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Amino Acid Composition of Cotton Nectar

Barbara W. Hanny* and Carroll D. Elmore

Amino nitrogen constitutes an average 0.04% (3.65 μmol/ml) of the extrafloral nectar of cotton (Gossypium hirsutum L.). Twenty-four amino acids were isolated from the nectar. Twenty of the amino acids were identified by gas chromatography of their n-acetyl n-propyl esters and thin-layer chromatography of their dansyl derivatives. Although the amino acid content of cotton nectar fluctuated quantitatively because of environmental conditions, the amino acid constituents were qualitatively consistent. The possibility of cotton nectar as a dietary source of amino acids for nectar-feeding insects is discussed.

The nectar from cotton (Gossypium hirsutum L.) has long been known as a source of food for many beneficial predator insects, as well as for parasites and insect pests of cotton (Trelease, 1879; Lukefahr and Rhyne, 1960; Butler, 1968; Butler et al., 1972). Thousands of cotton leafworm (Alabama argillacea Hubner) and American cotton bollworm (Heliothis armigera Hubner) moths have been observed feeding on the extrafloral nectaries of cotton (Trelease, 1879). The bollworm (Heliothis zea Boddie) and the honey bee (Apis mellifera L.) have also been reported as nectar feeders on cotton (Butler et al., 1972). Populations of cotton leafworm and cabbage looper (Trichoplusia ni Hubner) moths have been found to be seven to ten times higher on cotton with extrafloral nectaries than on nectariless cotton (Lukefahr and Rhyne, 1960).

It has been known since ancient times that nectar is used by anthophilous insects for the energy-providing sugars that it contains. It has been usually assumed that amino acids were obtained elsewhere. Recently, however, Baker and Baker (1973) surveyed nectar of 266 species of flowering plants growing in California and found that nectar contained sufficient concentrations of amino acids for the nutrition of certain insects. The fact that certain insects are known to feed on cotton nectar (Trelease, 1879; Lukefahr and Rhyne, 1960; Butler, 1968; Butler et al., 1972) suggested that cotton nectar should be examined as a potential source of amino acids. Clark and Lukefahr (1956) reported a partial analysis of cotton extrafloral nectar by paper chromatography. No amino acids were detected. Mound (1962) detected two ninhydrin-positive substances in cotton nectar, but amounts or identities were not determined.

This study was conducted to determine the kinds and amounts of amino acids present in cotton nectar and to discuss the implications of nectar as a source of amino acids for insect nutrition.

EXPERIMENTAL SECTION

Nectar Collection. Initially, separate analyses were made of extrafloral nectar collected from the Gossypium hirsutum L. cultivars Stoneville 213, Stoneville 7A, DPL-16, Stoneville 7A Frego, and Acala 1517-70, as well as nectar collected separately from subbracteal nectaries subtending bolls, squares, and flowers. Separate analyses of nectar collected from greenhouse- and field-grown cottons were also compared. No qualitative differences of amino acids were found among these various sources of extrafloral nectar. Therefore, G. hirsutum cv. Stoneville 213 was selected for replicate analyses, because it is the predominant cultivar in the immediate area. The nectar was taken directly from the extrafloral nectaries by 20-μl disposable glass micropipets. Analyses were performed immediately after collection. Separate analyses were performed on ten nectar samples collected at various times over a 4-month period.

Isolation. The cotton nectar was acidified, and the amino acids were isolated by chromatography on a miniature ion-exchange column of cationic resin (Harris et al., 1961). The eluted amino acid fraction was divided into two aliquots. One aliquot, to which a volume equivalent to 1 μmol/ml of phenylalanine (internal standard) was added, was used in the conversion of the amino acids to their stable volatile n-acetyl n-propyl esters. These were separated by gas chromatography (gc) (Graff et al., 1963; Coulter and Hann, 1968). The Perkin-Elmer Model 900 gas chromatograph used was equipped with dual 2 ft × $\frac{1}{8}$ in. stainless steel columns packed with Chromosorb G (H.P.), 80–100 mesh, coated with 0.7% PEG (6 M)–0.05% TCEPE, with flame ionization detectors and a Perkin-Elmer Model 56 recorder. The carrier gas (helium) flow was 60 ml/min, the injector temperature was 230°, the detector temperature was 260°, and the column temperature was programmed from 100 to 250° at 12°/min.

The second aliquot was used in the conversion of the amino acids to their stable dansyl derivatives, according to the method of Airhart et al. (1973). The dansylated amino acids were then separated by two-dimensional thin-layer chromatography (tlc) on Cheng-Chin polyamide sheets, according to the method of Woods and Wang (1967).

U. S. Department of Agriculture, Agricultural Research Service, Mississippi Agricultural and Forestry Experiment Station Cooperating, Southern Region, Cotton Physiology Laboratory, Stoneville, Mississippi 38776.

Table I. Concentration of Amino Acids in Cotton Nectar

Identity	Concentration, μmol/ml	
	Average[a]	Range
Cysteine	8.7×10^{-1}	7.9×10^{-1}–9.8×10^{-1}
Glutamic acid + glutamine[b]	6.7×10^{-1}	5.0×10^{-1}–8.0×10^{-1}
γ-Aminobutyric acid	1.9×10^{-1}	8×10^{-2}–3.0×10^{-1}
Glycine	1.2×10^{-1}	5×10^{-2}–2.7×10^{-1}
Methionine	1.2×10^{-1}	4×10^{-2}–2.1×10^{-1}
2,4-Diamino-butyric acid	5×10^{-2}	1×10^{-2}–1.1×10^{-1}
α-Aminoadipic acid	3×10^{-2}	8×10^{-3}–1.1×10^{-1}
Tyrosine	8×10^{-3}	2×10^{-3}–2×10^{-2}
Serine	8×10^{-3}	1×10^{-3}–2×10^{-2}
Ornithine	8×10^{-3}	1×10^{-3}–1×10^{-2}
Aspartic acid + asparagine[b]	6×10^{-3}	1×10^{-3}–1×10^{-2}
Proline	5×10^{-3}	1×10^{-3}–1×10^{-2}
Alanine	1×10^{-3}	6×10^{-4}–4×10^{-3}
Isoleucine	1×10^{-3}	8×10^{-5}–4×10^{-3}
Leucine	1×10^{-4}	6×10^{-5}–2×10^{-4}
Valine	1×10^{-4}	1×10^{-5}–2×10^{-4}
Lysine	1×10^{-4}	1×10^{-5}–2×10^{-4}
Arginine[b]	?	
Ammonia[b]	?	
4 unknowns	?	

[a] Ten separate nectar collections. [b] Detected by tlc.

Identification. Identity was established by cochromatography of n-acetyl n-propyl amino acid standards (obtained from Gallard-Schlesinger) with the n-acetyl n-propyl nectar amino acids by gc (Graff et al., 1963; Coulter and Hann, 1968). The n-acetyl n-propyl esters of arginine and histidine can be formed only after enzymatic and ozonolysis conversion processes, respectively; glutamine and asparagine are hydrolyzed by the acetylation procedure and are detected as their respective acids. Therefore, to determine if these amino acids were present in nectar, the dansyl derivatives of nectar amino acids were cochromatographed with dansyl amino acid standards (obtained from Sigma) by tlc (Woods and Wang, 1967; Airhart et al., 1973). Also, since certain amino acids were found to have similar gc retention times and overlapping tlc R_f values, both methods were necessary for identification. Several protein and nonprotein amino acid standards were tested, without success, in both identification systems in an attempt to identify the four amino acids listed as unknowns (Table I).

Quantitation. Amino nitrogen was determined by the ninhydrin assay of Yemm and Cocking (1955). Proteins were not detected by polyacrylamide gel electrophoresis of several random nectar samples.

Gc quantitation of the individual amino acids was performed according to the methods of Coulter and Hann (1968). Phenylalanine was not found in the nectar; therefore it was selected as the internal standard. Relative molar response of the individual amino acids to the internal standard was obtained with an Infotronics Automatic Digital Integrator Model CRS-208.

Quantitation of those amino acids detected only by tlc could not be made because of the lack of a spectrofluorometer (Table I).

RESULTS

Identities of the amino acids of cotton nectar were established by gc of their n-acetyl n-propyl esters and tlc of their dansyl derivatives (Figure 1).

Amino nitrogen was found to constitute an average 0.04% (3.65 μmol/ml) of the extrafloral nectar of cotton. Table I lists the average concentrations (micromoles/mil-
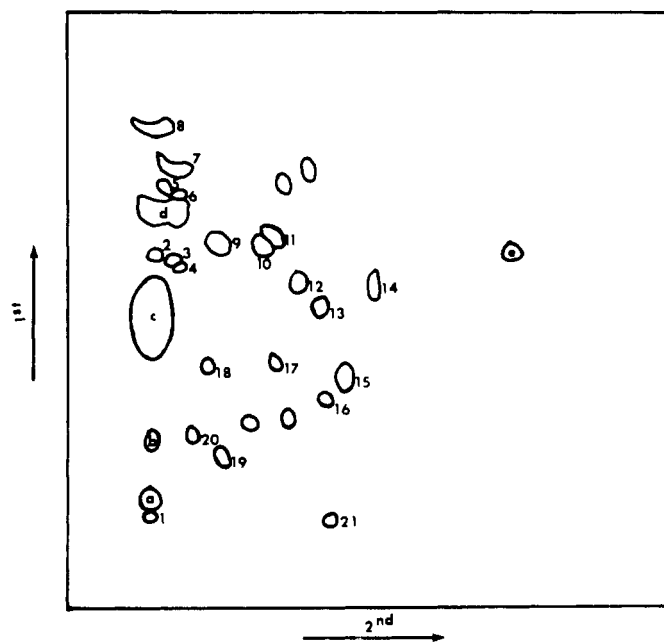
Figure 1. Tlc pattern of the dansylated amino acids of cotton nectar on Cheng-Chin polyamide. The coordinate labels 1st and 2nd indicate the direction of solvent running for formate-H_2O (3:200) and benzene–acetate (90:10), respectively. All derivatives detected under uv light: (a–e) dansyl reactants; (1) cysteine; (2) glutamic acid; (3) aspartic acid; (4) α-aminoadipic acid; (5) glutamine; (6) serine; (7) asparagine; (8) arginine; (9) glycine; (10) ammonia; (11) alanine; (12) γ-aminobutyric acid; (13) valine; (14) proline; (15) isoleucine; (16) leucine; (17) methionine; (18) 2,4-diaminobutyric acid; (19) lysine; (20) ornithine; (21) tyrosine.

liliter) of the individual amino acids found in the nectar. Concentration averages are given because of the quantitative variations of nectar collected at different times. These variations are attributed to fluctuations in water content, once nectar has been secreted and exposed to the environment. Although amino acid concentration fluctuated with time of nectar collection, no qualitative differences were found.

DISCUSSION

Very little qualitative and quantitative data are available concerning the amino acid requirements of adult insects. House (1968) reported that, in general, insects, like other animals, require the ten essential amino acids for normal growth. However, depending on the insect species, requirements vary for specific amino acids. Our understanding of insect nutritional requirements is limited by a lack of knowledge of the content of natural foodstuffs. Vanderzant (1958) found that Pectinophora gossypiella (Saunders) grew and developed better on an amino acid mixture equivalent to a protein of its natural food, cotton, than on a mixture like casein, which is satisfactory for other species. Gilbert (1972) reports that the Heliconius butterfly, unique in that it collects pollen, steeps it in nectar, and subsequently ingests the amino acids that diffuse from the grains, obtains about 840 nmol of free amino acids from a 1-day harvest of pollen (1.5 mg). This ingestion of free amino acids apparently has a profound effect on the Heliconius butterfly's life span and reproductive output.

The studies of Baker and Baker (1973) led them to conclude that "the occurrence of significant concentrations of amino acids in nectar is the rule." Therefore, the amino acid composition of nectar could play a significant role in the diet of at least some nectar-feeding insects. This study has revealed the presence of 24 amino acids in cotton nectar, including some of rather limited distribution. Thus it

is probable that the nutrition of cotton nectar feeding insects is significantly influenced by this array of amino acids. It is also possible that some of the less common acids may act as phagostimulants.

Unfortunately, the dietary requirements and preferences of adult cotton insects are, at best, imperfectly known. The fact that cotton nectar is a relatively rich source of amino acids should stimulate investigations in this direction. Such knowledge should have basic importance in the realm of biological control of these insects.

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Analysis of Fatty Acid Esters in Processed Raisins by Gas Chromatography

Allan E. Stafford,* Glenn Fuller, Harold R. Bolin, and Bruce E. Mackey[1]

An analysis procedure for the determination of the fatty acid ester residue on processed raisins was developed. Gas chromatographic analysis of the dipping oils which were used to increase the drying rate of grapes showed the presence of two or more of the following fatty acid esters: oleate, stearate, palmitate, and linoleate, with oleate accounting for the largest percentage. The esters were removed from the raisins by extraction with chloroform and the hexane-soluble fraction which contained the esters was analyzed by gas chromatography. Grapes dipped in 0.5-6.0% water emulsions of a commercial Australian ethyl ester dipping oil produced residues on the processed raisins of 41-397 ppm of total fatty acid esters, as measured by gas chromatography.

Fatty acid esters have been used for many years in Australia and the Middle East to reduce the drying time of grapes. This shorter drying time is caused by the interaction of esters in the dip with the waxy surface of the grapes to increase the rate of water loss during drying (Grncarevic, 1963). In contrast, most California raisin grapes are sun dried without the use of a drying aid, which results in a raisin with a blue-black color, darker than that of Australian raisins. Because of the long drying time required, sun-dried California raisins are very susceptible to rain damage.

Ponting and McBean (1970) at this laboratory determined the effect on the drying rate of dipping waxy skin fruits in water emulsions of ethyl esters of fatty acids in the C_{10}-C_{18} range. Other pretreatment processes were also studied but it was found that dipping waxy skin fruit in an emulsion of ethyl oleate was the most effective and convenient. This work has continued with expanded field studies by Petrucci *et al.* (1973), at California State University at Fresno. These studies have included on-the-vine dried and tunnel dehydrated raisins using methyl and ethyl fatty acid ester mixtures of plant and animal origin, to reduce drying time and produce a lighter colored raisin

without the use of sulfur dioxide. Taste threshold determinations have indicated that there is a wide variation in the thresholds for the fatty acid esters mixtures used (Guadagni, 1972). This work indicated a need for a method of determining the quantity of esters left on raisins after processing, in order to adjust the concentration of esters in the emulsion for optimum drying time without exceeding the taste threshold. This paper describes a procedure for analysis of fatty acid esters on processed raisins.

EXPERIMENTAL SECTION

The grapes used in these experiments were machine harvested Thompson seedless from the Fresno area. For de-

Table I. Per Cent Composition of Fatty Acid Ester Mixtures from Different Sources

	Methyl ester dipping oils		Ethyl ester dipping oils	
	A	B	A	B
Oleate	65.7	54.3	29.1	86.6
Stearate	1.1	23.7	12.1	
Palmitate	4.6	3.1	12.6	3.3
Linoleate	12.5	9.7		
Others[a]	16.1	9.2	46.2	10.1

[a] By subtraction. Precision: each value in the table is a mean of duplicates; standard deviation between duplicates (13 degrees of freedom) = 0.97%; 95% confidence interval (mean of duplicates) = ±1.5%.

Western Regional Research Laboratory, Agricultural Research Service, U. S. Department of Agriculture, Berkeley, California 94710.
[1] Present address: Biometrical Services, Agricultural Research Service, Western Regional Office, Berkeley, Calif. 94710.