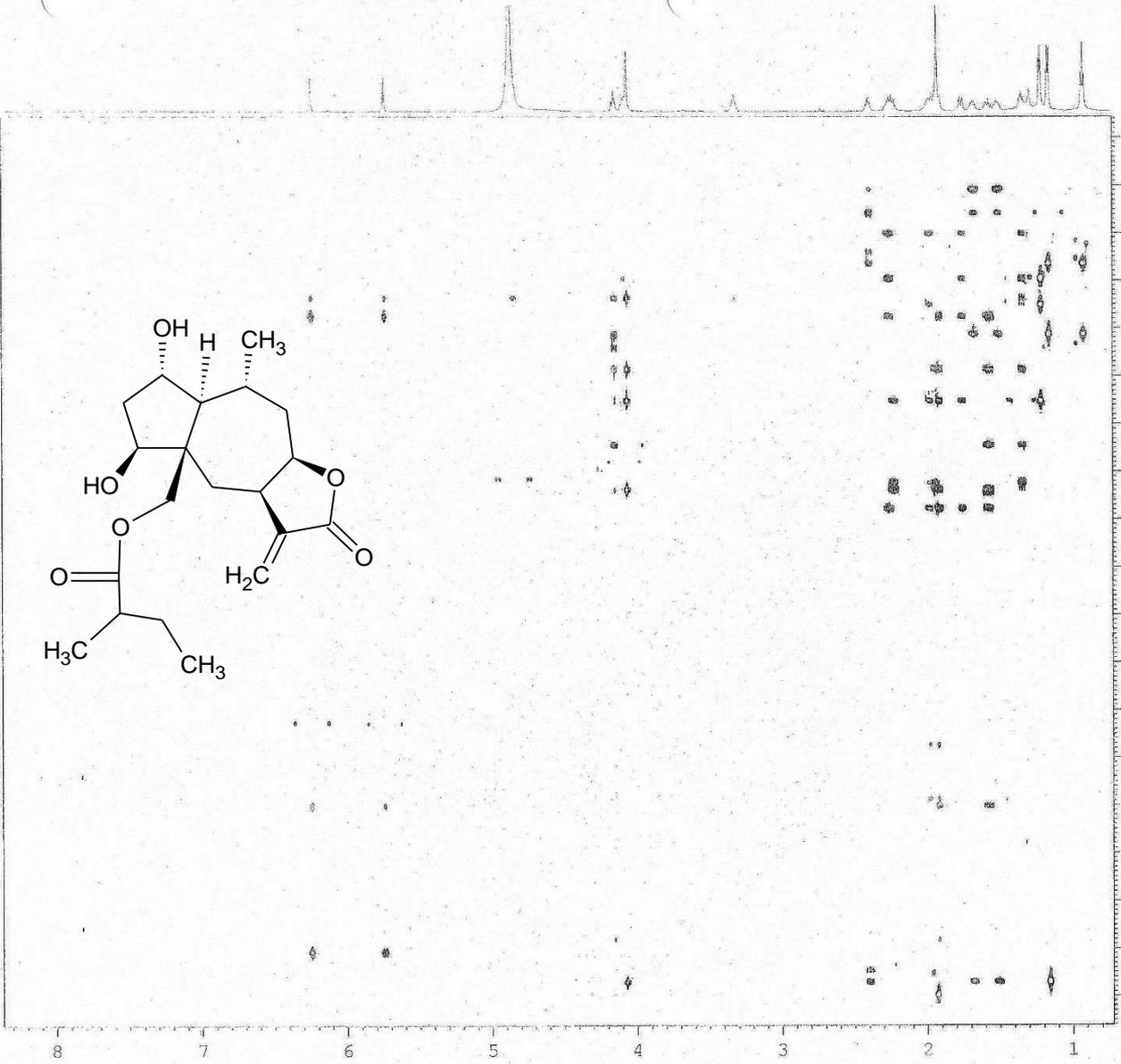
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GPZ3 40.10 %
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FAMODE QF

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SSB 0
LB 0 Hz
GB 0
PC 1.40

F1 - Processing parameters
SI 1024
VC2 QF
SF 176.0981190 MHz
WDW SINE
SSB 0
LB 0 Hz
GB 0



5

Conclusion

- Sesquiterpene lactones are ubiquitous, yet there is little research done on this class of compounds. Sesquiterpene lactones are most prevalent in the Asteraceae family; Asteraceae plants are in turn the most diverse and plant family in the world.
- Sesquiterpene lactones from Asteraceae play a highly significant role in human health. As pharmaceutical agents, sesquiterpene lactones have been found to sensitize tumor cells to conventional drug treatments.
- Clinical trials on sesquiterpene lactones have found these compounds to have high anti-carcinogenic potential, and high anti-inflammatory potential.⁸

- 8. Chadwick, M., Trewin, H., Gawthrop, F., & Wagstaff, C. (2013). Sesquiterpenoids Lactones: Benefits to Plants and People. *International Journal of Molecular Sciences*, 14(6), 12780–12805. <http://doi.org/10.3390/ijms140612780>



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