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# Incorporation of $^{14}C$ from [$^{14}C$]Phenylalanine into Condensed Tannin of Sorghum Grain

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A procedure is described for obtaining condensed tannin from sorghum [*Sorghum bicolor* (L.) Moench] seeds metabolically labeled from [$^{14}C$]phenylalanine. The [$^{14}C$]tannin should be useful in determining the metabolic fate of dietary condensed tannin.

The antinutritional effects of dietary polyphenols such as condensed tannins are usually ascribed to inhibition of digestion and absorption within the digestive tract (Cheeke and Shull, 1985). Some of these effects seem to require absorption of metabolically inhibitory polyphenols from the digestive tract, and preliminary evidence for such absorption was obtained by feeding sorghum tannin labeled with $^{125}I$ (Butler et al., 1986).

More definitive evidence on the question of absorption of dietary polyphenols from the digestive tract could be obtained with polyphenols metabolically labeled with $^{14}C$. Condensed tannins such as those found in the grain of some cultivars of *Sorghum bicolor* (L.) Moench are polymeric flavonoids (Haslam, 1981). Phenylalanine is a metabolic precursor for 9 of the 15 carbon atoms of its flavonoid units (Haslam et al., 1977). Here we report a procedure for obtaining condensed tannins metabolically labeled from [$^{14}C$]phenylalanine supplied to developing panicles of sorghum cultivars whose mature seed is relatively rich in tannins. The [$^{14}C$]tannin should be useful in determining the metabolic fate of condensed tannin in foods and feedstuffs.

## MATERIALS AND METHODS

Uniformly labeled [$^{14}C$]phenylalanine (specific activity 410 mCi/mmol) in 2% ethanol was obtained from ICN Biomedicals, Inc., Irvine, CA. Sorghum cultivars IS 8768 (primary panicles) and IS 6881 (panicles from tillers) were field-grown open pollinated plants from the Purdue Agronomy Farm, West Lafayette, IN. IS 8768 is a group II type with respect to tannin, and IS 6881 is a group III type (Price et al., 1978).

At 4–5 days after half-anthesis, panicles were cut, leaving stems at least 12 in. long, and were rapidly transported to the laboratory with the stems immersed in water. The stems were cut under water at approximately 45° angle (to increase the contact area) about 4 in. below the base of the panicle and were immediately inserted into 16 mm × 100 mm test tubes containing 5 μCi of [$^{14}C$]phenylalanine in 5 mL of water. The panciles in their test tubes were placed in a rack inside a plastic bag, and air was pulled through the bag and then through a trap containing 1 M KOH to trap respired $CO_2$. Under these conditions (room temperature, continuous air flow) the panicles imbibed the aqueous solution of [$^{14}C$]phenylalanine at an average rate of approximately 1 mL/h. When the level of liquid in the test tube diminished to near the cut surface of the stem, additional water, without phenylalanine, was added. When the panicles stopped imbibing water (usually 50–55 h; 30–50 mL of water was taken up per panicle), they were dried at room temperature for at least 2 days to permit tannin biosynthesis to go as far as possible toward completion. We have previously shown that polymerization of sorghum seed tannins occurs mainly during drying of the seed (Butler, 1982).

For extraction of the tannins, seeds and associated glumes (approximately 30 g from two dry panicles) were soaked for 12 h at room temperature in methanol containing 1 mM ascorbate and 1% (v/v) concentrated HCl, 15 mL/g of dried tissue. The solvent was removed by filtration, an equal volume of 1% HCl in methanol was added, and the tissue was homogenized on a Polytron homogenizer (Brinkmann Instruments). The acidic

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Table I. Distribution of $^{14}C$

| fraction | dpm × $10^{-3}$ |
|---|---|
| not imbibed | 85.4 |
| KOH ($CO_2$ trap) | 9800 |
| stems | 9920 |
| seed residue [extracted] | 176 |
| ethyl acetate | 565 |
| ethanol [batchwise] | 424 |
| ethanol [column] | 45 |
| 60% acetone (tannin) | 51 |
| 80% acetone (tannin) | 54 |
| total dpm supplied | 22000 |

methanol was removed by filtration (and the residue was reextracted with fresh solvent until the extract was colorless.

Tannin was purified from the combined extracts by a modification of the method of Hagerman and Butler (1980). Solvent was removed by rotary evaporation, and the residue was dissolved in 150 mL of 10 mM acetate buffer, pH 4. The aqueous solution was extracted twice with equal volumes of ethyl acetate. The resulting aqueous layer was mixed with four volumes of Sephadex LH-20 suspended in absolute ethanol, to absorb tannin and other phenolic materials. The LH-20 was washed with water until the eluant was colorless and then washed sequentially with approximately 400 mL each of absolute ethanol, 60% aqueous acetone, and 80% aqueous acetone. After rotary evaporation to remove acetone from the combined aqueous acetone fractions, a minimum volume of ethanol was added to dissolve any insoluble material. The solution was applied to a 4 × 30 cm column of sephadex LH-20 equilibrated with ethanol and washed with ethanol until the eluant was colorless. Purified [$^{14}C$]tannin was then eluted with 60 and 80% aqueous acetone. Acetone was removed from the pooled aqueous acetone eluates by rotary evaporation; lyophilization of the water then yielded [$^{14}C$]tannin in the form of a brown powder.

## RESULTS

The yield of tannin by chemical assay (Price et al., 1978) from sorghum panicles collected 5 days after half-anthesis was at least 15% greater than that from earlier or later stages of development so this stage was utilized for $^{14}C$ labeling. Under the conditions described, approximately 99% of the $^{14}C$ was taken up by the panicles (Table I). Addition of 2% sucrose to the phenylalanine solution increased the amount of material eluting in ethanol but did not increase the yield of [$^{14}C$]tannin.

This labeling and purification procedure was carried out a total of six times with similar results each time. The data presented in Table I are the average of the last two isolations. Almost half of the $^{14}C$ was respired and almost the same amount was left in the nonseed portion of the panicles. About 0.5% of the $^{14}C$ supplied as [$^{14}C$]-phenylalanine was obtained as [$^{14}C$]tannin. The ethyl acetate and ethanol fractions may be of interest as a source of [$^{14}C$]-labeled flavonoids.

The purified [$^{14}C$]tannin obtained by this procedure did not move from the origin on TLC with silica gel plates with 60:60:10 (v/v) toluene–acetone–formic acid solvent (Lea, 1978); previously purified samples of sorghum tannin (Hagerman and Butler, 1980) gave the same result. Under these conditions, components of the ethyl acetate and ethanol fractions did migrate as expected for lower molecular weight flavonoids. In a protein precipitation assay with bovine serum albumin (Hagerman and Butler, 1978), 100% of the $^{14}C$ was found to be protein precipitable. By these tests the [$^{14}C$]tannin appears to be functionally equivalent to previously prepared nonradioactive samples of sorghum tannin (Hagerman and Butler, 1980).

This [$^{14}C$]tannin was prepared for in vivo feeding trials to trace its metabolic fate when consumed, but it could also be useful in other types of experiments.

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