2,3-Di-(*E*)-caffeoyl-(2*R*,3*R*)-(+)-tartaric Acid in Terminals of Peanut (*Arachis hypogaea* L.) Varieties with Different Resistances to Late Leaf Spot Disease [*Cercosporidium personatum* (Berk. & M. A. Curtis) Deighton] and the Insects Tobacco Thrips [*Frankliniella fusca* (Hinds)] and Potato Leafhopper [*Empoasca fabae* (Harris)]

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Peanut (*Arachis hypogaea* L.) terminals (partially opened vegetative quadrifoliate leaf buds) of varieties with varying levels of resistance to the late leaf spot disease (LLSD) fungus [*Cercosporidium personatum* (Berk. & M. A. Curtis) Deighton] and the insects tobacco thrips [*Frankliniella fusca* (Hinds)] and potato leafhopper [*Empoasca fabae* (Harris)] were analyzed for their polyphenolic content. All varieties contained only one major polyphenol, which was isolated and identified as 2,3-di-(*E*)-caffeoyl-(2*R*,3*R*)-(+)-tartaric acid (levorotatory chicoric acid), previously reported in only one other plant species. Levels of *l*-chicoric acid were highest in newly emerged terminals (0.25% fresh wt) of Florunner (susceptible to LLSD) and Southern Runner (resistant) varieties and showed a steady decline (to 0.019%) as the leaves matured over 18 days. Levels of chicoric acid did not correlate with resistance to late leaf spot disease, tobacco thrips, or potato leafhopper.

Keywords: *Peanut, Arachis hypogaea L., 2,3-di-(E)-caffeoyl-(2R,3R)-(+)-tartaric acid (levorotatory chicoric acid), chicoric acid, disease, insect resistance*

INTRODUCTION

The production of crops with natural pest resistance has assumed major importance in recent years. Identification of compounds responsible for the resistance can be of great value in developing resistant plant varieties. Peanut germplasm resistance to most major peanut pathogens has been identified, and the cultivar Southern Runner was released as a cultivar with levels of partial resistance to the late leaf spot disease fungus [*Cercosporidium personatum* (Berk. & M. A. Curtis) Deighton] (Gorbet et al., 1986). It has also been found to have partial resistance to peanut rust (*Puccinia arachidis* Speg.) (Gorbet et al., 1986), southern stem not (*Selerotium rolfsii* Sacc.) (Arnold et al., 1988; Brenneman et al., 1990), and tomato spotted wilt virus (Culbreath et al., 1992). Southern Runner has also been shown to exhibit resistance to the insects tobacco thrips [*Frankliniella fusca* (Hinds)] and potato leafhopper [*Empoasca fabae* (Harris)] (Lynch, 1990). Although much work has been done on the chemistry of peanut seeds (*Arachis hypogaea* L.) (Ahmed and Young, 1982), little has been done on leaf chemistry in relation to pest resistance. Phenolics have been frequently implicated in plant resistance (Harborne, 1988). Consequently, we expected to observe higher levels of phenolics in the resistant variety. Recently, phenolics of peanut leaves have been investigated in relation to peanut resistance to the tobacco caterpillar [*Spodoptera litura* (Fab.)] (Singh and Sachan, 1992) and aphids [*Aphis craccivora* (Koch)] (Grayer et al., 1992). Also, cuticular lipids from leaves were characterized for wild and cultivated peanut species in relation to resistance to the fall armyworm [*Spordoptera frugiperda* (J. E. Smith)] and thrips (Yang et al., 1993). We have investigated the foliar polyphenolic content of peanut varieties with different levels of resistance to late leaf spot disease, tobacco thrips, and potato leafhopper.

MATERIALS AND METHODS

All solvents were analyzed reagent grade. Plants were grown in 1992 at the Coastal Plain Experiment Station, Tifton, GA, under standard cultural practices of fertilizer and weed control recommended for peanut production in Georgia (Johnson et al., 1987). Approximately 60 days after planting, vigorously growing plants were selected and their terminals marked and dated at the first sign of development for later sampling. At the appropriate date, terminals (usually consisting of four leaflets) were removed, placed in scintillation vials, covered with methanol, capped (Teflon-lined cap), and frozen (0 °C). Before analysis, the terminal leaves were cut into small pieces with scissors and ground with a Virtis tissue grinder (Virtis Co., Gardiner, NY).

Isolation and Identification of 2,3-Di-(*E*)-caffeoyl-(2*R*,3*R*)-(+)-tartaric Acid (Levorotatory Chicoric Acid). Approximately 100 g of Florunner terminals were slurried in methanol and filtered. The methanol/water solution was concentrated to a volume of about 200 mL. The residual solution was extracted with methylene chloride to remove chlorophyll and then adjusted to a pH of 3.0 with concentrated H_3PO_4. The water layer was then submitted to preparative reversed-phase column chromatography. The packing material from a Waters PrepPAK 500 C18 cartridge (Millipore Corp., Milford, MA) was repacked into an open tube column (2.54 × 50 cm). After addition of sample, the column was eluted with 500 mL of H_2O followed by 100 mL of 50% MeOH/H_2O to elute the chicoric acid. The last fraction was evaporated to dryness, dissolved in water, and chromatographed on a glass Cheminert LC column (109 × 1.25 cm; Valco

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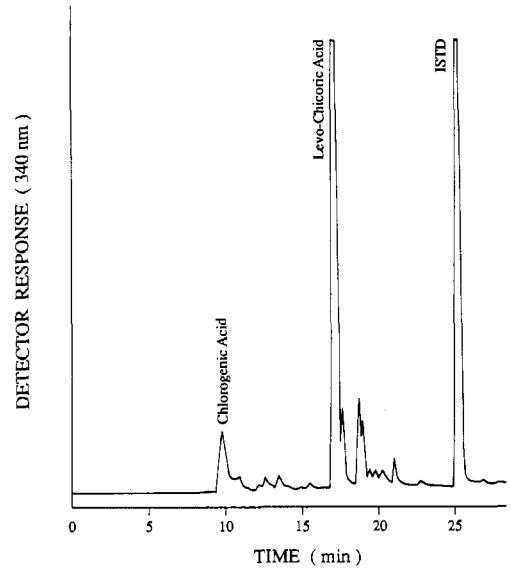

Figure 1. HPLC chromatogram of the polyphenols from Southern Runner peanut terminals; reversed-phase C18 column; 20–90% MeOH/H_2O (0.1% H_3PO_4) linear gradient in 35 min.

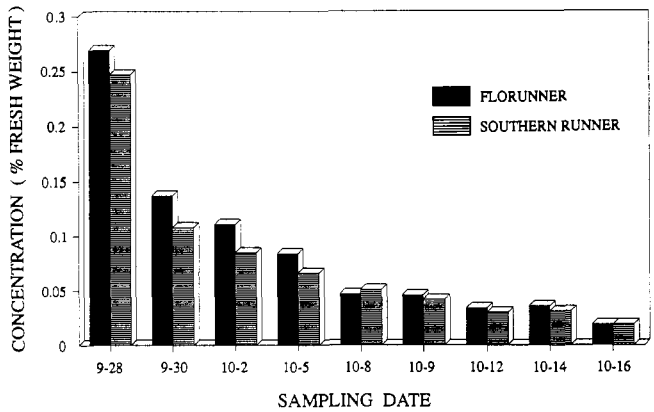

Figure 2. Levels of *l*-chicoric acid in terminals of late leaf spot disease resistant Southern Runner and susceptible Florunner at approximately 2-day intervals (average of 10 repetitions per variety).

Instruments Co., Inc., Houston, TX) packed with PrepPAK 500 C18 material. A linear gradient solvent program from water to methanol, over a period of 240 min, was used with a flow rate of 2 mL/min and effluent being monitored at 340 nm. Fractions containing the chicoric acid were evaporated to dryness, and the compound was recrystallized from water to yield 115 mg of 2,3-di-(*E*)-caffeoyl-(2*R*,3*R*)-(+)-tartaric (levorotatory chicoric acid): mp 203–204 °C (lit. mp 206 °C; Scarpati and Oriente, 1958); FAB-MS (negative ion mode) *m/z* 473 (M – H), 293 (M – H – caffeic acid), 179 (caffeic acid – H); optical rotation [α] –366.4° (*c*, 1.03 in methanol (lit. [α] –384.2°; Scarpati and Oriente, 1958). Partial acid hydrolysis (0.05 N HCl, 6 h at 110 °C) gave caffeic acid (UV spectrum identical to that of the authentic standard), with retention times correlated by HPLC and GC (silylated derivative TMS), tartaric acid, GC retention time (TMS derivative) correlated with that of the authentic standard, and monocaffeoyltartaric acid [GC/MS of TMS-caffeic acid *m/z* 396 (M^+); TMS-tartaric acid *m/z* 423 ($M^+ - CH_3$, spectra identical to those of the reference); TMS-monocaffeoyltartaric acid *m/z* 672 (M^+), 307 (caffeoyl acylium ion)]. The *l*-chicoric acid, found in methanolic extracts of terminals that were allowed to stand at room temperature for 1 week, was observed to produce a second compound, which eluted immediately after *l*-chicoric acid. Increased levels of the new compound were accompanied by a corresponding decrease in levels of *l*-chicoric acid, resulting in approximately equal concentrations of the two compounds. Although not isolated, it is likely that the second compound is the meso isomer of chicoric acid.

Table 1. Levels of Dicaffeoyltartaric Acid in Recommended Peanut Varieties versus Insect Resistance

variety	dicaffeoyltartaric acid[a] (% fresh wt)	mean insect rating[b] thrips	leafhopper
Florunner	0.330	4.2	5.8
Georgia Runner	0.259	4.0	6.8
NC-7	0.255	4.6	6.0
Southern Runner	0.227	4.0	5.7
Sunrunner	0.201	4.0	6.4
GK-7	0.201	4.0	6.4
GK-3	0.196	4.6	5.8
Florigant	0.189	4.8	6.2
NC-9	0.165	4.2	6.8
correlation coefficients[c]		–0.14 (ns)[d]	0.14 (ns)[d]

[a] 45-day-old leaves. [b] Insect damage rating scale (average of five repetitions): 1, no damage; 10, extensive damage. [c] Insect rating versus level of dicaffeoyltartaric acid. [d] Not significant.

Table 2. Levels of Dicaffeoyltartaric Acid in 46 Peanut Advanced Plant Resistance Varieties versus Insect Resistance

mean insect rating[a] thrips	leafhopper	dicaffeoyltartaric acid (% fresh wt)
3.50 (3)[b]	2.50 (1)[b]	>0.100–0.150 (1)[b]
3.75 (19)	3.00 (1)	>0.150-0.200 (8)
4.00 (17)	3.50 (2)	>0.200–0.250 (22)
4.25 (4)	3.75 (5)	>0.250–0.300 (10)
4.50 (1)	4.00 (5)	>0.300–0.375 (5)
4.75 (2)	4.25 (8)	
	4.50 (8)	
	4.75 (6)	
	5.00 (3)	
	5.25 (3)	
	5.50 (4)	
Correlation Coefficients		
0.007 (ns)	0.015 (ns)	

[a] Insect damage rating scale (average of four repetitions): 1, no damage; 10, severe damage. [b] Number of entries (of 46 total) with rating; 30-day-old leaves.

HPLC Analysis of *l*-Chicoric Acid in Peanut Terminals. Methanol extracts of peanut terminals were analyzed on a Beckman Ultrasphere C18 reversed-phase column, linear solvent gradient from 20% MeOH/H_2O to 90% MeOH/H_2O in 35 min (0.1% H_3PO_4 in both solvents, pH 2.5). Column effluent was monitored at 340 nm. Quantitation was done by the internal standard method using 5,7-dimethoxycoumarin.

GC Analyses. Gas chromatographic analyses were performed with an immobilized SE-54 (30-m X 0.3-mm i.d.) capillary column: injector, 250 °C; detector, 350 °C; temperature program, 100–300 °C at 8 °C/min. Analyses of liberated caffeic (retention time, 8.8 min) and tartaric acids (retention time, 14.8 min) were performed on trimethylsilylated derivatives (TMS) prepared from BSTFA/DMF (1:1) and heated at 75 °C for 30 min.

RESULTS AND DISCUSSION

Varieties of peanuts identified as resistant or susceptible to the late leaf spot disease fungus and the insects tobacco thrips and potato leafhopper were analyzed by HPLC for their polyphenolic content. Surprisingly, peanut terminals were found to contain only one major polyphenol in relatively large abundance (Figure 1). This compound was isolated by preparative HPLC and identified as dicaffeoyltartaric acid by UV spectrometry, FAB-MS, and hydrolysis and analysis of liberated caffeic and tartaric acids. The compound's optical rotation and melting point indicated it was 2,3-di-(*E*)-caffeoyl-(2*R*,3*R*)-(+)-tartaric acid (levorotatory chicoric acid). To our knowledge, the only other report of the levorotatory compound in nature is from *Echinacea purpurea* (Soicke et al., 1988). Singh and Sachan (Singh and Sachan, 1992) previously found

simple phenols, phenolic acids (including caffeic acid), and the polyphenol chlorogenic acid (caffeoylquinic acid) in 50-day-old, air-dried peanut leaves. Dicaffeoyltartaric acid was not reported by these authors, possibly due to its destruction during air drying.

Our previous studies have shown that caffeoyl esters have fungicidal activity (Snook et al., 1991, 1992). Therefore, levels of *l*-chicoric acid were determined in newly emerged terminals of resistant (Southern Runner) and susceptible (Florunner) varieties (to late leaf spot disease fungus) as well as in progressively older terminals (approximately 2-day intervals) for 18 days (Figure 2). Levels of the compound were similar in the terminals of resistant and susceptible varieties. Both varieties exhibited equal and maximum levels of *l*-chicoric acid (0.25% fresh wt) in newly emerged terminals followed by a steady decline as the leaves aged to the fully opened stage when levels fell to 0.019% fresh wt. Thus, elevated levels of *l*-chicoric acid were not found in Southern Runner and cannot account for the observed resistance. Similar decreases of chicoric acid with leaf age have been found in *Posidonia oceanica* (Cariello and Zanetti, 1979) and *Equisetum arvense* (Veit et al., 1991).

Various peanut lines have also been rated for resistance to tobacco thrips and potato leafhopper (Lynch, 1990). A number of these were analyzed for *l*-chicoric acid content. Levels of the compound in eight recommended peanut varieties are given in Table 1, while levels in 46 advanced plant resistant varieties are given in Table 2. Amounts of *l*-chicoric acid ranged from 0.375% to 0.141% fresh wt. However, levels of the compound were not found to correlate with resistance to tobacco thrips or potato leafhopper. Therefore, more research is needed on other chemical constituents of peanut leaves to define host-plant resistant factors.

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