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EFFECT OF BORON DEFICIENCY ON POLYPHENOL PRODUCTION IN THE SUNFLOWER

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Abstract—Sunflower plants deficient in boron have been found to accumulate at least two major blue-fluorescing compounds. One of these has been identified as scopolin, and the other has been shown to be a glucose derivative of gentisic acid. Esculin, isoquercitrin, and scopoletin have also been identified for the first time in sunflowers.

INTRODUCTION

SIGNIFICANT accumulation of scopolin (7-glucosyloxy-6-methoxycoumarin) in tobacco tissues which had severe boron deficiency symptoms was demonstrated previously in this laboratory.[1] Other investigators[2, 3] have reported the accumulation of unidentified phenolic compounds in boron-deficient tissues. Perkins and Aronoff[4] reported the accumulation of caffeic and chlorogenic (3-caffeoylquinic acid) acids in boron-deficient tomato, lettuce, radish, and sunflower plants. The relationship between the accumulation of phenolic compounds and boron nutrition has not yet been clarified.

In order to obtain additional information on the phenolic glycoside accumulation in tissues exhibiting boron deficiency, polyphenols present in boron-sufficient and in boron-deficient sunflowers, *Helianthus annuus*, Russian Mammoth variety, were investigated.

RESULTS AND DISCUSSION

Comparison of chromatograms under u.v. light of extracts from low- and normal-boron tissues of the sunflower, clearly indicated that the low-boron tissue accumulated at least two major blue-fluorescing substances. One of these has been identified as scopolin, the same compound which accumulates in boron-deficient tobacco. The other compound has been tentatively identified as a glucose derivative of gentisic acid (2,5-dihydroxybenzoic acid). In addition to these two compounds, chlorogenic acid, esculin (6-glucosyloxy-7-hydroxycoumarin), isoquercitrin (3-monoglucoside of 3,3′,4′,5,7-pentahydroxyflavone), and scopoletin (7-hydroxy-6-methoxycoumarin) have been identified. The last three have not been previously reported in sunflowers, although Urban[5] has reported the presence of caffeic acid, chlorogenic acid, scopolin, and an unknown flavonoid compound in normal sunflower leaves.

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The phenolic compounds identified in the boron-deficient sunflower tissues, their R_f values and u.v. absorption maxima are recorded in Table 1. With the exception of the gentisic acid–glucose derivative (for which no authentic reference compound could be found in the literature or obtained for comparison), all the compounds corresponded chromatographically and spectrally with authentic reference compounds.

The gentisic acid–glucose compound had a bright blue–white fluorescence on paper, a fluorescence maximum at 449 mμ and an activation maximum at 335 mμ in methanol, and from its high R_f values in polar solvents and low values in non-polar solvents, it may be a diglucoside. This supposition is supported by the observation that addition of alkali to an alcoholic solution of the unknown did not produce a spectral shift, indicating a possibility

TABLE 1. R_f VALUES AND λ_{max} OF SOME PHENOLIC COMPOUNDS FROM *Helianthus annuus*

Compound	R_f in Solvent system*					λ_{max} (mμ)
	1	2	3	4	5	
Unhydrolysed						
Scopolin	0·58	0·77	0·84	0·60		250, 287, 338
Esculin	0·59	0·67	0·79	0·22		250, 292, 339
Chlorogenic Acid	0·63	0·61, 0·81	0·76, 0·83			245, 302, 329
Isoquercitrin	0·44	0·16	0·47			258, 360
Gentisic Acid-Glucose Derivative	0·41	0·85	0·82	0·14		311
Hydrolysed						
Scopoletin	0·87	0·41	0·67			
Esculetin	0·81	0·35	0·63			
				decomp.		
Gentisic Acid	0·88	0·69	0·73	0·26	0·25	333
Quercetin	0·64	0·00	0·05			

* The solvent systems used were: (1) *n*-butyl alcohol–acetic acid–water (6:1:2 by vol.); (2) isopropyl alcohol–formic acid–water, (5:0·1:95 by vol.); (3) aq. 15% acetic acid; (4) *n*-butyl alcohol–aq. 2 N ammonia (1:1, v/v); (5) benzene–acetic acid–water (6:7:3, by vol. upper phase).

that both its phenolic groups are substituted. Treatment of the gentisic acid–glucose compound on the paper chromatogram with a 1% aqueous solution of emulsin did not however result in hydrolysis.

The aglycone was identified as gentisic acid by its R_f values, bright blue fluorescence, lack of reactivity toward diazotized spray reagents and spectral comparison with authentic material. Armstrong *et al.*[6] reported that gentisic acid did not react with diazotized reagents, but Ibrahim and Towers[12] reported a color development with subsequent fading.

Although the presence of gentisic acid in several species of plants has been reported previously,[7,8] this is the first report to our knowledge of the occurrence of a glucose derivative. Whether this derivative is produced only under boron-deficient conditions, or merely accumulates under this condition, will need further clarification.

The finding of an increase of scopolin in sunflowers indicates that its accumulation in boron-deficient plants is not limited to tobacco.

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MATERIALS AND METHODS

Plants were grown under controlled environmental conditions[9] at Argonne National Laboratory. The low-boron plants received a total of 50 μg of boron per plant and the normal plants received a total of 500 μg of boron per plant. The plants from both treatments were harvested when severe boron-deficiency symptoms appeared on the low-boron plants.

Approximately 300 g of fresh leaf tissue from each treatment were macerated in hot 80% isopropyl alcohol in a Waring blendor. The pulverized tissues were exhaustively extracted by percolating further portions of fresh solvent through the tissue at room temperature until the effluent was colorless. The extract was concentrated under reduced pressure and the aqueous residue extracted with benzene to remove lipid material. The final volume of the aqueous phase was adjusted so that 1 ml represented 2–3 g of tissue. A small aliquot of each extract was applied to separate sheets of Whatman No. 1 chromatography paper and developed in the first direction with the *n*-butyl alcohol–acetic acid–water (BAW) (6:1:2, v/v/v) solvent system and in the second direction with the isopropyl alcohol–formic acid–water (IFW) (5:0·1:95, v/v/v) solvent system. Examination of the dried chromatogram under u.v. light indicated that the low-boron extract contained two major blue-fluorescent spots not clearly visible on the chromatograms of the normal boron extract. Other minor differences were also apparent.

One-ml aliquots of the final extract from the low-boron tissues were applied as a band across 100 9-in. strips of paper and developed in the BAW solvent system. Examination under u.v. light revealed at least 9 fluorescent zones on the dried chromatograms. Each zone was eluted separately with either 50% isopropyl alcohol or with the IFW solvent. Each eluate was concentrated and purified by rechromatographing with one or more of the solvent systems listed in Table 1 until a single zone was obtained. Chromatographic and spectral analyses were used to determine purity of each eluate.

An aliquot of each purified eluate suspected of containing a glycoside was concentrated to dryness under reduced pressure and heated for 1 min with 1 ml of 1% HCl. The cooled solution was extracted with small volumes of ethyl acetate. The two phases were separated by freezing the aqueous phase. The acid in the aqueous phase was removed by concentrating the solution to dryness *in vacuo*. The sugars present in the concentrates were identified by chromatography alongside known sugars and development of color on the chromatograms with *o*-aminobiphenyl spray reagent.[10]

The ethyl acetate extracts were reduced to dryness and the compounds present were identified by chromatographic, spectral and chromogenic color comparison with known specimens.

All u.v. absorption spectra were determined on the Beckman DK-1 recording spectrophotometer. The activation and fluorescence spectra were measured on the Aminco–Bowman spectrophotofluorometer.

Scopoletin and scopolin were synthesized in this laboratory by Braymer[11] and Zane[12] respectively.

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