Effects of N^6-Benzyladenine on Glycoalkaloids in Potatoes as Determined by Thin Layer Chromatography

Throughout most of their history the *Solanum* glycoalkaloids have been treated as a single entity under the term 'solanine'. In 1955, a second component, chaconine, was separated from a glycoalkaloidal extraction of *Solanum chacoense*[1]. Since then, many other glycoalkaloids have been isolated from members of the Solanaceae family. ZITNAK[2] summarized 16 such members including α-, β-, and γ-variations of solanine and chaconine, plus solacauline, leptines I and II, and leptinines I and II, all of which exhibited modifications of the saccharide moiety on the alkaloid, solanidine. Additional individuals built on other aglycones were included in the listing. Procedures were proposed for thin layer chromatographic (TLC) analysis of glycoalkaloids using several different extraction methods[3]. Traditional ammonia precipitation was thought applicable only for extractions of α-solanine and α-chaconine, leaving other possible glycoalkaloids untouched. Additional methods proposed were warm carbonate, hot water, and ethyl acetate extractions which would separate glycoalkaloids whose glycosidic properties may prevail over their alkaloidal behavior.

Chemical treatments have been effective methods for reducing glycoalkaloid formation[4–8]. In previous experiments, preharvest spraying of N^6-benzyladenine were examined as to their effective ability to augment glycoalkaloidal formation[9].

Russet Burbank potatoes were used in this study. They were washed, towel dried, divided into 4 groups, and subjected to vacuum injection with water solution of N^6-benzyladenine. After drying, each of the 4 treatments (0, 250, 500, and 1000 ppm N^6-benzyladenine) were divided in half. One half of each was illuminated for 7 days at 200 ft-c, 16 °C, and 60% R.H., while the rest were stored in darkness at 5 °C for 7 days.

In preparation for extraction, each illuminated potato was peeled on its light-exposed side with a peeler. The peeler removed 2 mm of outer layer with each cutting. The tubers stored in darkness were similarly peeled. The peelings were cut into small pieces with a knife and were quickly weighed into 5 g amounts. Four 5 g portions were obtained from each of the 8 treatments (0, 250, 500, and 1000 ppm N^6-benzyladenine, half illuminated and half stored in darkness). Extractions of glycoalkaloids were made for thin layer chromatograph according to methods of ZITNAK[3].

Glass plates (5 × 20 cm) were coated with a 0.25 mm silica gel-G adsorbant and then etched to provide a uniform solvent movement distance of 15 cm. Following activation at 120 °C for 1 h, each plate was spotted in 2 places. The left hand spot contained a standard of solanine, solanidine, or chaconine, while the right hand spot contained an unknown glycoalkaloid mixture 1 of the 8 samples described above. The plates were then developed with a solvent system of methanol: 1-butanol: acetic acid: water (60:15:15:10). After developing and drying, plates were exposed to iodine fume in a glass tank. The resolved spots were graded according to visual intensity and the Rf values were calculated.

As shown in the Table, all samples extracted with ammonia revealed 2 spots unknown. In most cases these separations matched the Rf × 100 values for the solanine and chaconine controls. However, the spots representing potatoes from the 250 ppm N^6-benzyladenine dark treatment were more intense than those derived from the 100 ppm N^6-benzyladenine treatment. All 3 concentrations of N^6-benzyladenine in the tubers exposed to the light showed equal amount of solanine and chaconine. It would

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Hot water extraction									Ethyl acetate extraction								
Dark				Light				Dark				Light				Rf
ppm N6BA				ppm N6BA				ppm N6BA				ppm N6BA				×
0	250	500	1000	0	250	500	1000	0	250	500	1000	0	250	500	1000	100
–				–				–				–				100
	4	2	3	3					5	5	5	5				
–	2				–	3	3	3	4	–	5	5	5	5	–	5	4	5	5	–	80
	2		1	1				1	3							5				
			1			1	1		1			1			1			1	1	1	
–					–					–						–				–	60
–					–					–						–				–	40
–					–					–						–				–	20
–					–					–						–				–	0

[a] Charted numerals from 1 to 5 represent relative optical intensities of the TLC spot at that location [1 = least and 5 = most].

Summary of thin-layer chromatographic data obtained through 4 extraction procedures for potato tubers which were dipped by suction infiltration in various concentrations of N^6-benzyladenine (N6BA).

		Ammonia precipitation								Warm carbonate extraction							
Rf		Dark				Light				Dark				Light			
×		ppm N6BA				ppm N6BA				ppm N6BA				ppm N6BA			
100		0	250	500	1000	0	250	500	1000	0	250	500	1000	0	250	500	1000
100	–					–				–				–			–
										3		4					
											2						
80	–					–				–				–		4	3 –
														2	3		
					2			5		2		2	3				
		2[a]	4			3			5								
60	–			3	2	–	5	4	4	–				–		3	–
						1										4	1
		1	4	2			4					1			3		
										3			4	3	4		4
40	–					–				–				–	5		–
													4				
20	–					–				–				–			–
0	–					–				–				–			–

seem that in this case N^6-benzyladenine promotes the formation of glycoalkaloids in the dark and is even more influenced to do so in the light. Others have noticed the potentiation of a cytokinin effect by red light[10].

The non-ammonia precipitation extracts produced spot separations of several unidentified glycoalkaloids. The warm carbonate extraction usually consisted of 3 separations per treatment. The dark reaction promoted an intense, high Rf value spot at 500 ppm N^6-benzyladenine. Of the compounds extracted by hot water, the 250 ppm N^6-benzyladenine-dark treatment seemed most inhibitory to glycoalkaloid formation. The 1000 ppm N^6-benzyladenine-light treatment led to an increase in visual density of the highest Rf entity and the apparent origin of an additional compound. The individuals appearing in the ethyl acetate extraction were generally unchanged by either light or dark or N^6-benzyladenine treatments, excepting the 2 dense spots from light-treated potatoes receiving no N^6-benzyladenine treatment.

As indicated with the ammonia extractions, low concentrations of N^6-benzyladenine promote the formations of α-solanine and α-chaconine even in tubers stored in darkness. This situation was less apparent in extractions of unknown glycoalkaloids which would indicate an N^6-benzyladenine interference with solanidine formation and its incorporation into the glycoalkaloidal structure. The other unidentified glycoalkaloids demonstrate some differences in spot separation and intensity from dark to light treatments and when under the influence of N^6-benzyladenine. Perhaps the formation of the glycoside moieties in light and dark situations are also affected by this cytokinin.

Zusammenfassung. Behandlung der Sprossknollen mit Benzyladenin fördert die Glykoalkaloidbildung der Solanaceen. Je nach Extraktionsmethode können unidentifizierte Alkaloide nachgewiesen werden.

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Enhancement of the Blood Pressure Activity of VAL^5- and ILE^5-Angiotensin II by Sodium and Calcium Ions[1,2]

Enhancement by certain cations of several biological activities of Val^5-angiotensin II-Asp^1-β-amide (Asn^1, Val^5-A II) has previously been demonstrated. When the peptide is dissolved in hypertonic or isotonic NaCl solution rather than hypotonic solution or distilled water its dipsogenic, antidiuretic and natriuretic effects are enhanced when it is injected into the third brain ventricle in goats[3,4]. Similar studies include rat, guinea-pig, and bird pressor activities and stimulation of isolated rat uterus and guinea pig ileum[5–7]. Certain additional mono- and divalent cations were found to be effective in enhancing the rat blood pressure (bp) activity of Asn^1, Val^5-A II[6,7].

In the present study we investigate whether the effects of NaCl and $CaCl_2$ are restricted to the amide derivative of angiotensin II or whether they are also observed with the natural hormones, Asp^1,Val^5-angiotensin II (Val^5-A II) and Asp^1-Ile^5-angiotensin II (Ile^5-A II).

Methods. The rat bp assay was performed on nephrectomized rats as previously described[7], except that the