

Purification of Quebracho Tannin

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Purification of crude quebracho tannin to yield a condensed tannin (5-deoxyproanthocyanidin)-enriched fraction. Based on Asquith and Butler *J. Chem. Ecol.* 11: 1535-1544 (1985) and modified by Hagerman, 10/1/84.

Day 1

Suspend 1 g of quebracho in 10 mL of 80% ethanol (80 mL absolute etOH/20 mL water). Stir until solid no longer adheres to the bottom of the flask, then allow it to settle overnight without stirring in a refrigerator.

The Sephadex LH 20 is prepared in 80% ethanol instead of the usual 95-100% ethanol. Swell about 25 g of Sephadex in 80% ethanol to make 100 mL of slurry. Stir the beads gently with a stirring rod to facilitate moistening, then allow them to settle and decant off the supernatant and fines. Repeat this process three times. Store tightly capped in the refrigerator. This can be reused indefinitely if it is regenerated by completely washing away the acetone with 80% ethanol at the end of the purification process and storing tightly capped in the refrigerator. Before reusing old Sephadex LH20, several washes should be carried out with 80% ethanol as described above to remove fines and residual adsorbed materials.

Day 2

Filter the quebracho suspension through Whatman #40 (medium) filter paper, using a Buchner funnel and gentle vacuum if necessary. Add about 100 ml of a slurry of Sephadex LH20 in 80% ethanol to the filtrate, stir for 3 min with a stirring rod, then filter through a coarse sintered glass funnel. If the tannin is allowed to mix longer with the LH 20, brown tar which is difficult to remove from the Sephadex forms. Wash the LH20 with 95% ethanol until the absorbance at 280 nm starts to approach zero. It is best to wash fairly slowly, allowing the LH20 to equilibrate with the fresh ethanol at each step. Slow gravity filtration is better than vacuum filtration. The ethanol washes can be discarded. The final eluate should be colorless, and the Sephadex should be brown. Wash the Sephadex with 50% aqueous acetone (acetone:water, 1:1, v/v), combining and saving these washes. Continue to wash until the Sephadex is white and the eluate is nearly colorless. Refrigerate overnight (or continue with the next step if you have time to complete it).

Day 3

Rotary evaporate (evaporate under reduced pressure) the acetone washes to completely remove the acetone. It may also be desirable to reduce the volume of the aqueous sample, but do not bring the sample to dryness or concentrate it to the extent that material starts to precipitate. The temperature should be maintained at less than 30 C during the evaporation. The aqueous sample should be extracted three times with an equal volume of ethyl acetate, each time discarding the organic (upper) phase. After the third extraction, traces of ethyl acetate should be removed by rotary evaporation, again maintaining the temperature below 30C. The aqueous sample should be frozen and then lyophilized to yield a fluffy brown powder, which should be stored desiccated in a freezer. Yields are disappointingly small with this process, but the purified material is a much better standard than the crude preparation.

Commercial suppliers of quebracho (large quantities)

Trask Chemical Corporation
3200 W. Somerset Court
Marietta, Georgia 30067
(404)955-9190
[Cabeza de Caballo is on the bag we obtained]
(material from L. Butler is from Trask)

Tannin Corporation
60 Pulaski St.
P.O. Box 606
Peabody, MA 01960
(617)532-4010
(Charlie Robbins obtained quebracho from Tannin Corp.)

Small quantities of crude quebracho tannin can be obtained from
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