

# Sorghum Phenolic Acids, Their High Performance Liquid Chromatography Separation and Their Relation to Fungal Resistance<sup>1</sup>

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## ABSTRACT

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A high performance liquid chromatographic (HPLC) method was developed for the separation and identification of substituted benzoic and cinnamic acids (phenolic acids) of *Sorghum bicolor* (L.) Moench. Eight phenolic acids were identified in sorghum extracts. In order of decreasing polarity they were: gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, ferulic, and cinnamic acids. In addition to these eight compounds, 12 other peaks were separated from the grain extracts, but these have not been identified. Protocatechuic, *p*-hydroxybenzoic, caffeic, *p*-coumaric, and ferulic acids were found in the free form in all seven sorghum varieties with means of 8.9, 5.9, 6.4, 37.6, and 34.7  $\mu\text{g/g}$ , respectively. Protocatechuic, *p*-hydroxybenzoic, caffeic, *p*-coumaric, and ferulic acids were also found in the bound form in all seven sorghum varieties with means of 82.0, 18.3, 30.8, 82.1, and 163.2  $\mu\text{g/g}$ , respectively. Gallic acid was found only in the bound form with a mean of 23.6  $\mu\text{g/g}$ . Vanillic acid was found in six varieties in the free form with a mean of 31.8  $\mu\text{g/g}$  and in four varieties in the bound form with a mean of 38.4  $\mu\text{g/g}$ .

Cinnamic acid was found in four varieties in the free form with a mean 6.7  $\mu\text{g/g}$ . Cinnamic acid was found in the bound form in only one variety at a concentration of 19.7  $\mu\text{g/g}$ . Free phenolic acids were extracted from ground sorghum by shaking in methanol. The crude extracts were cleaned up before chromatography by passage through C18 Sep-Pak cartridges (Waters Associates). Bound phenolic acids were released from the residue of the free phenolic acid extraction by hydrolysis in 2*N* HCl. The phenolic acids were removed from the hydrolysate by an ethyl acetate partition. Mean recoveries for the extraction of free and bound phenolic acids were 94.4 and 72.1%, respectively. Sorghum grain resistant to fungal attack contained both a greater variety and larger amounts of the identified phenolic acids and unknown compounds than did susceptible varieties. Resistant sorghums also had a greater percentage of their total, identified phenolic acids in the free form. However, resistance to fungal attack could not be attributed solely to phenolic acid content or profile. Tannin content and physical characteristics of the grain also contribute to fungal resistance.

Before harvest, sorghum can be attacked by a variety of fungi. The most frequently encountered fungi are from the genera *Fusarium*, *Curvularia*, *Alternaria*, *Aspergillus*, and *Phoma* (Williams and Rao 1980). Infestation before grain maturity is referred to as molding, and after maturity as weathering. Both conditions reduce grain yield and viability. The quality of the affected grain for both feed and food is reduced.

Williams and Rao (1980) observed no apparent correlation between evident panicle and/or grain characteristics and the ability to resist becoming severely molded in the several thousand diverse sorghum lines at ICRISAT (Patancheru, India). Brown sorghums (with pigmented testa) with high tannin content were relatively more resistant to molding and weathering than nonbrown sorghums. However, tannins produce astringent flavor and reduce the grain's nutritional value. There is no real difference in tannin content among nonbrown sorghums, but some of these sorghums do have appreciable resistance to molding and weathering. Nonbrown sorghums do have polyphenolic compounds that may be important in fungal resistance. Glueck and Rooney (1980) found that water extracts of resistant nonbrown sorghums retarded the growth rate of sorghum seedlings, indicating that inhibitory compounds were present. Paper chromatography of the water extracts resulted in a great variability in number and quantity of the separated compounds among the sorghums. This suggests that there is probably a chemical component in the resistance mechanism of sorghum to attack by molds.

In low-tannin sorghums, many of these compounds are likely to be derivatives of benzoic and cinnamic acid. Many of the substituted benzoic and cinnamic acids have shown antifungal activities in other plant systems (Baranowski et al 1980, Byrde et al 1960, Goodman et al 1967, Harding and Heale 1980).

Mold growth occurs in two stages: germination of the spores followed by mycelial growth. Plant phenolics are known to inhibit both stages (Friend 1977). In addition to direct inhibition of fungal growth, protective activity can be caused by the inhibition of fungal extracellular enzymes, inhibiting cell-wall degradation and penetration of the mycelia (Hunter 1974). Protective activity can

also be conferred by the oxidation of pre-existing phenolic compounds (Walker 1969). Ferulic, *p*-coumaric, vanillic, *p*-hydroxybenzoic, cinnamic, and protocatechuic acids inhibit the growth of several fungal species (Baranowski et al 1980, Byrde et al 1960, Friend 1977, Leungchaikul 1982).

Thirteen of the commonly occurring benzoic and cinnamic acids have been successfully separated (Murphy and Stutte 1978). Eight benzoic and cinnamic acids were chosen as standards for this study: gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, *p*-coumaric, ferulic, and cinnamic acids. This study had three objectives: to develop a rapid, reproducible procedure for the extraction of the low molecular weight phenolics from sorghum; to optimize high performance liquid chromatography (HPLC) separation procedure capable of identifying and quantifying the phenolic compounds extracted; and to apply the extraction and separation schemes to several varieties of sorghum with varying degrees of resistance to grain molding and weathering fungi.

## MATERIALS AND METHODS

### Sorghum Samples

Seven sorghum varieties that varied in kernel characteristics and degree of resistance to weathering and molding were grown at Halfway, TX, in 1980 (Table I). Samples were stored at  $-4^{\circ}\text{C}$  until analysis.

### Chemicals

Gallic, protocatechuic, vanillic, caffeic, *p*-coumaric, and ferulic acids were obtained from Aldrich Chemical Company (Milwaukee, WI), *p*-hydroxybenzoic acid from Eastman Organic Chemicals (Rochester, NY), and cinnamic acid from Nutritional Biochemicals (Cleveland, OH). None of the compounds contained impurities when assayed alone by HPLC. Caffeic, *p*-coumaric, and ferulic acids each exhibited two peaks when chromatographed singly, one each for the *cis* and *trans* isomers. High-purity glass-distilled solvents were obtained from Alltech Associates (Houston, TX).

### Instrumentation

A Beckman model 334 gradient liquid chromatograph (Beckman Instruments, Palo Alto, CA) was used in the studies. The column was a nonpolar Rsil, C18 HL (10- $\mu$  diameter particles), of 4.6 mm (i.d.)  $\times$  5 cm slurry-packed guard column (C18 packing, 10- $\mu$

<sup>1</sup> Presented at the AACC 66th Annual Meeting, Denver, CO, October 1981.

TABLE I  
Genotypes and Kernel Structure of Seven Sorghum Varieties with Varying Resistance to Attack by Mold

Sorghum Variety	Genotype <sup>a</sup>	Testa	Pericarp Color	Mesocarp Thickness	Endosperm	Endosperm <sup>b</sup> Texture	Resistance to Molds <sup>c</sup>	
							Molding	Weathering
IS2327	RRyyIISSb <sub>1</sub> b <sub>1</sub> B <sub>2</sub> B <sub>2</sub> ZZ	—	white	...	white	2	1.7	3.1
CS3541	RRyyIISSb <sub>1</sub> b <sub>1</sub> B <sub>2</sub> B <sub>2</sub> ZZ	—	white	thin	white	3	2.5	3.3
SC0748	rrYYIISSb <sub>1</sub> b <sub>1</sub> B <sub>2</sub> B <sub>2</sub> ZZ	—	lemon yellow	thin	white	3	2.3	2.5
SC0630	RRYYIISSb <sub>1</sub> b <sub>1</sub> B <sub>2</sub> B <sub>2</sub> ZZ	—	red	thin	white	3	2.0	1.5
Tx2536	RRyyiiSSb <sub>1</sub> b <sub>1</sub> B <sub>2</sub> B <sub>2</sub> ZZ	—	white	thin	yellow	3	4.3	5.0
Tx623	RRyyiiSSb <sub>1</sub> b <sub>1</sub> B <sub>2</sub> B <sub>2</sub> ZZ	—	white	thick	white	3	3.0	3.8
SC0719	RRYYIISSB <sub>1</sub> B <sub>1</sub> B <sub>2</sub> B <sub>2</sub> ZZ	+	red	thick, chalky	white	5	1.5	1.0

<sup>a</sup>This is the currently proposed genotype for kernel characteristics. These may change slightly as new information is obtained.

<sup>b</sup>1 (corneous) to 5 (floury).

<sup>c</sup>1 (excellent resistance) to 5 (very poor resistance).

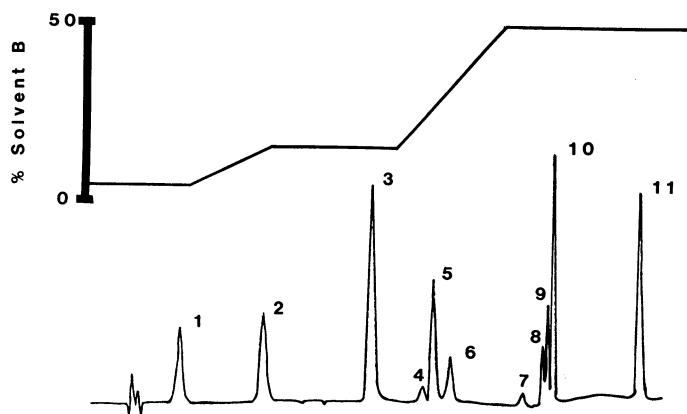


Fig. 1. Chromatographic conditions and the separation of the phenolic acid standards. 1, Gallic acid; 2, protocatechuic acid; 3, *p*-hydroxybenzoic acid; 4, vanillic acid; 5, 6, caffeic acid; 7, 8, *p*-coumaric acid; 9, 10, ferulic acid; 11, cinnamic acid. Solvent A = Acetic acid-water (2:98); solvent B = butanol-methanol (8:92).

diameter particles) from Alltech Associates. Detection was by ultraviolet absorption at 254 nm using a Beckman model 153 analytical UV detector. Retention times and peak areas were obtained with a Hewlett Packard 3390A reporting integrator (Avondale, PA).

#### Separation Procedure

To optimize separation and to calculate chromatographic parameters, each benzoic and cinnamic acid standard was dissolved in methanol at  $1.0 \times 10^{-4} M$ . Samples of 10  $\mu$ l were chromatographed singly and as mixtures at a flow rate of 1 ml/min.

Optimum separation was obtained by a multistep gradient (Fig. 1) of the following solvent mixtures: A, acetic acid-water (2:98); and B, butanol-methanol 8:92). The separation was programmed isocratically for 10 min at 5% solvent B, followed by a 7.5 min linear gradient to 15% solvent B. This intermediate mixture was then programmed isocratically for 13.5 min, followed by a 10-min linear gradient to 50% solvent B. Acetic acid was present in the solvent to lower the pH and, thereby, suppress ionization of the carboxyl hydrogens of the benzoic and cinnamic acid nuclei.

#### Sample Extraction for Free Phenolic Acids

Each sample was ground in a Udy laboratory mill (Udy Company, Boulder, CO) to pass through a 1.0-mm-diameter screen. Five grams of ground sample were extracted by vigorous shaking for 30 min in 20 ml of 100% methanol. The solid material was removed by centrifugation and the extraction repeated. The pooled extracts were reduced to near dryness under vacuum at 30–35°C and dissolved in 102 ml of spectral grade methanol. This extract was applied to a 1  $\times$  0.8 cm column (C18 Sep-Pak Cartridges, Waters Associates). High molecular weight polyphenols in the sample were absorbed and retained as a yellow-

brown band in the top few millimeters of the column. After applying the sample, the column was washed with 1.5 ml of methanol to elute cinnamic and benzoic acids. The final volume of the extract was measured and filtered through a 0.45- $\mu$ m pore (millipore) size filter. A 10- $\mu$ l sample was applied to the HPLC column for analysis.

#### Sample Extraction for Bound Phenolic Acids

The residue from the free phenolic acid extraction was hydrolyzed for 1 hr with 20 ml of 2N HCl in a boiling water bath. The hydrolysate was allowed to cool to room temperature and was centrifuged to remove insoluble residue. Both the supernatant and the residue were extracted twice with 20 ml of ethyl acetate to selectively remove phenolic acids, and the extracts were pooled. The pooled extracts were vacuum-evaporated to dryness and dissolved in 2 ml of spectral-grade methanol. The resulting extract was filtered and chromatographed as above.

#### Colorimetric Analysis of Levels of Phenolic Compounds

Total free phenolic compounds were determined by the method of Price and Butler (1977). Total bound phenolic compounds were determined by acid hydrolysis as described above. The hydrolysate was neutralized to a pH of 7.0 with 0.1N NaOH. The resulting solution (0.1 ml) was analyzed by the Prussian blue method of Price and Butler (1977). Protocatechuic acid was used as a standard for determining phenol levels.

## RESULTS AND DISCUSSION

#### HPLC Separation of Substituted Benzoic and Cinnamic Acids

Figure 1 illustrates the chromatographic separation of the eight standards. At a flow rate of 1 ml/min, analysis time was approximately 52 min. Baseline resolution was achieved with most compounds. No appreciable baseline shift occurred due to absorbance by solvents.

Table II lists the retention data and the calculated resolution achieved by the separation. An  $R_s$  value of 1 (2% band overlap) is routinely accepted as satisfactory separation (Johnson and Stevenson 1978). The compounds investigated have  $R_s$  values of or greater than 1, with the exception of the *cis* isomers of *p*-coumaric and ferulic acids. In a multicomponent system, capacity factor ( $k'$ ) values should cluster around the optimum, ie,  $1 \leq k \leq 10$  (Murphy and Stutte 1978). Only three of the 11 peaks (27%) fall outside of this range, and then only slightly ( $1.01 \leq k \leq 12.36$ ).

Elution was in order of decreasing polarity, typical of reverse-phase chromatography (Johnson and Stevenson 1978). Figure 2 shows the relationship between the structure of the benzoic and cinnamic acids and their order of elution. The addition of a methoxy group or the loss of a hydroxy group decreases polarity within each class (benzoic vs cinnamic). The presence of the ethylenic side chain of cinnamic acid also decreases polarity. The  $R_s$  of related pairs was: 7.18 for protocatechuic acid vs caffeic acid; 4.12 for *p*-hydroxybenzoic acid vs *p*-coumaric acid; and, 4.09 for vanillic acid vs ferulic acid. Separation was also dependent on the number of hydroxyl groups on the aromatic nucleus, gallic

acid (3-OH) vs protocatechuic acid (2-OH),  $R_s = 6.80$ , and, protocatechuic (2-OH) vs *p*-hydroxybenzoic acid (1-OH),  $R_s = 7.95$ . Reproducibility of the separation was acceptable. The coefficient of variation (CV) for 10 of the peaks was 2.6 or less (Table II). These values are only slightly higher than those reported by Murphy and Stutte (1978) with similar samples and chromatographic conditions. The CV decreased as retention time ( $t_R$ ) increased because of the sharper peaks obtained by the use of gradient elution (increase in solvent strength over time). The reproducibility of peak areas (Table III) was not as good as that of retention time, but was acceptable. For eight of the peaks, CV for the area was less than 10% (2.0–9.3%). Of the remaining three standards, two were near 10% (11.6–12.9%), and the third had a CV of 17.7%. All three of these peaks correspond to compounds with low molar absorptivities at 254 nm. This resulted in small peaks, which were affected more by detector noise.

### Extraction Efficiency

Extraction recoveries of the benzoic and cinnamic acid standards by both the free and bound acid extraction procedures are shown in Table IV. A known amount of each compound was added, as a standard addition, to a ground sample of CS3541 grain (for free acid extraction) or to the residue of a methanol-extracted sample of CS3541 (for bound acid extraction). Spiked samples were then processed as previously described. The percent recovery of each standard was calculated as the amount recovered divided by amount added.

Extraction of free phenolic acids gave recoveries of 83.0–105.1% with CVs of 1.1–14.9%. In general, the recoveries for the extraction of free phenolic acids were good. The recovery of ferulic and cinnamic acids was lower than the other standard compounds. This may be because they are destroyed during acid hydrolysis (Krygier et al 1982). It was not possible to calculate the recovery of *p*-coumaric acid added to ground grain because it was eluted on the upslope of a large, broad peak caused by a number of unknown compounds present in the grain extract. This, along with its low molar absorptivity did not allow the area to be determined accurately. However, recovery of *p*-coumaric acid was good (90% with a CV of 4.7%) when the extractions were performed on a solution of the acid alone, rather than as a standard addition to a grain sample.

Subjecting spiked samples to the extraction procedure for bound

phenolic acids resulted in recoveries of 56.3–99.8% with CVS of 1.0–9.4%. These recoveries are lower than those from the extraction of free phenolic acids. The larger amount of sample manipulation required by the bound acid extraction procedure decreased precision. A flocculant precipitate that is not soluble in either phase is present at the interface of ethyl acetate and water, which makes them difficult to partition cleanly. Some of the phenolic acids may be caught in this precipitate. The lower recoveries may also be the result of losses due to acid hydrolysis (Krygier et al 1982).

### Phenolic Acids in Sorghum

The amounts of the eight standard phenolic acids are shown in

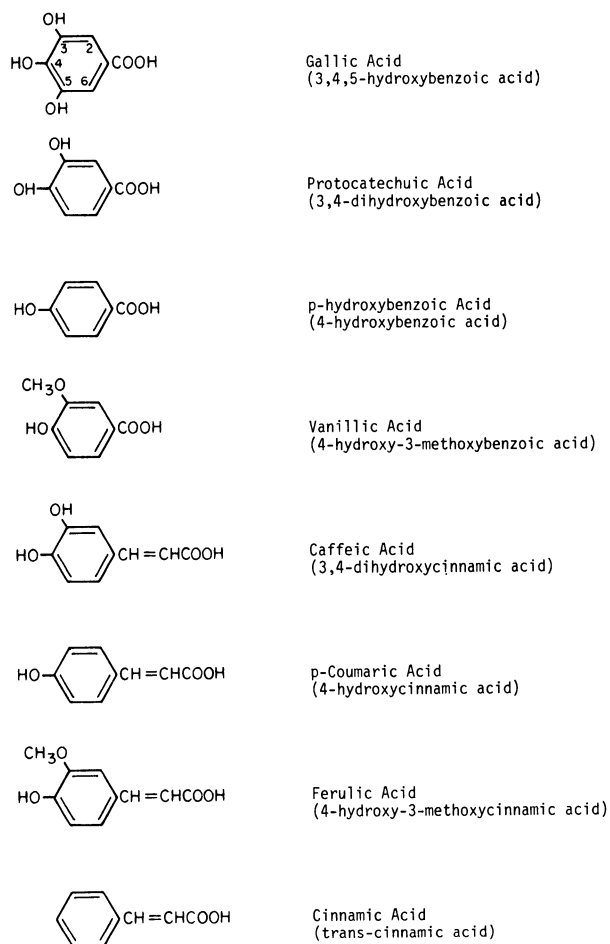


Fig. 2. The structural relationship of the benzoic and cinnamic acid standards to their elution order.

TABLE II  
Retention Times ( $t_r$ ) with Coefficients of Variation (CV),  
Capacity Factors ( $K'$ ), Separation Factors ( $\alpha$ ), and Resolution ( $R_s$ )  
of Substituted Benzoic and Cinnamic Acids

Acid Compound	$t_r^b$ (min)	CV (%)	$k'$	$\alpha$	$R_s$
Gallic	7.92	5.2	1.01	2.92	6.80
Protocatechuic	15.60	2.6	2.95	1.89	7.95
<i>p</i> -Hydroxybenzoic	25.97	2.6	5.57	1.24	2.34
Vanillic	31.29	1.3	6.92	1.04	0.47
Caffeic	32.34	1.4	7.19	1.06	0.69
	33.95	1.4	7.59	1.24	2.42
<i>p</i> -Coumaric	41.15	0.5	9.42	1.06	0.71
	43.33	0.3	9.97	1.01	0.12
Ferulic	43.74	0.2	10.07	1.01	0.12
	44.30	0.3	10.22	1.20	2.14
Cinnamic	52.52	0.3	12.30		

<sup>a</sup>Flow rate of 1 ml/min;  $t_0 = 3.95$  min.

<sup>b</sup>Average of eight runs.

TABLE III  
Integrated Peak Areas and Coefficients of Variation (CV)  
of HPLC-Separated Phenolic Acids

Acid Compound	Area <sup>a</sup>	CV (%)
Gallic	58,099	9.3
Protocatechuic	91,424	2.0
<i>p</i> -Hydroxybenzoic	159,190	4.9
Vanillic	12,563	17.7
Caffeic <sup>b</sup>	84,527	8.6
	35,520	3.9
<i>p</i> -Coumaric <sup>b</sup>	6,714	12.9
	18,818	7.8
Ferulic <sup>b</sup>	32,209	11.6
	86,598	6.1
Cinnamic	111,418	3.4

<sup>a</sup>Average of four runs.

<sup>b</sup>Both *cis* and *trans* isomers.

Table V. In addition to these phenolic acids, there were 12 additional peaks present in the grain extract that were not identified. Five were found only in the free extract, two only in the bound extract, and five in both extracts. There is a difference in both distribution and amounts of both the identified and unknown compounds between sorghum varieties (Fig. 3).

### Phenolic Acids and Resistance to Fungal Attack

The relative ratings of resistance to molding and weathering shown in Table I are subjective measurements taken in the field by experienced personnel.<sup>2</sup> Sorghum varieties rated resistant to molding and weathering contained more of the eight phenolics for which we had standards. There was little difference in the distribution or amounts of those eight compounds in the bound form.

Castor and Frederiksen (1980) observed that sorghums resistant to one genus of fungus or mode of fungal attack were not necessarily resistant to other genera or modes of attack. This suggests that the resistance mechanisms in sorghum are specific to the genus of fungi and/or mode of fungal attack. The sorghums in this study may have different resistance mechanisms. This would explain the variation in phenolic acid content among resistant varieties. There was no difference in the total free or bound phenols measured by the method of Price and Butler (1977) between nonbrown sorghums (Table VI). This may indicate that specific compounds, the release of bound phenols upon fungal invasion, or both may be important in imparting resistance.

SC0719 and SC0630 are both relatively resistant to molding and weathering yet contain low levels of phenolic acids. In fact, the phenolic acid distribution and content of these varieties were similar to the susceptible varieties analyzed. The resistance of SC0719 may be explained, in part, by the presence of a pigmented testa layer containing condensed tannins. The method of Price and

Butler (1977) shows that it has a high level of phenolic compounds, most of which are not phenolic acids. The ability of tannins to bind and precipitate proteins (Butler et al 1979) and inhibit enzyme activity may give them antifungal properties. The resistance in SC0719 is, therefore, probably due to the presence of tannins and other phenolic compounds rather than phenolic acids.

Like SC0719, SC0630 has a red pericarp, but it has no testa layer and contains low levels of tannins. Its resistance does not appear to be due to tannins or phenolic acids. The pericarp does contain red pigments that may be flavonoid compounds that inhibit fungal

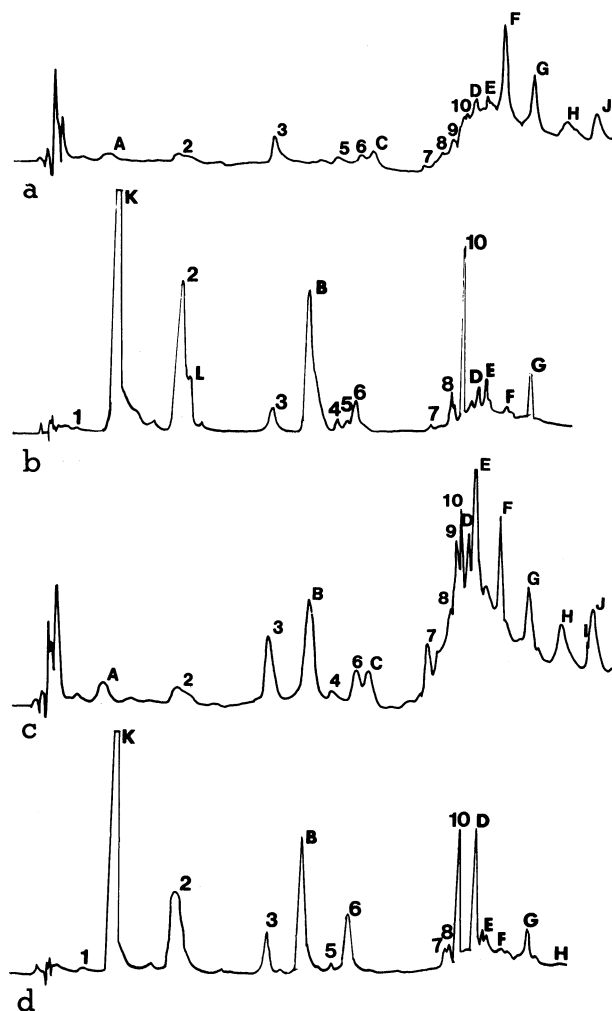


Fig. 3. Chromatograms of the free and bound phenolic acids of two sorghum varieties. a, Free phenolic acids of Tx2536; b, bound phenolic acids of Tx2536; c, free phenolic acids of SC0748; d, bound phenolic acids of SC0748. The numbers 1-11 denote the standards in Figs. 1 and 2. A-K denote major peaks that have not been identified.

<sup>2</sup>Personal communications. 1981. F. R. Miller, sorghum breeder, Texas A&M University, College Station, and D. T. Rosenow, Texas Agricultural Experiment Station, Lubbock.

TABLE IV  
Recovery of Standard Phenolic Acids When Added to Ground Grain

Acid Compound	Free Extraction <sup>a</sup> Procedure		Bound Extraction <sup>b</sup> Procedure	
	Recovery (%)	CV (%)	Recovery (%)	CV (%)
Gallic	93.5	14.9	56.3	4.4
Protocatechuic	100.5	7.0	69.0	9.3
<i>p</i> -Hydroxybenzoic	101.2	6.2	76.7	3.9
Vanillic	89.5	12.2	99.8	9.4
Caffeic	105.1	2.7	58.6	2.0
<i>p</i> -Coumaric	... <sup>c</sup>	... <sup>c</sup>	79.2	1.0
Ferulic	87.8	2.0	64.7	2.9
Cinnamic	83.0	1.1	72.6	1.0

<sup>a</sup>Average of six values.

<sup>b</sup>Average of four values.

<sup>c</sup>Peak area could not be determined accurately.

TABLE V  
Free and Bound Phenolic Acid Composition of Several Sorghum Varieties

Peak No.	Acid Compound	Sorghum Varieties ( $\mu\text{g/g}$ , dry weight basis)													
		SC0748		IS2327		CS3541		SC0719		SC0630		Tx623		Tx2536	
		Free	Bound	Free	Bound	Free	Bound	Free	Bound	Free	Bound	Free	Bound	Free	Bound
1	Gallic	...	13.2	...	...	...	19.7	...	26.1	...	46.0	...	12.9	...	23.5
2	Protocatechuic	11.0	11.5	7.0	98.4	7.4	133.9	8.0	15.8	13.0	83.0	6.3	13.2	9.7	218.3
3	<i>p</i> -Hydroxybenzoic	10.1	23.7	2.3	23.6	4.0	11.4	9.3	24.2	6.7	16.0	2.4	14.2	6.7	15.2
4	Vanillic	15.5	...	126.7	...	8.3	...	23.3	27.4	7.7	19.2	9.2	41.5	...	65.6
5,6	Caffeic	6.0	44.6	6.3	17.0	3.4	22.2	8.7	26.8	4.1	48.0	10.5	20.4	5.8	36.9
7,8	<i>p</i> -Coumaric	109.1	123.0	47.4	53.5	45.7	138.5	6.4	79.9	13.5	72.5	34.7	38.0	6.4	69.3
9,10	Ferulic	74.0	213.0	18.2	139.0	45.4	297.2	26.0	91.9	8.9	95.7	45.7	136.5	25.5	169.5
11	Cinnamic	4.7	...	2.0	...	9.4	...	...	19.7	10.7	...	...	...	...	...

**TABLE VI**  
**Total Free and Bound Phenolic Content of Several Sorghum Varieties<sup>a</sup>**

	Phenolic Content	
	Free <sup>b,c</sup>	Bound <sup>b,c</sup>
SC0748	733 <sup>b</sup>	1,280 <sup>c</sup>
IS2327	683 <sup>b</sup>	1,210 <sup>c</sup>
CS3541	558 <sup>b</sup>	1,160 <sup>c</sup>
SC0719	2,892 <sup>d</sup>	820 <sup>c</sup>
SC0630	850 <sup>b</sup>	950 <sup>c</sup>
Tx623	... <sup>d</sup>	1,240 <sup>c</sup>
Tx2536	592 <sup>b</sup>	1,170 <sup>c</sup>

<sup>a</sup> As measured by the method of Price and Butler (1977).

<sup>b</sup> Measured as protocatechuic acid equivalents.

<sup>c</sup> Those with the same letter are not significantly different at  $\alpha = 0.05$ .

<sup>d</sup> Value not available.

growth (Bell and Stipanovic 1979, Friend 1977). These compounds may be responsible for the fungal resistance of this variety.

The phenolic acids analyzed for in this study did not correlate well with fungal resistance. The ability of sorghum to resist fungal attack does not appear to be due to a single factor, but is most likely the result of the combination and interaction of many factors. These factors include both the chemical and physical properties of the grain.

#### ACKNOWLEDGMENTS

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