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Short Communication

Leakage of Dhurrin and *p*-Hydroxybenzaldehyde from Young Sorghum Shoots Immersed in Various Solvents[1]

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ABSTRACT

Spectral scanning was used to provide estimates of the leakage of the cyanogenic glucoside, dhurrin (*p*-hydroxy-[*S*]-mandelonitrile-β-D-glucoside), and its metabolite, *p*-hydroxybenzaldehyde (*p*-HB), from young light-grown shoots of Atlas sorghum (*Sorghum bicolor* [L.] Moench) when these shoots were immersed in water, toluene, chloroform or mixtures of water and toluene or water and chloroform. Minimal leakage of dhurrin and virtually no leakage of *p*-HB occurred with water as the solvent. The 0.5% concentration (v/v) of both toluene and chloroform was more effective than either the 1.0 or 2.0% concentrations in effecting leakage of the two solutes. With either 0.5% toluene or 0.5% chloroform as the solvent, 80 to 90% of the total dhurrin was extracted from shoots in a 3-hour period. Breakdown of dhurrin during extraction was much more extensive with 0.5% chloroform than with 0.5% toluene. Some loss of *p*-HB occurred during 3- or 6-hour extractions in the water-organic solvent mixtures; spectral and chromatographic evidence suggested partial conversion of *p*-HB to *p*-hydroxybenzoic acid. With undiluted toluene or chloroform as solvents, extracts contained appreciable amounts of free *p*-HB but essentially no dhurrin. These solvents were less effective than the water-organic solvent mixtures in extracting the solutes from the shoot issue.

Weimberg *et al.* (6) observed that low mol wt solutes (sugars, amino acids, and inorganic ions) readily leaked from roots and leaves of young sorghum (*Sorghum bicolor* [L.] Moench) plants when the tissues were immersed in water containing a small amount (0.2 to 2% by volume) of toluene. With 0.5% toluene at 25°C, quasiquantitative leakage of the small mol wt solutes occurred in 3 h, but macromolecules were retained in the plant tissues. The authors concluded that toluene was able to diffuse rapidly into the plant cells and to render the membranes permeable to small molecules and ions.

Sorghum leaves, especially those of young seedlings, are characterized by the presence of the cyanogenic glucoside, dhurrin (*p*-hydroxy-[*S*]-mandelonitrile-β-D-glucoside), which is localized in the vacuoles of epidermal cells (4). The enzymes, dhurrin β-glucosidase and hydroxynitrile lyase, catalyze the breakdown of dhurrin to HCN, glucose, and *p*-HB;[2] these enzymes occur primarily in the mesophyll cells (4) and are thus separated from dhurrin in intact leaves. The leakage of dhurrin and its metabolite, *p*-HB, from young sorghum shoots immersed in water, toluene, chloroform, or mixtures of water and toluene or water and chloroform was investigated in the experiments reported in this paper.

MATERIALS AND METHODS

Seeds of the forage sorghum cultivar, Atlas, were planted in Hoagland solution-saturated vermiculite and were incubated at 27°C under continuous cool-white fluorescent light (about 150 $\mu E \cdot m^{-2} \cdot s^{-1}$). Water was added as needed to keep the vermiculite moist. Seven d after the seeds were planted, shoots were excised at the cotyledonary node and were sorted at random into groups of 25 or 30 shoots. Each group was weighed; the fresh weight per shoot for all experiments was 63 ± 1 mg (mean ± SE). Groups of shoots were then assigned to treatments which were imposed in 125-ml Erlenmeyer flasks. Shoots were cut transversely into two pieces to facilitate their introduction into the flasks. Solvents (100 ml except as noted) were then added to the flasks. For those flasks receiving mixtures of water and toluene or chloroform, 100 ml of water was first added to the tissue in the flask, then the organic solvents were added. As in the report of Weimberg *et al.* (6), solvents consisting of 0.5, 1.0, or 2.0 ml of toluene or chloroform in 100 ml of water are referred to as 0.5, 1.0, or 2.0% toluene or chloroform, respectively. Filled flasks were immediately placed on a reciprocating shaker (about 100 cycles per min, 5-cm stroke) where they were incubated at room temperature (about 27°C). One-ml samples were removed at specified times for spectral scanning. For those treatments involving pure toluene or chloroform, 1.0 ml of water was added to each 1.0-ml sample, and the organic solvent was then evaporated by placing the tube in a water bath at about 55°C and directing a gentle stream of air on the liquid while constantly rotating the tube. Evaporation was stopped when the organic phase appeared to be completely vaporized. Each 1-ml sample was diluted with 9 ml 0.1 N NaOH and immediately scanned from 400 to 240 nm with a Perkin-Elmer Lambda 3 spectrophotometer equipped with a Model 561 recorder.[3] The basic solutions were then held at room temperature for 3 h to permit hydrolysis of any dhurrin present

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[2] Abbreviations: *p*-HB, *p*-hydroxybenzaldehyde; *p*-HBA, *p*-hydroxybenzoic acid; A_m, molar absorbance.

[3] Mention of a trademark or proprietary product does not constitute a guarantee or warranty of the product by United States Department of Agriculture or the University of Nebraska, and does not imply its approval to the exclusion of other products that may also be suitable.

in the extracts, after which spectra were again scanned. Because the volume removed in sampling was small relative to the total volume of liquid in the flasks, no attempt was made to correct for volume changes.

At the completion of incubation, solvents were decanted, and the tissue residue in each flask was washed by suspending it in 100 ml of water for about 5 min and then decanting the water. Washed residues were suspended in 100 ml of water and were autoclaved at about 120°C for 30 min to extract and hydrolyze any dhurrin remaining in the tissue (2). Also, for all solvents except pure toluene and chloroform, 20-ml portion of the final extract was autoclaved 30 min to hydrolyze the dhurrin contained therein, to permit a comparison of this method of hydrolysis with basic hydrolysis at room temperature.

RESULTS AND DISCUSSION

The spectra shown in Figure 1 are typical of extracts prepared by incubating 7-d-old Atlas shoots in water, 0.5% toluene, or 0.5% chloroform for 1 h. Dhurrin in basic solution has an absorbance maximum and an A_m of 1.51×10^4 at 255 nm; *p*-HB in base has a maximum and an A_m of 2.74×10^4 at 330 nm (5). Each initial scan (Fig. 1) had a peak near 255 nm, suggesting the presence of dhurrin. Little if any free *p*-HB was present initially in the water extract, as shown by the very low A_{330} reading and the absence of a peak at this wavelength. Also the dhurrin content of this extract was low as shown by the relatively

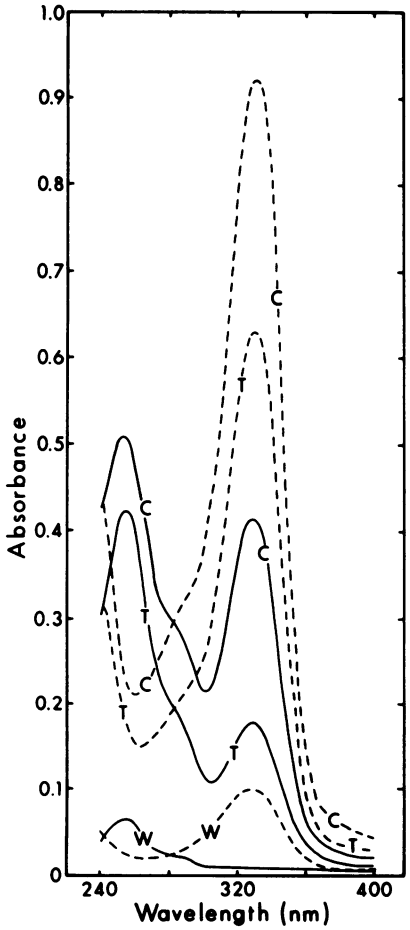

FIG. 1. Absorbance spectra of water (W), 0.5% toluene (T), and 0.5% chloroform (C) extracts of 7-d-old Atlas sorghum shoots. Extraction time, 1 h; solid lines, scans made immediately after extracts were diluted 1:10 in 0.1 N NaOH; dashed lines, same solutions scanned 3 h after dilution in base. See text for further details.

low peak at 255 nm before hydrolysis and 330 nm after hydrolysis. Both 0.5% toluene and 0.5% chloroform extracted dhurrin much more effectively than water, and some of the dhurrin was degraded when these solvents were used, as shown by the 330-nm peaks in the initial scans. Dhurrin extraction and breakdown were most extensive when 0.5% chloroform was used.

Results of extractions with three concentrations of toluene and three of chloroform are shown in Table I, experiment 1. All values shown in Table I are based on A_{330} readings from spectral scans, and all are expressed in relative terms with a value of 100 assigned to the sum of the content of the final water extract ('total' value for this extract, hydrolysis by autoclaving; values derived from alkaline hydrolysis were very similar to those based on autoclaving) plus the content of the residue remaining after this extraction. For both toluene and chloroform, the 0.5% level was the most effective in extracting dhurrin and *p*-HB. This observation agrees generally with the report of Weimberg *et al.* (6) who found that rates of leakage of low mol wt solutes were highest at toluene concentrations between 0.5 and 1.0%. Dhurrin breakdown was more extensive in the chloroform-containing solvents than in those containing toluene, but in both types of solvent, most of the dhurrin remained intact. More dhurrin was left in the tissue residue by the toluene-containing solvents than by those containing chloroform. However, total values (residue plus extract) were somewhat less for the chloroform-containing solvents, especially at the 1.0 and 2.0% concentrations.

In experiment 2 (Table I), shoots were extracted with water, 0.5% toluene, and 0.5% chloroform, and samples for assay were withdrawn at various times. With water as the solvent, contents of both free and total *p*-HB in the extract rose steadily throughout the 6-h incubation period. However, only 18% of the total *p*-HB was extracted. When either 0.5% toluene or 0.5% chloroform was the solvent, contents of free *p*-HB in the extract rose to a maximum at about 1 h and then declined, suggesting the conversion of *p*-HB into other products. At most sampling times, contents of free *p*-HB in the extracts were two to three times as great with 0.5% chloroform as with 0.5% toluene. The fraction of total *p*-HB remaining in the tissue residue after 6 h was not more than about 10% with either of these two solvents, agreeing in general with the conclusion of Weimberg *et al.* (6) that with 0.5% toluene as the solvent, leakage of small mol wt solutes was quasiquantitative in 3 h at 25°C. Weimberg *et al.* also called attention to the possibility that during the 3-h extraction period some degradation of the solutes by cellular enzymes might occur. The presence of free *p*-HB in extracts and the apparent loss of free *p*-HB during the longer extraction periods indicate that solute modifications indeed occurred in the present experiments.

Further evidence of solute modification was seen in the fact that in many of the water-toluene and water-chloroform extracts resulting from incubation periods longer than 1 h, a spectral peak near 280 nm was observed (data not shown). Paper chromatographic evidence indicated that the substance responsible for this peak was *p*-HB, which was probably formed by the oxidation of *p*-HB. This conversion may account for some of the decline in free *p*-HB observed in the time series with 0.5% toluene or chloroform.

Undiluted toluene and chloroform also were used as solvents (Table I, experiment 3). With the pure organic solvents, almost all of the *p*-HB that appeared in the extracts was present in the free form rather than as dhurrin, and larger amounts of *p*-HB and/or dhurrin were left in the tissue residue than when the water-organic solvent mixtures were used. These results suggest that the organic solvents, especially chloroform, provided effective contact between dhurrin and its degradative enzymes, but that, not surprisingly, they were not effective in extracting dhurrin from the plant tissue. There was extensive loss of *p*-HB during incubation periods exceeding 30 min, especially when chloro-

Table I. *Relative contents of p-Hydroxybenzaldehyde (p-HB) in Various Extracts and Tissue Residues of 7-Day-Old Atlas Sorghum Shoots*

Each experiment was replicated three times. Values shown are means ± SE, with the total content of the water extract plus residue taken as 100 in each experiment. Observed total *p*-HB concentrations (μmol/g fresh tissue, mean ± SE) for the water extractions were: experiment 1, 23 ± 1; experiment 2, 25 ± 1; experiment 3, 25 ± 1.

Experiment	Solvent	Extraction Time	Relative *p*-HB content		
			In extract		In tissue residue
			Free	Total	
	%	*min*			
1	Water	180	1.1 ± 0.2	11 ± 4	89 ± 4
	Toluene				
	0.5	180	10.0 ± 0.9	84 ± 2	20 ± 1
	1.0	180	9.2 ± 0.7	79 ± 4	27 ± 3
	2.0	180	7.4 ± 0.3	70 ± 1	30 ± 1
	Chloroform				
	0.5	180	28 ± 3	90 ± 4	8.1 ± 0.7
	1.0	180	26 ± 4	72 ± 2	6.1 ± 0.7
	2.0	180	21 ± 3	65 ± 3	9.4 ± 0.9
2	Water	0	0.3 ± 0.1	1.6 ± 0.2	
		15	0.6 ± 0.1	6.3 ± 0.6	
		30	0.8 ± 0.1	8.2 ± 0.7	
		60	0.9 ± 0.2	9.8 ± 0.8	
		180	1.2 ± 0.1	13 ± 1	
		360	1.3 ± 0.1	18 ± 2	82 ± 2
	Toluene				
	0.5	0	0.4 ± 0.1	2.3 ± 0.2	
		15	6.6 ± 1.2	24 ± 2	
		30	9.8 ± 2.3	38 ± 2	
		60	11.1 ± 2.4	54 ± 1	
		180	8.0 ± 0.2	82 ± 5	
		360	7.0 ± 0.9	95 ± 7	9.8 ± 1.9
	Chloroform				
	0.5	0	0.6 ± 0.1	3.2 ± 0.2	
		15	24 ± 3	45 ± 4	
		30	31 ± 3	65 ± 4	
		60	33 ± 2	82 ± 4	
		180	25 ± 1	88 ± 5	
		360	15 ± 2	83 ± 6	7.2 ± 1.2
3	Water	0	0.4 ± 0.03	1.2 ± 0.2	
		1	0.4 ± 0.1	2.2 ± 0.4	
		15	0.7 ± 0.1	5.3 ± 0.5	
		30	0.8 ± 0.1	6.5 ± 0.3	
		60	0.9 ± 0.1	8.2 ± 0.4	
		180	1.3 ± 0.1	14 ± 0.4	86 ± 1
	Toluene				
		0	2.3 ± 1.8	1.2 ± 0.1	
		1	1.2 ± 0.6	0.7 ± 0.3	
		15	7.1 ± 1.2	5.4 ± 0.6	
		30	7.3 ± 0.5	7.1 ± 0.4	
		60	4.7 ± 0.7	5.0 ± 0.5	
		180	1.0 ± 0.1	1.7 ± 0.1	73 ± 3
	Chloroform				
		0	0.6 ± 0.2	1.6 ± 0.4	
		1	6.4 ± 1.1	7.0 ± 1.1	
		15	52 ± 1	52 ± 1	
		30	47 ± 1	47 ± 1	
		60	33 ± 1	33 ± 1	
		180	4.5 ± 0.4	4.8 ± 0.5	39 ± 3

form was used. Spectral evidence (data not shown) did not suggest extensive conversion of *p*-HB to *p*-HBA in these extracts.

Woodhead *et al.* (7) reported that *p*-HB was a significant component of the epicuticular wax of sorghum seedlings, and that it occurred to some extent in the wax of all cultivars tested at all stages of growth up to flowering. Atkin and Hamilton (1) reported results similar to those of Woodhead *et al.* Immersion times were not clearly specified in either of these papers. On the basis of the present study as well as previous work (3) from this laboratory, it appears highly probable that the *p*-HB found in

chloroform extracts of Atlas and several other sorghum cultivars was not derived from epicuticular wax but rather resulted from the degradation of dhurrin from within disrupted epidermal cells. A very recent paper by Woodhead *et al.* (8) presents further evidence of free *p*-HB in wax from young shoots of sorghum cultivar 65D, but the report also states that 'this is clearly a restricted phenomenon with many cultivars having little or no free *p*-HB.

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