ball. The wheat protein concentrate and the commercial vital gluten formed a reasonably strong and elastic gluten ball but not quite so good as that from laboratory NE 701136 gluten. The devitalized gluten could not form a gluten ball. Vital gluten (ability to form a gluten ball) is preferred for baked goods. Since the protein concentrate is about equal to the commercial vital gluten in gluten ball forming ability, likely no appreciable denaturation of the protein resulted from alkaline extraction, and the concentrate can be used in baked goods.

Potential Uses of Protein Concentrate and By-Products. Wheat protein concentrate may find application in conventional wheat foods to improve protein levels in the final product. Other possible uses of this protein concentrate include meat extenders and protein fortification of beverages. Currently, wheat starch has acceptable outlets. Further research will be necessary to evaluate the products from wheat for different food and industrial applications.

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Condensed Tannins in Grain Sorghum: Isolation, Fractionation, and Characterization

David H. Strumeyer* and Michael J. Malin[1]

A new procedure was developed for the isolation of condensed tannins. Separation from non-tannins was based upon the finding that tannins were adsorbed to Sephadex LH-20 in 95% ethanol. Following exhaustive washing with ethanol, the tannins were eluted with 50% aqueous acetone. By applying this procedure, highly purified condensed tannins were isolated from grain sorghum. Tannins from Leoti red sorghum and Georgia 615 were further fractionated by gel filtration on Sephadex LH-20 using 50% aqueous acetone. The tannins from both strains were shown to consist of a series of polymeric polyphenols which upon acid hydrolysis generated cyanidin, exclusively. Hydrolyzable tannins were apparently absent since neither glucose nor other sugars were found after hydrolysis.

The hydrolyzable and condensed tannins are two groups of polyphenols widely distributed in the plant kingdom which may be differentiated by their structure and reactivity toward hydrolytic agents. The hydrolyzable tannins are readily cleaved by enzymes as well as by dilute acids, into a sugar such as glucose, and a phenolcarboxylic acid such as gallic acid. In contrast, the condensed tannins are resistant to enzymatic degradation. Upon acid treatment, this group of polyphenolics not only decomposes with the liberation of a small amount of anthocyanidins, but also progressively polymerizes to yield amorphous phlobaphens or tannin reds (Haslam, 1966; Ribereau-Gayon, 1972).

The tannins are characterized by their ability to interact with and precipitate proteins such as gelatin. They appear to be responsible for the astringency of many plant materials and decrease nutritive value when added to the diet or when found naturally in high levels in certain foodstuffs. The presence of tannins in grain cereals is relatively rare, occurring only in selected strains of sorghum and barley. Although the toxic properties of tannins in sorghum have been well documented (Chang and Fuller, 1964; Fuller et al., 1966; McGinty, 1969; Harris et al., 1970), there have been relatively few characterizations of these tannins. Several types of anthocyanidin-generating compounds have been identified in different varieties of sorghum. Blessin et al. (1963) used a procedure based on the adsorption on a strongly basic ion-exchange resin and extracted profisetinidin as the main pigment in Martin sorghum. (The prefix pro is used rather than leuco to designate those colored, polymeric flavonoids which generate anthocyanidins when heated with hot acid (Freudenberg and Weinges, 1960).) In a further report of the chemical nature of the pigment of sorghum, Yasumatsu et al. (1965) obtained propelargonidin by using methanol extracts of seed coats of commercial sorghum. However, neither the profisetinidin nor the propelargonidin was tested for tanning properties.

Bate-Smith and Rasper (1969) reported that the principal tannin of sorghum, based upon their work with the Kafir variety, was a proanthocyanin which, when heated with mineral acid, yielded the very uncommon luteolindin. They also found procyanidin in a rose-brown variety of the grain but not in the red grains examined.

Many of the usual methods for the isolation of tannins depend on the differential solubility of tannins in various solvents (Roux, 1953; Roux and Maihs, 1960; Haslam,

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1966) while fractionation of condensed tannins has been achieved in the past several years by use of polydextran gels and aqueous alcohol or aqueous acetone systems. Gel filtration has been used successfully for the fractionation of tannins from apple peel (Durkee and Jones, 1969), oak leaf (Feeny and Bostock, 1968), tea (Lewak, 1968), carobpod (Tamir et al., 1971), *Pinus radiata* (Porter and Wilson, 1972), as well as from sorghum (Strumeyer and Malin, 1969).

In the course of a study with tannins from sorghum, we observed that the tannins were strongly adsorbed to polydextran gels. In this paper we describe a new procedure for the isolation of tannins using Sephadex LH-20 as adsorbant and characterize the tannins present in two varieties of grain sorghum, Leoti red and Georgia 615.

EXPERIMENTAL SECTION

Materials. Leoti Red Cane sorghum seeds, purchased from the Bowman Seed Co., Concordia, Kan., were fractionated by a wet milling process at the Ohio Agricultural Research and Development Center of the USDA, Wooster, Ohio. The "shorts over 40W" were powdered on a Wiley Mill using a no. 60 mesh. Georgia 615 sorghum seeds were obtained from Prichard Seed Farms, Louisville, Ga.

Cyanidin chloride, catechin, and other flavonoids were the products of K&K Laboratories Inc., Plainfield, N.Y. Other reagents were of the best commercial grade available.

Chromatography. Paper chromatograms were developed by the ascending technique at room temperature using either Whatman no. 1 or 3MM paper (40 × 40 cm). Thin-layer plates (18 × 18 cm) were prepared with either silica gel G or H (Merck) according to the method of Randerath (1966). Ascending chromatograms were developed in glass tanks lined with filter paper and saturated with solvent. For column chromatography, Sephadex gels were allowed to swell in the appropriate solvent for a minimum of 5 hr. Sephadex columns were calibrated with blue dextran (V_0) and *p*-nitrophenol (V_i). Flow rates of eluting solvents were maintained constant by means of a Mariotte flask. Fractions were collected in a Buchler fraction collector.

Detection Reagents. After development of the chromatograms, anthocyanidins could be detected without a spray reagent because of their easily observable color. Their location was confirmed by exposing the paper to ammonia fumes which causes the color to change. Cyanidin appears as a vivid red spot which turns blue upon exposure to ammonia. The following reagents were employed as detection spray reagents in conjunction with thin-layer chromatography (TLC): 50% sulfuric acid for detection of organic compounds (Randerath, 1966); 5% phosphomolybdic acid for phenols (Stahl and Schorn, 1961); ninhydrin for amines and amino acids (Clark, 1964); 3% *p*-toluenesulfonic acid (TSA) for proanthocyanidins and catechin; bisdiazotized benzidene (BDB) for phenols (Roux and Maihs, 1960); 2,6-dichlorobenzoquinone chloroimide (Gibb's reagent) for phenols (Haslam, 1966); ultraviolet light following exposure to ammonia for flavonoids (Britton and Haslam, 1965); aniline–phthalate for sugars (Block et al., 1958).

Spectrophotometry. The absorbance of column chromatographic fractions was ordinarily determined with a Beckman DU spectrophotometer using 1-cm cuvets. Occasionally the absorbance of the column effluent was continuously monitored by passage through a flow cell housed in a Beckman DB recording spectrophotometer. All spectra were obtained with the Beckman DB recording spectrophotometer using 1-cm cuvets. For anthocyanidin spectra, the instrument was calibrated in the 500–550 nm region with potassium permanganate (Cooper, 1953).

Chemical Analysis. Elemental analysis was performed by Dr. R. Rittner, New Haven, Conn.

Enzymatic Methods. The enzymatic methods involved in this study have been described elsewhere (Strumeyer and Malin, 1969).

Extraction of the Tannins from Leoti Sorghum. The powdered Leoti fraction (100 g) was defatted by shaking with peroxide-free ether (3 × 125 ml). The residue (89 g) was then extracted with 95% ethanol (2 × 125 ml) for 1 hr at 25° in the dark. Evaporation of the solvent under reduced pressure yielded 6.8 g of solid material. Two-dimensional TLC on silica gel H was conducted with *n*-hexane–ether–acetic acid (80:20:1) and acetone–water (9:1) referred to as HEA and AW, respectively. Visualization by charring with sulfuric acid revealed five mobile spots and one spot at the origin.

Isolation of Tannins by Adsorption on Sephadex LH-20. A sample of the dried extract, dissolved in 95% ethanol (110 mg/0.5 ml), was applied to a column of Sephadex LH-20 (2.2 × 27 cm) equilibrated with 95% ethanol. The column was eluted with ethanol (400 ml) at a flow rate of 24 ml/hr and 2.0-ml fractions were collected. The absorbance at 280 nm was determined with a Beckman DU spectrophotometer. The column was then eluted with 50% acetone–water (200 ml) at a flow rate of 30 ml/hr. Fractions of 3.0 ml were collected and the absorbance at 400 nm was determined. The fractions were assayed for tannins by the gelatin precipitation test and by a microanalytical method involving the inhibition of amylase activity (Strumeyer and Malin, 1969). Only the acetone eluate contained compounds with tannin-like properties. The purified tannin was examined by TLC on silica gel-H as described in the preceding paragraph. Only one spot at the origin was detected with each solvent.

Chromatography on Sephadex G-100. A sample of tannin from the acetone fraction (25 mg/10 ml in 33% ethanol) was applied to a column of Sephadex G-100 (2.5 × 35 cm) previously equilibrated with 33% ethanol. The column was eluted at a flow rate of 8.3 ml/hr and 6.4-ml fractions were collected. The elution pattern, produced by monitoring the effluent at 500 nm, was continuously determined by passage through a flow cell in a Beckman DB spectrophotometer, the output of which was coupled to a recorder. The fractions were assayed for tanning properties as described above.

Fractionation of Purified Tannin on Sephadex LH-20. A sample of tannin from the acetone fraction (300 mg/3.0 ml of 50% acetone) was applied to a column of Sephadex LH-20 (3.5 × 43 cm) which had been equilibrated with 50% acetone. The column was eluted at a flow rate of 38 ml/hr and 2.6-ml fractions were collected. The absorbance at 540 nm was then determined for each fraction. Samples obtained from fractions under each peak were tested for their ability to form a precipitate when added dropwise to 1% gelatin.

Extraction of Tannin from Georgia 615 Sorghum. The seeds (2.5 kg) were powdered in a Waring Blendor and extracted at room temperature with petroleum ether (bp 60–80°, 2 × 5 l.), ethyl acetate (2 × 5 l.), acetone (2 × 5 l.), and ethanol (4 × 5 l.). The ethanol extract was filtered and concentrated under reduced pressure to a volume of 3 l. Ether (3 l.) was then added giving a precipitate (13.2 g) of tannins representing 0.52% of the powdered seed.

Chromatography of Georgia 615 Sorghum Tannins on Sephadex LH-20. A sample of Georgia 615 tannin (0.550 g/10 ml of 50% acetone) was applied to a column of Sephadex LH-20 (7.1 × 55 cm) equilibrated in 50% acetone. The column was eluted and 18-ml fractions were collected. The effluent was continuously monitored by following the absorbance at 460 nm. Each of the resulting components yielded precipitates when added dropwise to 1% gelatin. Fraction B contained 165 mg or 30% of the sample applied to the column.

Non-Tannin Component of Leoti Sorghum Ethanol

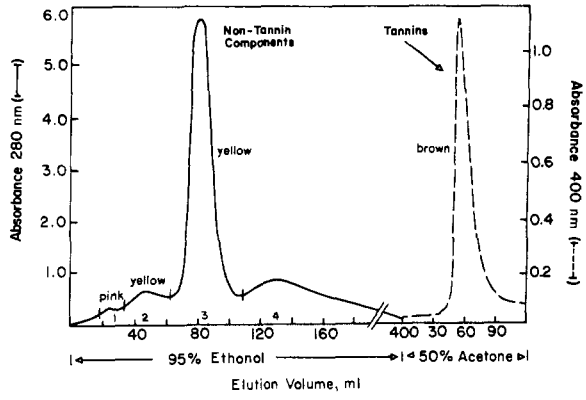

Figure 1. Separation of tannins from non-tannins by adsorption on Sephadex LH-20, and elution with 95% ethanol followed by 50% acetone.

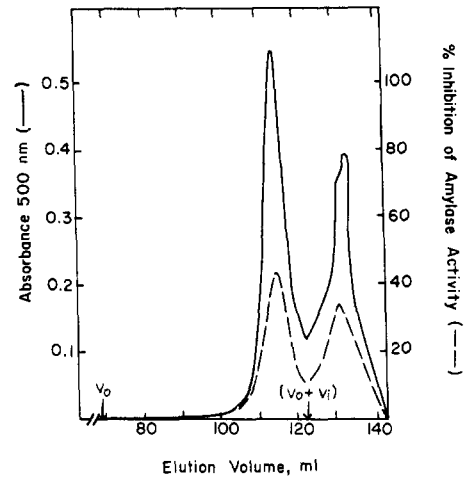

Figure 2. Fractionation of purified Leoti tannin by gel filtration on Sephadex G-100 using 33% ethanol.

Extract. The non-tannin components resulting from Sephadex LH-20 chromatography were examined for the presence of phenolic compounds by TLC analysis and exposure to a variety of spray reagents. Solvent systems used to develop the chromatograms were as follows: (1) benzene–methanol–acetic acid (45:8:4), chloroform–ethyl acetate–formic acid (5:4:1), and acetone–water (9:1).

Degradation of Sorghum Tannins with Alcoholic HCl. Samples of the tannin (40 mg) from both Leoti and Georgia 615 were each dissolved in 32 ml of propan-2-ol–water (1:1) and 2 ml of 3 *N* HCl was then added. The final concentration of HCl was 0.17 *N*. The system was refluxed at 100° for 30 min. During this time, the original amber color changed to scarlet red. After cooling, the reaction mixture was extracted with 120 ml of ethyl acetate (organic phase). The aqueous phase was washed with 10-ml portions of *n*-amyl alcohol–benzene (1:1), benzene, and ether, and evaporated to dryness under reduced pressure, and the residue dissolved in 0.1 ml of water (washed aqueous phase).

Chromatography of Acid-Hydrolyzed Tannin. (a) *Organic Phase.* The red ethyl acetate extract was evaporated to dryness under reduced pressure and the residue dissolved in 0.5 ml of methanol, streaked on Whatman no. 3MM paper, and developed with acetic acid–HCl–water (30:3:10) (Forestal solvent) along with a marker of authentic cyanidin. The red zone with R_f 0.50 was cut out, eluted with 50 ml of methanol–0.01% HCl, and filtered. The eluate was then concentrated under reduced pressure and rechromatographed in the Forestal system. After elution and evaporation to dryness, the residue was dissolved in ethanol–0.01% HCl. As a control, authentic cyanidin was treated in an identical fashion.

To confirm the identification of the red pigment, samples of the red extract along with authentic cyanidin were chromatographed on Whatman no. 1 paper using the Forestal, formic acid–HCl–water (5:2:3) and *m*-cresol–5.5 *N* HCl–water (1:1:1) systems and with authentic catechin using the Forestal and 1-butanol–acetic acid–water (4:1:5) (BAW) systems.

(b) *Aqueous Phase.* Samples of the washed concentrated aqueous phase were spotted on Whatman no. 1 paper along with glucose, galactose, and ribose (5 μl of 1% solutions). The chromatograms were developed with BAW and 1-butanol–95% ethanol–water (4:2:2.2) solvent systems. After drying, the papers were sprayed with the aniline–phthalate reagent.

The absorption spectrum of the ethanolic HCl solutions of the reaction product and of authentic cyanidin was determined with the recording spectrophotometer. Shifts in the spectrum were recorded after adding 6 drops of $AlCl_3$ (2.5% in 50% ethanol) to the sample cuvette (Geissman et al., 1953).

RESULTS

Separation of Tannins from Non-Tannins. The red-brown ethanol extract of the defatted Leoti sorghum fraction contained numerous mobile phenolic compounds when examined by thin-layer chromatography and visualized by selective spray reagents. On the other hand, the tannin phenolics remained at or near the origin in all the usual solvent systems. For the isolation and purification of the tannin fractions from Leoti sorghum, a number of techniques were explored. These included thin-layer and column chromatography using cellulose, nylon, polyamide, and Sephadex. Although resolution could be achieved in most cases, the tannin was found to be very tightly bound to the support substances and could not be recovered. However, by utilizing Sephadex LH-20, it was possible to both separate the tannins from non-tannin compounds and recover approximately 90% of the original material applied to the column. As illustrated by the chromatogram in Figure 1, when a 95% ethanol extract was applied to a 2.2 cm × 27 cm column of Sephadex LH-20, the non-tannins were removed by exhaustively washing with 95% ethanol. These non-tannin components were resolved into a series of fractions visually distinguishable by color. Each fraction contained numerous components which were subsequently resolvable by TLC.

The tannins, on the other hand, remained tightly adsorbed to the top of the column in 95% ethanol and could be observed as an immobile, orange-brown band. Elution of the column with 50% aqueous acetone subsequently released the tannins as a symmetrical peak. Approximately 58% of the dry matter from the ethanol extract was found in the non-tannin fraction while 31% was obtained as tannin. Together they represent a total recovery of 89% of the material applied to the column.

After removing the acetone solvent, dissolving the tannin residue in 95% ethanol, and repeating the adsorption–elution procedure, there was no 280-nm absorbing material eluted by 95% ethanol. With aqueous acetone, a single, symmetrical band was obtained which exhibited a constant ratio of absorbance at 400 nm to tanning activity as measured by the enzymatic assay. Examination by TLC with the HEA and AW solvent systems on silica gel H revealed that the tannin fraction contained no mobile components, but gave a positive reaction only for a material at the origin. Evidently all low molecular weight, alcohol-soluble substances had been effectively eliminated by this procedure, leaving only the purified tannin fraction free of non-tannin components.

Gel Filtration of Tannin Fractions. In an attempt to resolve the components of the tannin fraction recovered

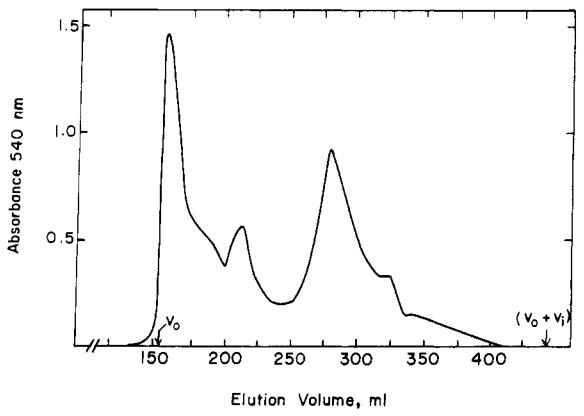

Figure 3. Gel filtration of Leoti tannins on Sephadex LH-20 using 50% aqueous acetone.

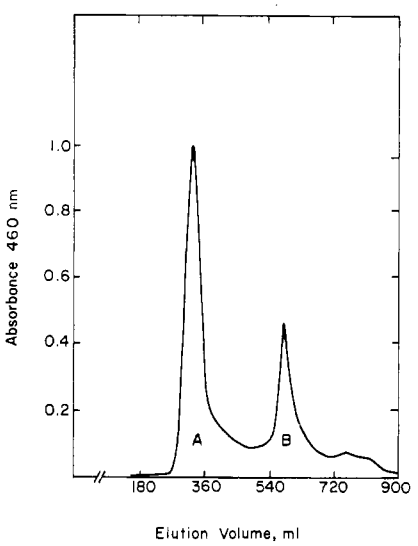

Figure 4. Gel filtration of Georgia 615 sorghum tannins on Sephadex LH-20 using 50% acetone as eluent.

from Sephadex LH-20, a 25-mg sample was applied to columns of Sephadex G-100 using 33% ethanol as eluent as described by Somers (1966) for wine tannins. As shown in Figure 2, two major peaks were resolved by this method, but there was an indication of several other partially resolved components. With this procedure, however, a portion of the tannin fraction was retarded on the column and was eluted later than expected due to adsorptive effects. To obtain V_i, the column had been calibrated with *p*-nitrophenol which is itself retarded compared to glucose. The strength of adsorption of the tannins due to interaction of the polyhydroxylic phenols with Sephadex G-100 in the 33% ethanol system is reflected by the long delay in elution.

When the same tannin fraction was passed through a column of Sephadex LH-20 using 50% aqueous acetone (Figure 3) as eluent, a significantly improved resolution pattern was achieved and at least five components were observed. In this system there was no evidence for binding of the polyphenolic tannins to Sephadex LH-20. All components were eluted within the expected liquid bed volume (V_0 + V_i).

Tannin Fraction from Georgia 615 Sorghum Grain. A tannin fraction was isolated from pulverized seeds of Georgia 615 using a traditional extraction procedure (Roux, 1953). After extracting the ground seeds with petroleum ether, ethyl acetate, and acetone, the subsequent concentrated ethanol extract contained a tannin fraction which was precipitated upon the addition of 3 vol of ether. From 2.5 kg of seeds, 13.2 g of tannin, equivalent to 0.53% of the powdered seeds, was obtained.

The tannin isolated by this procedure was chromatographed on a column of Sephadex LH-20 using aqueous acetone as the eluent. The pattern obtained by monitoring the effluent at 460 nm is shown in Figure 4. Two major peaks were resolved which were shown to precipitate gelatin. These components did not migrate on polyamide sheets using solvent systems composed of dimethylformamide mixed 1:1 with acetone, methanol, or ethanol, or when 1:1 acetone–water was used. No mobile components were observed on paper using 2% acetic acid or water-saturated butanol as irrigants.

Properties of the Tannins. The Leoti fractions obtained after chromatography on Sephadex LH-20 were subjected to a series of qualitative chemical tests, the results of which are summarized in Table I. The color reactions as well as spectral properties of the fractions are completely consistent with those of polyphenolic substances possessing neither nitrogen nor sulfur. The appearance of a precipitate with gelatin as well as the inactivation of several enzymes reflect the tanning potential of these polyphenols which were also shown to turn distinctly red when HCl was added.

Degradation of the Tannins with Alcoholic Hydrochloric Acid. In order to determine the composition of the tannins from Leoti sorghum and from Georgia 615, the products of acid hydrolysis were characterized by paper chromatography and spectrophotometry.

Tannin fractions obtained from Leoti and Georgia 615 after Sephadex LH-20 chromatography were treated with alcoholic hydrochloric acid as described above. The results, which are summarized in Table II, show that in each case a single red pigment was generated which migrated with R_f values identical with authentic cyanidin in several solvent systems. In addition, the color of the spot at the cyanidin position turned from red to blue when exposed to NH_3 vapor as expected and found from an authentic sample of cyanidin.

Comparison of the absorption spectra of the eluted pigment with that of authentic cyanidin revealed no differences. Confirmation of the identification of the pigment as cyanidin was obtained by the observation of a bathochromic shift (Table II) in the spectral maximum following the addition of $AlCl_3$.

Other components at the expected catechin position and elsewhere on the paper were not detected even after spraying with *p*-toluenesulfonic acid. Apparently then, cyanidin is the exclusive flavonoid degradation product from the tannins isolated both from the germ fraction of Leoti sorghum and from Georgia 615 seeds. Furthermore, after hydrolysis when the aqueous phase was examined by thin-layer chromatography there was no detectable glucose or other aniline–phthalate positive sugar released. Samples of glucose, galactose, and ribose gave the expected R_f values in the two solvent systems.

Non-Tannins of Leoti Sorghum. The non-tannin components of the ethanol extract of Leoti sorghum were separated from the tannins by adsorbing the tannins on Sephadex LH-20. The non-tannin components were eluted with ethanol, and the pattern obtained is shown in Figure 1. Fractions were combined where indicated on the curve. Fractions 1 and 3 were subjected to TLC on silica gel G using the benzene–methanol–acetic acid (45:8:4) solvent system. Each fraction contained numerous phenolic and flavonoid components as detected by selective spray reagents such as BDB, Gibbs reagent, TSA, and exposure to ammonia (Roux and Maihs, 1960; Seikel, 1962; Jurd, 1962). Of interest were the pink compounds of fraction 1 (R_f 0.08, 0.14, 0.23, and 0.30) which turned orange after exposure to ammonia. These pink pigments were probably not anthocyanins since the change with ammonia would have been to

Table I. Properties of the Leoti Tannins

Property or test	Result
Solubility	Soluble: alcohol, water Insoluble: acetone, ether
Elemental analysis	No nitrogen or sulfur
Ferric chloride	Green color
Bisdiazotized benzidine	Orange color
Basic lead acetate	Precipitate
Gelatin	Precipitate
Inhibited action of enzymes	α-Amylase, pectin esterase, polygalacturonic acid lyase, ribulose diphosphate carboxylase
Absorption spectrum	$\lambda_{max}(H_2O)$ at 277 nm; $E_{cm}^{1\%} = 162$ λ_{max} (0.1 N NaOH) at 292 nm; $E_{cm}^{1\%} = 279$
Color of aqueous solution at 25°	Yellow in acidic solution, red in basic solution; color change was reversible

Table II. Paper Chromatography of Products Extracted into Ethyl Acetate following Degradation of Tannins by Alcoholic HCl

Degraded tannin[a] or compd	R_f for solvent			Absorption spectrum	
	Forestal[b]	FHW[c]	CHW[d]	λ_{max},[e] nm	$AlCl_3$ added shift, Δ nm
Leoti	0.50	0.26	0.72	546	+16
Georgia 615	0.50			546	+16
Cyanidin	0.50	0.26	0.72	546	+16, +18[f]
Catechin[g]	0.73				

[a] The Leoti and Georgia 615 tannins were purified fractions obtained after gel filtration on Sephadex LH-20 using 50% acetone. [b] Acetic acid-HCl-water (30:3:10). [c] Formic acid-HCl-water (5:2:3). [d] *m*-Cresol-5.5 N HCl-water (1:1:1). [e] 95% ethanol-0.1% HCl. [f] Jurd (1962). [g] Catechin was detected with the following reagents: uv light (purple), $FeCl_3$ (green), *p*-toluenesulfonic acid (brown), bisdiazotized benzidine (brown).

a blue color. The colors observed resemble those of betacyanins which have been reported to occur in the Centrospermae almost exclusively and would not be expected in sorghum (Swain, 1965). The peak absorption for the fraction containing these pigments was found at 536 nm which is in the same range for both anthocyanins and betacyanins. No conclusion can be made about the identity of these compounds without additional characterization. Neither fraction contained proanthocyanidins as evidenced by the absence of the formation of pink or red color when TSA was used as a color reagent (Roux and Maihs, 1960).

DISCUSSION

Sorghum. Sorghum is an important grain for human consumption in parts of Asia and Africa and, according to estimates by the Agency for International Development (AID), more than 300 million people depend on sorghum as their principal food. In the United States, however, sorghum is used primarily as an animal feed (Leonard and Martin, 1963; Wall and Ross, 1970). In the southwestern United States, for example, sorghum is replacing corn in poultry rations, especially since the grain sorghums are much more adaptable to the prevailing semi-arid growing conditions than is corn. Similarly, in the humid southeastern United States, brown-coated sorghum grains are proving to be more resistant to weathering and to attack by birds than are varieties with light-colored seed coats. The resistance of these sorghums has been attributed to their characteristically higher tannin content (Prine et al., 1967; Tipton et al., 1970). Except for the presence of tannin, sorghum grain is similar to corn in nutritional value. Just as corn was improved by breeding of high lysine varieties, a great deal of attention has been focused recently on finding or developing improved strains of sorghum (Singh and Axtell, 1973).

Tannins in Sorghum. Although several authors, such as McGinty (1969), Chang and Fuller (1964), Fuller et al. (1966), and Harris et al. (1970), have pointed out that nutritional quality decreases in grain sorghum containing high levels of tannin, descriptions of the chemical features of the tannins found in sorghum have been scarce. In the studies showing the relationship between tannin content and feeding value, no distinction was made between hydrolyzable and condensed tannins (Fuller et al., 1966). In other studies, tannic acid, a hydrolyzable tannin, was added to sorghum and shown to retard the rate of growth of young chicks (Chang and Fuller, 1964) leaving the impression that hydrolyzable tannins might be responsible for the toxic effects of high tannin sorghum. Similarly, in the paper by Tipton et al. (1970), regarding bird resistance of sorghum grains, the authors state that it is now generally considered that the bitterness in seed of some hybrids is caused by tannic acid and related astringents. They also reported that "the *tannic acid* content of the bird-resistant hybrid was approximately eight times higher than that of the bird-susceptible hybrids".

Although no serious attempt has yet been made to determine the relative levels of hydrolyzable and condensed tannins in sorghum grain, Maxson and Rooney (1972) compared seven methods of tannin analysis for their potential use on sorghum. Since each method measured different and not clearly definable substances, the values obtained were at best relative measures of tannin content. It can be gleaned from their data that the amount of tannin, determined as tannic acid by the ferric–ammonium sulfate method was very low and did not vary more than fourfold (0.04–0.16 mg/100 mg) when going from a low tannin to a high tannin variety of sorghum. On the other hand, the amount of condensed tannin determined as catechin equivalents with the vanillin-HCl method was 5 to 20 times as high and varied 15-fold (0.20 to 3.10 mg/100 mg) for the respective varieties. It seems logical to conclude from their data that the condensed tannins are the predominant type of tannin in sorghum grain.

The results presented in our paper, based upon the isolation and characterization of polyphenols with tannin properties, also show that condensed tannins are the primary, if not exclusive, tannin found in Leoti and Georgia 615 sorghums. In our studies, Leoti sorghum was selected because it was a pure bred, red-coated variety. Georgia 615 was used because it was a hybrid brown-coated variety, shown by Fuller et al. (1966) to contain 1.6% tannin as tannic acid equivalents and to significantly depress the rate of growth of chicks.

Identification of the Tannins. The polyphenols isolated from these grain sorghums were resolved into a tannin and non-tannin fraction by adsorption on Sephadex LH-20 and the tannins identified by virtue of their affinity for proteins. A positive reaction in the gelatin precipitation test and inhibition of several enzymes were obtained and used as indicators and monitors of tanning ability. When treated with hydrochloric acid, the tannins generated cyanidin as the apparently exclusive product of depolymerization. No evidence was found from paper chromatography or spectral analysis for the presence of other anthocyanidins

such as fisetinidin as found by Blessin et al. (1963) or pelargonidin as observed by Yasumatsu et al. (1965) in other varieties of sorghum. In our studies, the tannins isolated from both the Leoti and Georgia 615 sorghums behaved in essentially identical fashion suggesting that the natures of the tannin polyphenols in the two varieties are extremely similar. Differences observed in the chromatographic patterns of the two tannins may reflect the difference in the procedures used. The Leoti sample was initially treated with ether while the Georgia 615 sample was treated with petroleum ether followed by ethyl acetate and acetone. Some phenolic compounds will be extracted by each procedure, but not the same ones.

Since catechin was not detected as a degradation product of Leoti tannin, the possibility of a leucoanthocyanidin–catechin biflavonoid type of structure, analogous to the dimers isolated from avocado seed (Geissman and Dittmar, 1965) and cola nut (Weinges and Freudenberg, 1965), may be minimized. The absence of glucose or other aldose as a degradation product rules out the presence of an *O*-glycoside and, in addition, hydrolyzable tannins such as tannic acid. The latter type of tannin would be converted to glucose and gallic acid (Haslam, 1966) upon acid hydrolysis, but these products were not observed in our studies. Since the tannins from Leoti and Georgia 615 isolated in our study could be further fractionated by gel filtration on Sephadex, it may be argued that these tannins are a homologous series of oligomeric procyanidin tannins varying in degree of polymerization.

Isolation of Tannins by Adsorption on Sephadex LH-20. Various methods have been used by others for the separation of tannins from other substances but often these present serious problems in recovery of the tannins or do not yield preparations free of non-tannin phenolics. Such difficulties have long been associated with the fractionation of tannins (Haslam, 1966). In this paper we have presented a new and simple method for the separation of sorghum tannins from the non-tannin materials based upon the adsorption of the tannins on Sephadex LH-20 in 95% ethanol. These conditions seem to be suitable for the adsorption of tannins, specifically, and permit removal of phenolics and all other ethanol-soluble materials. The phenolic pigments in the non-tannin fraction can then be resolved further by thin-layer chromatography. The tannin polyphenolics can be removed from the column by eluting with 50% aqueous acetone after exhaustively washing with 95% ethanol which eliminated non-tannin components. Importantly, it is then possible to fractionate the tannins by filtration through Sephadex LH-20 using the aqueous acetone solvent.

The versatility of Sephadex LH-20 gels to effect further fractionation of both the Leoti and Georgia 615 tannins was demonstrated by the chromatograms shown in Figures 2 and 4. With the Sephadex LH-20 systems in which aqueous mixtures of organic solvents were used, separation occurred by the process of gel filtration in contrast to the adsorption procedure with 95% ethanol (Figure 1). When compared to the Sephadex G-100 system using aqueous ethanol, the Sephadex LH-20 procedure using 50% acetone offers the advantage that nonspecific adsorption of the tannins to the gel was eliminated. The use of Sephadex LH-20 appears to have great potential for the purification of other tannins as well. We have also applied this procedure for the purification of wattle tannin and will report those results in a subsequent communication.

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