

# ***PGG Preparation from Tannic Acid***

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## ***Introduction***

Pentagalloyl glucose is easily prepared from appropriate preparations of tannic acid. The method described here is from two sources:

Hagerman, A.E.; Zhao, Y.; Johnson, S. Methods for Determination of Condensed and Hydrolyzable Tannins. In ***Antinutrients and Phytochemicals in Foods*** (Shahadi, F., ed.). American Chemical Society, Washington DC, 1997; pp. 209-222.

Chen, Y.; Hagerman, A.E. Characterizing soluble non-covalent complexes between bovine serum albumin and  $\beta$ -1,2,3,4,6-penta-*O*-galloyl-D-glucopyranose by MALDI-TOF mass spectrometry. ***Journal of Agricultural and Food Chemistry*** 2004 52, 4008-4011.

## ***Reagents***

- Acetate buffer (0.1 M) pH 5. Add 0.57 mL glacial acetic acid to 80 mL distilled water, titrate to pH 5 with NaOH solution, then bring volume to 100 mL with distilled water.
- Methanolysis solution: Mix 70 mL methanol with 30 mL of the acetate buffer.
- Ethyl acetate
- Diethyl ether (peroxide-free)
- Methanol
- 2% Methanol (2 mL methanol plus 98 mL distilled water) (cold)
- Cold distilled water

## ***Method***

1. In a test tube, dissolve 0.5 g tannic acid in 10 mL of the methanolysis solution (can scale this to as much as 25 g of tannic acid, using proportional amount of methanolysis solution). Cover, and place in 65°C water bath for 15h. (Can methanolyze longer with possible improvement in yield).

2. Immediately raise pH to 6, while stirring, with 0.25 M NaOH.
3. Evaporate under reduced pressure (rotary evaporate) at temperatures below 30°C to remove methanol. As the methanol is removed, add water to maintain volume. Do not let the sample go to dryness.
4. After removing all of the methanol, extract the aqueous solution three times with diethyl ether (in a small separatory funnel). The PGG should be in the aqueous (bottom) layer. Strip off traces of the ether by rotary evaporation.
5. Extract the aqueous layer three times with ethyl acetate. Combine the three ethyl acetate fractions (top layer) and rotary evaporate to reduce the volume. Add water to maintain the volume, and continue to evaporate to remove all of the organic solvent.
6. The PGG should now be visible as white/tan material suspended in the water. Continue to evaporate until you have a cloudy solution.
7. Centrifuge at 10,000 rpm for 15 min at 4°C. Carefully remove the supernatants to new tubes, and centrifuge repeatedly until you have collected all the solids you can recover.
8. Resuspend all the pellets in a minimal amount of 2% methanol. You can also recover any traces of solid from the round bottom flask with 2% methanol. Combine all the 2% methanol containing PGG in a single container.
9. (You can freeze this PGG in 2% methanol for one night at this step).
10. Warm the PGG in 2% methanol to 60°C in a water bath. All the PGG should dissolve.
11. Cool the samples back to room temperature. The PGG should again precipitate, and should be centrifuged as above. The supernatants should be recentrifuged until they are clear.
12. Combine all the pellets, and wash with a small volume of ice cold 2% methanol. Mix the solid with the cold solution, centrifuge and remove supernatant. You can repeat this washing with ice cold methanol up to 3 times to increase purity.
13. Suspend the pellet in a small volume of ice cold water. Transfer to the freeze drying flask and freeze dry. Store in glass vials at -20 in dessicator. Stable for years under these conditions.

## ***Analysis***

The starting material, and the various fractions at each step of the procedure, should be monitored by HPLC to verify that methanolysis occurred (loss of late eluting peaks, large increases in methyl gallate and PGG). The final product purity should be assessed by HPLC, mass spec and by proton nmr in deuterated acetone. The assignment of peaks in the nmr will confirm that the product is PGG, possibly contaminated with a trace of methyl gallate and gallic acid. Proton nmr (from TMS): Glucose C-1, 6.3 ppm (d, 1H); glucose C-2, C-4, 5.6 ppm (q, 2H); glucose C-3, 6.0 ppm (t, 1H); glucose C-5, 4.5 ppm (d, 1H); glucose C-6, 4.4 ppm (dd, 1H); galloyl group, between 6.9-7.2 ppm, 5 singlets (2H).