ethyl levulinate, 539-88-8; methyl benzoate, 93-58-3; ethyl benzoate, 93-89-0; diethyl succinate, 123-25-1; ethyl 3-hydroxyhexanoate, 2305-25-1; benzyl acetate, 140-11-4; ethyl phenylacetate, 101-97-3; 2-phenylethyl acetate, 103-45-7; ethyl laurate, 106-33-2; ethyl myristate, 124-06-1; ethyl pentadecanoate, 41114-00-5; ethyl palmitate, 628-97-7; ethyl heptadecanoate, 14010-23-2; ethyl stearate, 111-61-5; ethyl oleate, 111-62-6; benzyl benzoate, 120-51-4.

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Occurrence of an Unusual Leucoanthocyanidin and Absence of Proanthocyanidins in Sorghum Leaves

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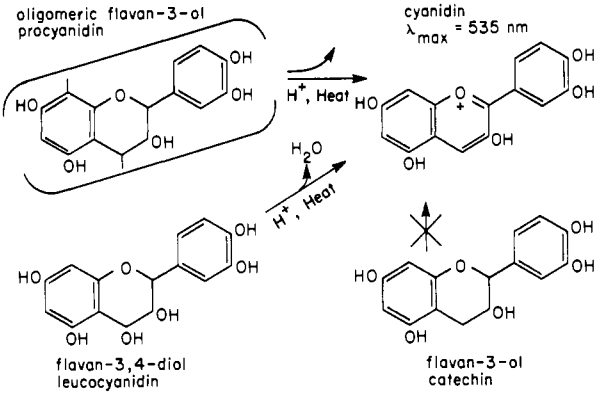
Condensed tannins of sorghum seeds could, if also present in vegetative tissue, lower the digestibility of the forage. In order to eliminate interference by pigments such as chlorophyll and anthocyanins in tannin assays of forage, we adsorb tannin and related flavanols on insoluble poly(vinylpyrrolidone) (PVP). After washing out interfering materials we heat the PVP in HCl/butanol to convert the bound tannins to anthocyanidins which are measured spectrophotometrically. By use of this assay, no tannins were found in leaf tissue of the 47 sorghum lines examined. However, leaf tissue from 16 of the lines was found to contain a PVP-binding material with properties corresponding to a monomeric flavan-4-ol (designated a leucoanthocyanidin). The leucoanthocyanidin, which is also present in seed of these varieties, has been tentatively identified as apiforol (4',5,7-trihydroxyflavan-4-ol). This unusual monomeric flavan-4-ol, which has not been previously reported from plants, yields the yellow anthocyanidin, apigeninidin, when heated in aqueous acid and at low temperatures in acid/alcohol mixtures is converted to an unidentified unstable pink anthocyanidin.

Tannins are the most important group of secondary metabolites involved in plant defense (Swain, 1979). Tannins present in the grain of certain sorghum varieties confer agronomic benefits such as resistance to bird depredation (McMillian et al., 1972) and to preharvest seed molding (Harris and Burns, 1973). Tannins bind certain proteins very strongly (Hagerman and Butler, 1981) and thus diminish the digestibility and nutritional value of high tannin sorghum grain (Price et al., 1979). Sorghum seed tannins are of the "condensed" type (Strumeyer and Malin, 1975), oligomers of flavan-3-ols. Because of their propensity for depolymerization in acid solution to yield anthocyanidin pigments, condensed tannins are classified as proanthocyanidins; those from sorghum form cyanidin and are therefore called procyanidins (Gupta and Haslam, 1978). Certain monomeric flavanols such as flavan-3,4-diols and flavan-4-ols can also give rise to anthocyanidins (Scheme I) and are therefore distinguished from the oligomeric flavan-3-ols by the name "leucoanthocyanidin" (Weinges et al., 1969).

Relatively little is known about the occurrence and distribution of proanthocyanidins and leucoanthocyanidins in sorghum tissues other than grain. As a result of injury or physiological stress, sorghum leaf tissue frequently develops red coloration due to anthocyanidins and/or anthocyanins (Bate-Smith, 1969). Leucoanthocyanidins

Scheme I



could be the precursors of these pigments. If proanthocyanidins serve as a deterrent to herbivores and pathogens (Swain, 1979), they might be expected to occur in the vegetative tissue of the plant, as well as the seed. Proanthocyanidins present in forage could diminish its nutritional value. Jung and Fahey (1981) reported that removal of unidentified phenolic materials from alfalfa increases its digestibility.

We have adapted an anthocyanidin formation assay to vegetative tissue, eliminating interference by chlorophyll and endogenous anthocyanin or anthocyanidin pigments. We found a leucoanthocyanidin in the leaf tissue of 12 of the 43 sorghum lines we examined but no proanthocyanidins in the leaf tissue of any of the lines.

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EXPERIMENTAL SECTION

Sorghum and other crop plants were grown at the Purdue Agronomy Farm, West Lafayette, IN, under the direction of Dr. John Axtell, Department of Agronomy, Purdue University. Samples of leaf tissue from other plants were obtained from Horticulture Park, Purdue University. Poly(vinylpyrrolidone) (PVP) (Polyclar AT) was obtained from GAF Corp.; it was boiled for 10 min in 10% HCl, fines were decanted, and the residue was filtered and dried. Condensed tannin from sorghum (BR 54), purified by our standard procedure (Hagerman and Butler, 1980), was provided by A. Hagerman.

Extraction of Tissue. The youngest fully developed leaves (from several different plants of each variety to minimize sampling error), 3.0 g fresh weight, were cut into small pieces and homogenized in a 40-mL centrifuge tube with 20 mL of methanol on a Polytron homogenizer (Brinkmann Instruments, Inc., Westbury, NY 11590) at $^1/_2$ full speed for 1 min. The homogenizer was rinsed with 10 mL of methanol, which was mixed with the original homogenate. After centrifugation for 5 min at 12000*g*, the supernatant was carefully decanted and used for assays. Methanol extracts of mature seeds were prepared as previously described (Price et al., 1978).

HCl/Butanol Assay for Anthocyanidin Formation. One milliliter of extract was added to approximately 0.5 g of treated PVP in a screw-top test tube. After occasional stirring for 5 min at room temperature, 5 mL of methanol was added with vigorous mixing, and the mixture was centrifuged briefly in a table top centrifuge. The chlorophyll-containing supernatant fluid was discarded and the PVP was washed once more with 5 mL of methanol. Then 7.0 mL of 30% HCl/70% (v/v) butanol was added to the PVP pellet, and the mixture was slowly agitated on a Lab-Quake mixer (Labindustries, Berkeley, CA) for 1 h at room temperature. After centrifugation for 5 min the absorbance of the supernatant was measured at 550 nm. The supernatant was then remixed with the PVP and heated on a boiling water bath for 1.5 h. After centrifugation the absorbance of the supernatant was again measured at 550 nm. For blanks, a duplicate sample adsorbed on PVP was suspended in 7.0 mL of 15% (v/v) 0.1 N acetic acid/15% methanol/70% 1-butanol and treated as described, without heating. No anthocyanidins form in these samples, so that absorbance of the extract can be measured and subtracted from the samples in HCl/1-butanol. For fresh green leaf tissue the absorbance of the blank was insignificant; readings could be made against butanol or water.

RESULTS AND DISCUSSION

Development of the Assay for Anthocyanidin Formation in Extracts of Leaf Tissue. The presence of chlorophyll in leaf extracts seriously interferes with spectrophotometric assays for anthocyanidins. Proanthocyanidins are known to be strongly adsorbed from aqueous solution by insoluble polymeric materials such as Dowex anion-exchange resin (Lam and Shaw, 1970) and PVP (Loomis and Battaile, 1965) which are often used, in extraction of plant enzymes, to minimize binding by endogenous polyphenols (Loomis, 1974). Little work has been done on the adsorbed materials, although McFarlane (1961) utilized adsorption on Nylon 66 to eliminate interferences with "anthocyanogen" (proanthocyanidin) assays in beer. We found that chlorophyll does not bind to PVP under conditions in which proanthocyanidins are quite strongly bound, and we utilized this differential binding in developing an assay for proanthocyanidins and leucoanthocyanidins in leaf tissue.

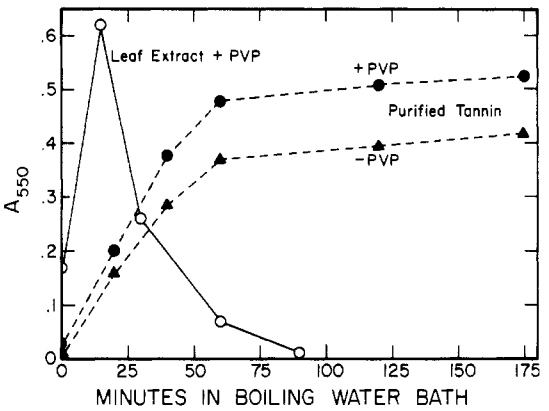


Figure 1. Time course of anthocyanidin development on heating. Leaf extract was from sorghum variety BR 54. Purified tannin, also from BR 54, was 0.1 mg/mL in methanol. Samples containing PVP were washed and developed as described in the text. Washing was omitted for the sample assayed in the absence of PVP. After the indicated times in a boiling water bath, samples were cooled and centrifuged before the absorbance of the supernatant at 550 nm was determined.

We found that insoluble PVP strongly and completely adsorbs purified condensed tannin (Hagerman and Butler, 1980) not only from aqueous solution but also from methanol and methanol/H_2O mixtures. An advantage of this PVP technique is that adsorption can be accomplished by simply adding dry washed PVP to the tannin solution. Proanthocyanidins in crude methanol extracts of sorghum seed are adsorbed equally well, and monomeric flavan-3-ols such as catechin are also strongly bound. Anthocyanidins such as cyanidin are not bound.

Adsorbed proanthocyanidins could not be eluted from PVP in good yield by any solvents tested, including acetone/water mixtures and *N*-methylpyrrolidone (Loomis and Battaile, 1965). Up to 50% of the proanthocyanidins bound to PVP were eluted with a 1/1 (v/v) mixture of water and dimethyl sulfoxide, but this solvent interferes with measurements of anthocyanidin formation and is difficult to remove. We therefore sought to assay the material while it remains bound to PVP.

The vanillin reaction as modified by Price et al. (1978), a widely used assay for sorghum tannin, is not suitable for assaying bound proanthocyanidins because the reagent simply adds to the tannin oligomer without depolymerization (Singleton, 1972). The color produced by the vanillin reaction all remains bound to the PVP and therefore cannot be readily measured. The Prussian Blue assay for total phenolics (Price and Butler, 1977) can be used for PVP-bound materials, but only 25% as much color is formed as in solution, possibly due to restricted accessibility of phenolic groups in the bound form.

As shown in Figure 1, purified condensed tannin from sorghum seed is converted on a boiling water bath in HCl/butanol to anthocyanidin pigments, largely cyanidin (Gupta and Haslam, 1978), at a rate which is similar whether the tannin is bound to PVP or is in solution. Surprisingly, the sample bound to PVP gave approximately 30% more product than in the absence of PVP. This enhancement of anthocyanidin formation by adsorption on PVP was also observed in crude extracts of seed from many sorghum lines, although the degree of enhancement varied severalfold. Washing the PVP-bound proanthocyanidin may remove components which interfere with the complex conversion process and thus result in the observed enhancement.

Application of the Assay to Leaf Extracts. Also shown in Figure 1 is the time course of color development

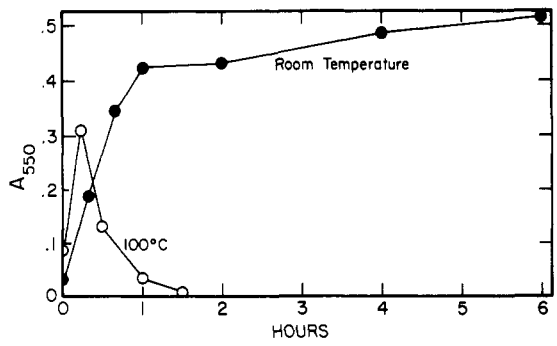


Figure 2. Effect of temperature on time course of color development of leaf extracts of sorghum variety BR 54. Data at 100 °C were taken from Figure 1. The same extract was developed first at room temperature as described in the text.

when a methanol extract of mature leaf tissue of sorghum (BR 54) is adsorbed on PVP, washed with methanol to remove chlorophyll, and suspended in HCl/butanol on a boiling water bath. The characteristic pink color (λ_{max} 550 nm) develops rapidly, being apparent even before heating, and then quickly declines to near zero. This response cannot be attributed to adsorption on PVP. Similar results, though obscured by chlorophyll, are obtained with leaf extracts not adsorbed on PVP. This leaf tissue does not contain detectable amounts of proanthocyanidins such as those present in the seeds of many lines of sorghum which, on heating in HCl/butanol, form stable anthocyanidin pigments. However, it apparently does contain a different material which is readily converted to a labile pigment. Time courses of color development of PVP-bound leaf extract from sorghum in HCl/butanol at room temperature and in a boiling water bath support this suggestion. As shown in Figure 2, the conversion to pink pigment (λ_{max} 550 nm) occurs quite readily in HCl/butanol at room temperature, and the product is stable for several hours at this temperature. However, the colored product is labile at 100 °C. In contrast, at room temperature purified sorghum seed tannin (proanthocyanidin) is only slightly (<4%) converted to anthocyanidin in 1 h (data not shown). We therefore routinely measure color (A_{550}) production twice in the same HCl/butanol mixture. We first measure the labile pigment after 1 h in HCl/butanol at room temperature and then heat on a boiling water bath for 1.5 h to destroy this labile material and convert any condensed tannin to the heat-stable anthocyanidin which we then measure.

Identification of the Leucoanthocyanidin from Leaf Tissue. The pink pigment obtained by HCl/butanol treatment of PVP-adsorbed leaf extracts is too unstable for extensive characterization. Its spectral characteristics and its ready extraction into isoamyl alcohol from aqueous acids (to give an unstable cherry red solution) are consistent with an anthocyanidin structure (Riberêau-Gayon, 1972).

The rate of anthocyanidin formation is influenced by the structure of the proanthocyanidin/leucoanthocyanidin precursor (Bate-Smith, 1975). The facile conversion to an apparent anthocyanidin in HCl/butanol without heating suggests that the precursor is a monomeric flavan-4-ol (Singleton, 1972) rather than an oligomeric flavan-3-ol. Like condensed tannins but unlike monomeric flavan-3-ols, monomeric flavan-4-ols are converted to anthocyanidins in acid solutions, although the latter reaction is a dehydration rather than depolymerization (Singleton, 1972). Flavanols of this type are rare, but one such compound, luteoforol (3',4',5,7-tetrahydroxyflavan-4-ol), has previously been reported from sorghum seed (Bate-Smith and Rasper, 1969) and other tissues of sorghum (Bate-Smith, 1969). Luteoforol in sorghum seed was assayed by agitating whole kernel grain in 43% (v/v) H_2SO_4 in methanol at 0 °C for 24 h (Bate-Smith and Rasper, 1969). The pigment formed has a major absorption peak at 550 nm, which is similar to that of the labile pigment we obtain.

Table I. Pigment Produced from Sorghum Seed without Heating

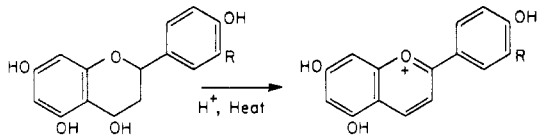
line	A_{550}/g, Bate-Smith assay[a]	A_{550}/g, our assay[b]
IS 0469	0	0
IS 0893	1.08	2.8
IS 3974	0	0
IS 4225	3.0	8.5
IS 6881	0.27	0.9
IS 12281	0	0
IS 15455	5.1	16
P 954035	0.4	6.5
P 954063	0	0
P 7210	0	0
RS 610	1.52	1.8
Savanna III	0.66	3.7

[a] Bate-Smith and Rasper (1969). Whole seeds agitated in 43% (v/v) H_2SO_4 in methanol at 0 °C for 24 h. [b] Polyphenols extracted in methanol, adsorbed on PVP, and suspended in 30% (v/v) HCl in 1-butanol for 1 h at room temperature.

We applied Bate-Smith's assay for luteoforol to the seed from 12 different lines of sorghum and obtained a positive test with 7 of them. We then tested these seeds with our room temperature anthocyanidin formation assay, with and without adsorption on PVP, and observed a positive result on the same lines (Table I). Differences in the ratio of values obtained by the two methods may be due to differences in extractability from whole grain (Bate-Smith's assay) and ground grain (our assay). The results are consistent with the detection of the same leucoanthocyanidin component(s) by both assays.

For further characterization of the unknown leucoanthocyanidin, 1.0 g of dry ground mature leaf tissue of a sorghum line in which it is especially abundant (IS 9958) was extracted with 50 mL of methanol and then absorbed on Amberlite XAD-2 resin. It was found that the unknown could be much more readily eluted from this polystyrene resin than from PVP. The adsorbed material was washed free of chlorophyll with two methanol washes (100 mL each) and eluted with 100 mL of 1/1 (v/v) acetone/H_2O. This material was taken to dryness on a rotary evaporator and redissolved in methanol, in which it is stable, as judged by its capacity to be converted at low temperature in HCl/butanol to the unstable pigment with λ_{max} 550 nm. On thin-layer chromatography on microcrystalline cellulose (Anasil type C, Analabs, Inc., North Haven, CT) in formic acid/HCl/H_2O (10/1/3), with color development by spraying with HCl/butanol, this material produced a single discrete pink spot with R_f = 0.87. The high mobility and apparent homogeneity of this material suggests that it is not polymeric. As expected for a monomeric flavanol, it did not precipitate significant amounts of protein, despite doubling the usual amount of sample in either of two protein precipitation assays (Hagerman and Butler, 1978; Schultz et al., 1981), even after 4 days in solution, during which polymerization might occur. On comparison to epicatechin in the Prussian Blue assay for total phenols (Price and Butler, 1977) and in the vanillin assay (Price et al., 1978) it was found that the unknown produces relatively little color in the vanillin reaction. The ratio of vanillin color to total phenols for the unknown was only 4% of that for epicatechin. This suggests either the

Scheme II



R	Name
OH	luteoferol
H	apiforol

R	Name	λ_{max}
OH	luteolinidin	495 nm
H	apigeninidin	485 nm

presence of a double bond between C_2 and C_3 (Sakar and Howarth, 1976) or a deactivating group such as a carbonyl at C_4 (Ribereau-Gayon, 1972).

Many of the properties of the leucoanthocyanidin we observe in leaf tissue of sorghum, and which also occurs in the seed of these lines, correspond to those expected of a flavan-4-ol such as luteoforol (Scheme II). Like our unknown, luteoforol is readily converted, at low temperature in acidic alcohol solution, to an unstable anthocyanidin-like pigment with λ_{max} 545–550 nm and in dilute mineral acids to a stable anthocyanidin with λ_{max} 480–500 nm (Stafford, 1965; Bate-Smith, 1969). The stability of anthocyanidins lacking a hydroxyl group on C_3 is quite different from that of other anthocyanidins (Ribereau-Gayon, 1972). The unknown leucoanthocyanidin we observe cannot be luteoforol, which is an *o*-diphenol, because in contrast to epicatechin and catechin controls, it gave a clearly negative result in the Waite and Tanzer (1981) *o*-diphenol assay.

When heated for 15 min on a boiling water bath in 2 N HCl, conditions in which luteoforol is converted to luteolinidin (λ_{max} 495 nm) (Stafford, 1965), its corresponding anthocyanidin (Bate-Smith and Rasper, 1969), the unknown gave a stable yellow pigment (λ_{max} 485) which readily and completely extracts into isoamyl alcohol, as expected for an anthocyanidin. No shift in λ_{max} was observed on addition of $AlCl_3$, again suggesting that *o*-diphenol groups are absent. On addition of NaOH the λ_{max} shifted to 555 nm.

The stable yellow anthocyanidin produced from the unknown as described above was subjected to thin-layer chromatography on glass plates precoated with a 0.25-mm layer of silica gel, No. 60 F-24, from E. Merck, Darmstadt, West Germany, in ethyl acetate/formic acid/H_2O/concentrated HCl (85/6/8/1) (Stafford, 1965). The major band, R_f = 0.42, turned from yellow to pink on exposure to ammonia vapors. Under these chromatographic conditions the unknown leucoanthocyanidin remained at the origin. After spraying with HCl/butanol to convert it to the unstable pink anthocyanidin, it then chromatographed on the same plate with the same solvent with an R_f of approximately 0.33.

These results suggest that our unknown leucoanthocyanidin is 4′,5,7-trihydroxyflavan-4-ol, apiforol (Bate-Smith, 1969); the corresponding anthocyanidin produced from it is apigeninidin (Scheme II). Apigeninidin, which has previously been reported from 4-day-old first internodes of sorghum (Stafford, 1965) and in purple glumes (Misra and Seshadri, 1967), has λ_{max} of 483 nm and λ_{max} does not shift with $AlCl_3$ since it is not an *o*-diphenol (Harborne, 1958). When fumed with ammonia, apigeninidin turns from yellow to pink, as we observed, whereas luteolinidin turns violet to purple (Stafford, 1965). Several unidentified forms of apigeninidin, presumably glycosides, have been reported to be present in sorghum seeds (Nip and Burns, 1969, 1971), but to our knowledge its colorless precursor apiforol has not been reported from any sorghum tissue. Bate-Smith (1969) found no evidence for it in glumes in which he found luteoforol, luteolinidin, and apigeninidin.

Table II. Proanthocyanidin and Leucoanthocyanidin Distribution in Sorghum Lines

	anthocyanidin formation, A_{550}/g				
	room temperature, leucoanthocyanidin (monomeric flavan-4-ol)		boiling water, proanthocyanidin (oligomeric flavan-3-ols)		vanillin assay, catechin equiv, g/100 of seed,
line	leaf	seed	leaf	seed	seed
(1) IS 0071	0	0	0	0	0
(2) IS 0014	0	0	0	0	0
(3) IS 0135	0	0	0	1.4	0
(4) IS 0409	0	0	0	1.7	0
(5) IS 0466	0	0	0	0	0
(6) IS 0469[a]	0	0	0	0	0
(7) IS 0893	14	2.8	0	32	2.9
(8) IS 2319	0	0	0	2.3	0
(9) IS 2830	0	0	0	26	1.7
(10) IS 2863	0	0	0	0	0.3
(11) IS 2939	0	0	0	0	0
(12) IS 3441	0	0	0	6.8	0.8
(13) IS 3974	0	0	0	1.8	0
(14) IS 4225	5.1	8.5	0	29	1.9
(15) IS 6881	0	0.9	0	50	5.3
(16) IS 7579	0	5.0	0	37	3.7
(17) IS 8070	0	0	0	38	3.0
(18) IS 8248	28	1.7	0	1.1	0
(19) IS 8330	0	0	0	1.5	0
(20) IS 8361	19	0.7	0	0	0
(21) IS 8377	0	0	0	0	0
(22) IS 8583	0	1.1	0	19	2.5
(23) IS 8614	0	0	0	38	4.5
(24) IS 8768	0	0	0	1.3	0.2
(25) IS 9576	0	0	0	1.2	0
(26) IS 9750	0	0	0	11	1.6
(27) IS 9958	39	3.6	0	0	0
(28) IS 10354	0	0	0	0	0
(29) IS 12281	0	0	0	0	0
(30) IS 15067	17	7.8	0	46	7.9
(31) IS 15455	15	16	0	30	6.1
(32) P 121087	0	0	0	0	0
(33) P 121089	0	0	0	0	0
(34) P 232067	0	0	0	0	0
(35) P 932075	0	0	0	0.8	0.1
(36) P 954035	47	6.5	0	1.0	0
(37) P 955014	23	1.2	0	1.3	0
(38) P 955026	0	0	0	1.4	0.1
(39) BR 64	8.0	2.2	0	44	4.7
(40) P 721 N	0	0	0	0	0
(41) P 721ϕ	0	0	0	0	0
(42) RS 610	4.8	1.8	0	1.3	0
(43) Savanna III	9.1	3.7	0	44	3.8

[a] Tan plant.

Distribution of Leucoanthocyanidins and Proanthocyanidins. Mature leaf tissue from 43 lines of grain sorghum was assayed with the PVP adsorption/anthocyanidin formation assay. Of these, 12 gave a positive test at room temperature, indicating the presence of a leucoanthocyanidin (Table II). Similar assays of mature seed samples from the same lines showed that lines which give a positive test in leaf tissue invariably test positive in the seed, although the amount present in seed is usually much less than in the leaf. In three lines the seeds gave a positive test although the leaf tissue was negative.

When heated in HCl/butanol at 100 °C all leaf samples tested negative, indicating the absence of proanthocyanidins (condensed tannins) characteristic of seeds. Proanthocyanidins of seeds were found to occur independently of leucoanthocyanidins (Table II). Except for

the lowest values, the PVP adsorption/heated anthocyanidin formation assay of seeds gave values approximately proportional to those obtained with the vanillin assay (Table II). This suggests that both assays measure the same proanthocyanidin components, as expected.

It should be noted that flavan-4-ols form anthocyanidins in the methanol/HCl solvent of the vanillin assay and can therefore give false high values unless a vanillin-free blank is run under the same conditions (Price et al., 1978). Likewise, use of an unheated control in the assay for leucoanthocyanins as recommended by Swain and Hillis (1959) could cause problems if flavan-4-ols were present.

Leaves of two varieties of forage sorghums were found to contain the leucoanthocyanidin in both the normal and brown midrib forms (Porter et al., 1978) in amounts comparable to that found in leaf tissue of the grain types. Analysis of glume and rachis tissue of the sorghum BR 54 gave values of A_{550} per gram which were somewhat higher than those for leaf and seed tissue and only traces within stem and root tissue.

The 43 lines listed in Table II were analyzed thoughout the growing season. No leucoanthocyanidin was detected until the plants were approximately 50 cm tall (extended leaf height). The later the leaf develops, the greater its initial content of apiforol. Apiforol content then diminishes as the leaf ages.

Under conditions of plant stress or disease, apiforol may be metabolized to another, as yet unidentified, leucoanthocyanidin. BR 64, in which apiforol occurs, and P 954063, in which it does not, both developed considerable senescent brown tissue on mature leaves of greenhouse-grown plants. Brown tissue of BR 64, but not of P 954063, when subjected to the PVP binding/anthocyanidin formation assay *without* heating, formed large amounts of an apparent anthocyanidin pigment with λ_{max} about 505 nm. Similarly, field-grown leaves of BR 54 infected with *Helminthosporium sorghicola* exhibited extensive purple coloration surrounding infection sites. When leaves were dried and separated into purple (infected) and green (uninfected) tissue and assayed as above, the infected tissue gave an apparent anthocyanidin with λ_{max} about 500 nm. The uninfected tissue gave the unstable pink anthocyanidin (λ_{max} 550 nm), as expected. The pigment with λ_{max} near 500 nm was in both above cases stable on heating in HCl/butanol, in contrast to the pink pigment formed from apiforol without heating. We hope in future studies to elucidate the role of sorghum flavan-4-ols in disease and stress resistance.

Methanol extracts of leaf tissue from 20 lines of corn and from oats, wheat, and a wide variety of other plants including gymnosperms and ferns were tested for flavan-4-ols in the PVP binding/anthocyanidin formation assay at room temperature. Likewise, seeds of corn, wheat, millet, beans, and amaranthus were tested. Only in sorghum was a positive test observed, confirming the reported rarity of flavan-4-ols and their anthocyanidin products (Jurd, 1972).

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Registry No. Apiforol, 55167-29-8.

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