

2<sup>nd</sup> July, 2013

### Smith degradation protocol

- (1) Oxidize 20g of Gum Arabic in 2000 ml of 0.1 M periodate (40 g) at 4°C in the dark for 7 days
- (2) Terminate the reaction by addition of 10 ml of ethylene glycol, and neutralize with 5 N NaOH to pH 7.0
- (3) Condense by evaporator, and then dialyze against water at 4°C for 2 days
- (4) Add 10 g of NaBH<sub>4</sub>, and keep at room temperature over night
- (5) Neutralize with 6 N acetic acid to pH 7.0, condense by evaporator (to app. 300 ml), then dialyze against water for 2 days
- (6) Add 100 ml of 4 N TFA (1/3 volume of sample, final 1 N) to hydrolyze at room temperature over night
- (7) Centrifuge at 9000 rpm, 10 min to remove insoluble compound
- (8) Precipitate the galactan by addition of 800 ml of ethanol (2 volume of sample), and collect the precipitate by centrifugation at 9000 rpm, 10 min
- (9) Wash with 70% ethanol (800 ml), then ethanol (800 ml) (in each wash, supernatant after centrifugation at 9000 rpm for 10 min should be removed)
- (10) Wash with acetone (800 ml), then petrol ether (800 ml) by suction filtration
- (11) Lyophilize in desiccator, about 7.4 g beta-galactan will be obtained.  
To get beta-1,3-galactan, at least thrice Smith degradation will be necessary.

### References

- Goldstein IJ, Hay GW, Lewis BA, Smith F** (1956) Controlled degradation of polysaccharides by periodate oxidation, reduction, and hydrolysis. *Methods Carbohydr Chem* **5**, 361-370.
- Tsumuraya Y, Hashimoto Y** (1987) An L-arabino-D-galactan and an L-arabino-D-galactan-containing proteoglycan from radish (*Raphanus sativus*) seeds. *Carbohydr Res* **161**, 113-126.