CHARLES E. SCHMIDT COLLEGE OF SCIENCE

New Briareolate esters isolated from *Briareum asbestinum*

Rian J. Meginley and Prasoon Gupta, Thomas C. Schulz, Allan J. Robins, and Lyndon M. West

Department of Chemistry and Biochemistry, Charles E. Schmidt College of Science, Florida Atlantic University, Boca Raton, FL

The gorgonian *Briareum asbestinum* is widely studied because it possesses highly oxygenated novel structures, many of which exhibit useful biological activities. Recently, two new briarane diterpenoids, briareolate esters J and K, together with two known briareolate esters have been isolated from a specimen of *Briareum asbestinum* collected off the coast of Boca Raton, Florida. The method used was a 96-well plate real-time cell electronic sensing (RT-CES) system to discover compounds that impact human embryonic stem cell growth. The compounds were isolated using reversed phase polystyrene divinylbenzene chromatographic support HP20ss followed by normal phased HPLC using a luna silica column. The structures of the compounds were established though the interpretation of spectroscopic data. Activity testing was conducted against hESCs (BG02) with briareolate ester J showing no inhibition activity and briareolate ester K showing mild activity with an EC_{50} value of 25μM. These results confirm that the exact confirmation and existence of the (*E,Z*)-dienone is related to the activity that was observed with the previously isolated briareolate esters L and M.

New briareolate esters from *Briareum asbesintum*

Rian J. Meginley[1], Prasoon Gupta[1], Thomas C. Schulz[2], Allan J. Robins[2], and Lyndon M. West[1]

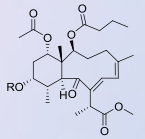
[1] Florida Atlantic University, 777 Glades Road, Boca Raton, Florida 33431

[2] Viacyte Inc., 111 Riverbend Road, Athens, Georgia 30602

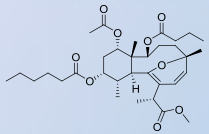
Introduction:

- *Briareum asbestinum.*
- Marine gorgonian.
- Posses highly oxygenated diterpenoids.
- Contains a unique group of briarane diterpenoids containing C-19 methyl esters.
- Numerous biological activities including cytotoxicity against hESCs (human embryonic stem cells) and anti-cancer.

- Previously, briareolate esters L, M and N were isolated from *B. asbestinum* using real-time cell electronic sensing (RT-CES) from initial cytotoxicity screening, followed by HPLC.
- Briareolate esters L and M containing an (*E,Z*) – dieneone were biologically active against hESCs (BG02) and pancreatic cancer (BxPC-3). Briareolate ester L exhibits EC_{50} values of 2.4 and 9.3 μ M against hESCs and pancreatic cancer respectively. Briareolate ester M exhibits an EC_{50} value against hESCs of 8.0 μM and 13.0 and 17.0 μM against pancreatic cancer.



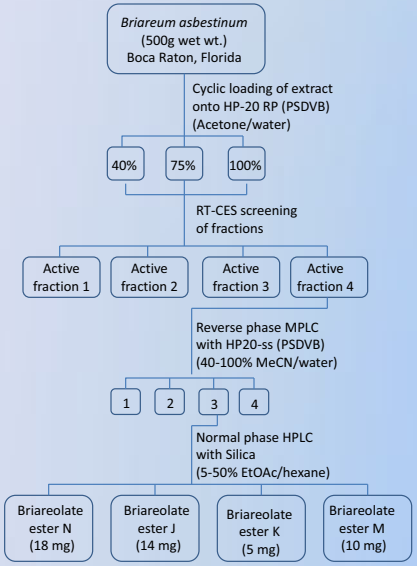
R = H (Briareolate ester L)
R = -CO(CH$_2$)$_4$CH$_3$ (Briareolate ester M)



Briareolate ester N

- The objective of this project was to identify compounds that are cytotoxic against hESCs using a RT-CES (real-time cell electronic sensing) system.

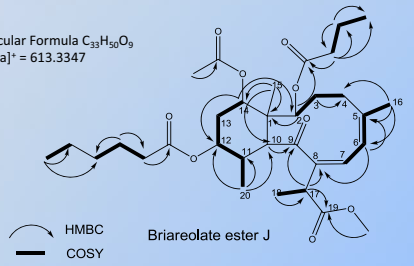
Isolation:



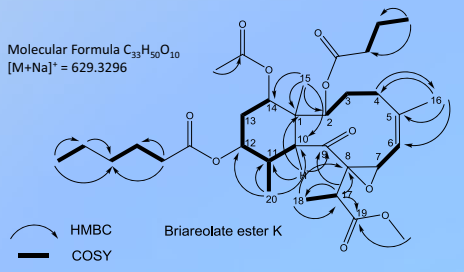
Structural Elucidation:

- HRESIMS was used to determine the molecular formulas of briareolate esters J and K.
- 1D and 2D NMR experiments were used to determine the connectivity and relative stereochemistry.

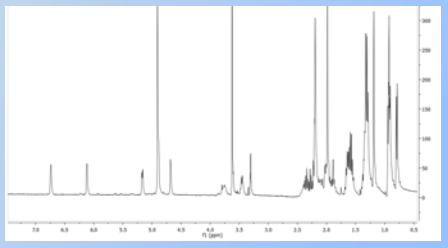
Molecular Formula $C_{33}H_{50}O_9$
$[M+Na]^+$ = 613.3347



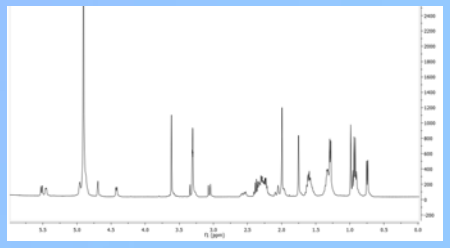
Molecular Formula $C_{33}H_{50}O_{10}$
$[M+Na]^+$ = 629.3296



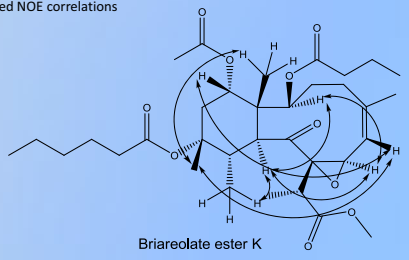
^{1}H NMR spectrum of briareolate ester J (400MHz in CD_3OD)



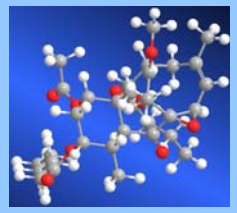
^{1}H NMR spectrum of briareolate ester K (400MHz in CD_3OD)



Selected NOE correlations

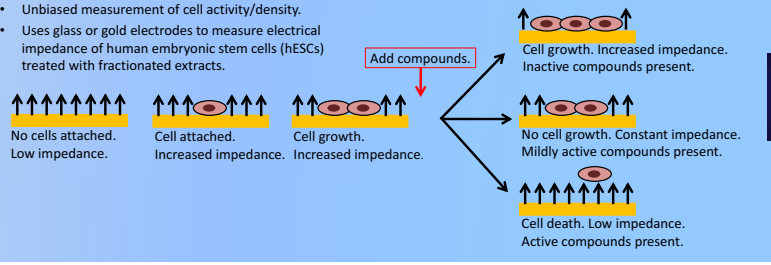


3D structure of briareolate ester K



Real-time cell electronic sensing (RT-CES) Bioassay

- Unbiased measurement of cell activity/density.
- Uses glass or gold electrodes to measure electrical impedance of human embryonic stem cells (hESCs) treated with fractionated extracts.



NMR Data Table:

^{1}H and ^{13}C NMR data for Briareolate esters J and K (400MHz in CD_3OD)

	Briareolate ester J		Briareolate ester K	
Position	δ ^{1}H	δ ^{13}C	δ ^{1}H	δ ^{13}C
1		46.4		48.8
2	5.17	73.7	5.51	80.5
3α	1.89	31.3	1.60	31.1
3β			1.29	
4α	2.20	30.2	2.28	34.1
4β	2.08		2.53	
5		146.3		147.1
6	6.12	125.3	5.47	116.2
7	6.74	140.9	4.42	64.3
8		146.3		71.3
9		207.0		211.5
10	3.79	48.3	3.05	44.7
11	2.19	38.4	2.34	36.2
12	4.89	73.7	4.96	72.62
13α	1.86	30.2	2.05	29.9
13β	2.05		1.99	
14	4.68	75.6	4.69	74.6
15	1.19	14.9	0.99	12.8
16	2.19	26.3	1.75	26.8
17	3.45	46.4	2.39	40.8
18	1.32	19.8	1.29	13.5
19		176.7		175.3
20	0.79	17.6	0.75	16.8
OMe	3.62	52.8	3.61	52.5
1′ C-2 ester		175.1		175.4
2′	2.19	37.7	2.38	37.9
3′	1.57	19.2	1.60	19.7
4′	0.92	14.5	0.93	14.5
1″ C-12 ester		175.1		174.3
2″	2.19	36.1	2.32	35.9
3″	1.63	26.3	1.64	26.4
4″	1.31	33.0	1.33	33.0
5″	1.31	24.0	1.33	24.0
6″	0.92	17.6	0.95	14.8
1‴ C-14 ester		172.1		172.6
2‴	1.98	22.1	1.99	22.3

Biological Activity:

- Briareolate ester J was found to be inactive against hESC (BR02 cell line).
- Briareolate ester K was found to be mildly active exhibiting an EC_{50} value 25 μM against hESC (BR02 cell line).

Conclusion:

- Two new briareolate esters J and K were isolated from a sample of *Briareum asbestinum* that was collected off the coast of Boca Raton, Florida.
- The bioactivity test results of briareolate ester K indicate that not only the (*E,Z*)-dieneone is required for activity.

References:

- Berrue, F.; Kerr, R. G. *Nat. Prod. Rep.* **2009**, *26*, 681-710.
- Gupta, P.; Sharma, U.; Schulz, T. C.; Sherrer, E. S.; McLean, A. B.; Robins, A.J.; West, L.M. *Org. Lett.* **2011**. *13*. 3920-3923.

Acknowledgments:

- This research was supported by the National Institutes of Health grants (P41GM079597 and P01GM085354).