Gossypol and Some Other Terpenoids, Flavonoids, and Phenols that Affect Quality of Cottonseed Protein

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ABSTRACT

Recent interest in gossypol, the biologically active phenol characteristic of the cotton genus *Gossypium*, has been generated by concerns beyond its physiological effects on animals. These concerns are primarily investigations into possible natural pest resistance factors in cotton and were prompted by differences noted in glanded and glandless varieties. This work has led to the characterization of gossypol-like compounds in other parts of the plant as well as in the seed. Likewise, work on the investigation of color and flavor components of cottonseed, carried out in an effort to characterize the product for food use, has led to observations on the nature of flavonoids. Phenolic acid fractions have also been indicated in flour preparations of both glandless and glanded cottonseed.

INTRODUCTION

Any listing of the constituents of a living organism soon expands to amazing numbers and cotton is no exception. Hedin et al. (1) enumerated the constitutents of bolls, lint, and seed from the cotton plant, which possess economic value or biological activity, and arrived at a total list of 252 compounds. Table I presents the pigments Hedin et al. (1) list as being present in cottonseed or the glands contained in the seed. Not all of these compounds are of interest or practical concern, while others have been the subject of much investigation and even controversy. This paper deals mainly with those compounds that affect the quality of cottonseed flour or other protein products obtained from it in an important manner.

Functionality, color, flavor, and of course, nutritional value, as influenced by the presence of biologically active compounds, are of primary importance in the consideration of cottonseed protein for human use. Indeed, all of these points have come under scrutiny in the development of cottonseed protein for food use (2,3).

TERPENOIDS

Gossypol is biologically active, but also presents problems with regard to color. Casual inspection of cottonseed meal reveals that the yellow pigments must be contended with.

The terpenoid compound, gossypol, historically has been the compound of greatest concern in cottonseed. The chemical characteristics have been outlined by Berardi and Goldblatt (4). Gossypol is markedly reactive and shows strongly acidic properties. It can act as a pehnolic and as an aldehydic compound. The phenolic groups react readily to form esters and ethers. The aldehyde groups react with amines to form Schiff bases and with organic acids to form heat labile compounds (5). The reaction with aromatic amines such as aniline is important in analysis. Gossypol, with a molecular weight of 518.5, is soluble in a number of organic solvents, and is insoluble in low boiling petroleum ether (bp 30-60 C) and is also insoluble in water. Gossypol of mp 184 C is obtained upon crystallization from ether, of mp 199 C from chloroform, and of mp 214 C from ligroin. Such a wide range of melting temperatures is attributed by Campbell et al. (6) to the polymorphism of gossypol.

The postulation of the three tautomeric forms of gossypol as proposed by Adams et al. (7) was necessary to explain many of the reactions of the compound. As shown in Figure 1, (a) represents the hydroxy aldehyde tautomer, (b) the lactol tautomer, and (c) the cyclic carbonyl tautomer.

The reaction of native gossypol from the seed glands with other seed components in the oil extraction process is very important to the practical use of the meal in animal feeding. The free gossypol reacts with, among other things, available amino residues of the protein, especially the epsilon amino groups of lysine, and in this state is much less physiologically active in the animal gut (8). The gossypol that is thus reacted is called "bound gossypol" and that gossypol that is still not bound to protein by the heat and moisture of processing is designated as "free gossypol." Thus, the main concern of nutritionists is the amount of free, or unreacted, gossypol rather than the total amount of gossypol that is consumed by an animal.

One of the bases for interest in gossypol is the physiological activity of the pigment. There is a rather extensive body of evidence detailing the toxicity of gossypol to different animal species (4,9,10). Swine, guinea pigs, and rabbits are the most sensitive to gossypol, while poultry, mice, and rats are intermediate in their sensitivity, although the effect of gossypol on egg damage and on yolk discoloration of stored eggs is the most sensitive biological indicator of gossypol activity (4). Functional ruminants have a very high degree of tolerance to gossypol due to the action of the rumen. The toxicological effects of gossypol on simple stomached animals has been classified in three levels. Acute

TABLE I

Some Pigments of Cottonseed from Hedin et al. (1)

Gossypetin	alpha Carotene	Violaxanthin
Leucodelphinidin	beta Carotene	Auroxanthin
Vitamin A_1	Phytoene	Neoxanthin
Gossypitrin	Phytofluene	Neochrome
Gossypol	Lutein	Gossyverdurin
Gossycaerulin	Isolutein	Gossypurpurin
Gossyfulvin	Flavoxanthin	

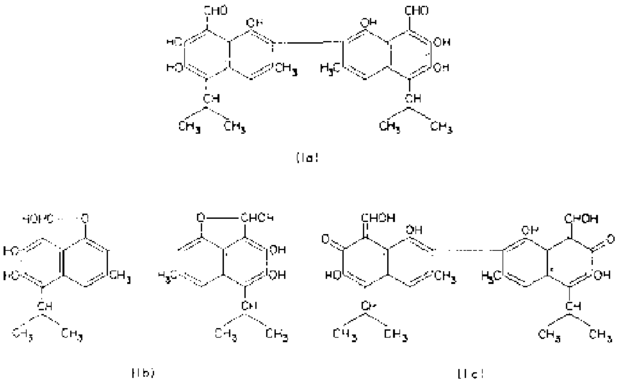

FIG. 1. Structures of the various tautomeric forms of gossypol where (a) represents the hydroxy aldehyde tautomer; (b) the lactol tautomer; and (c) the cyclic carbonyl tautomer.

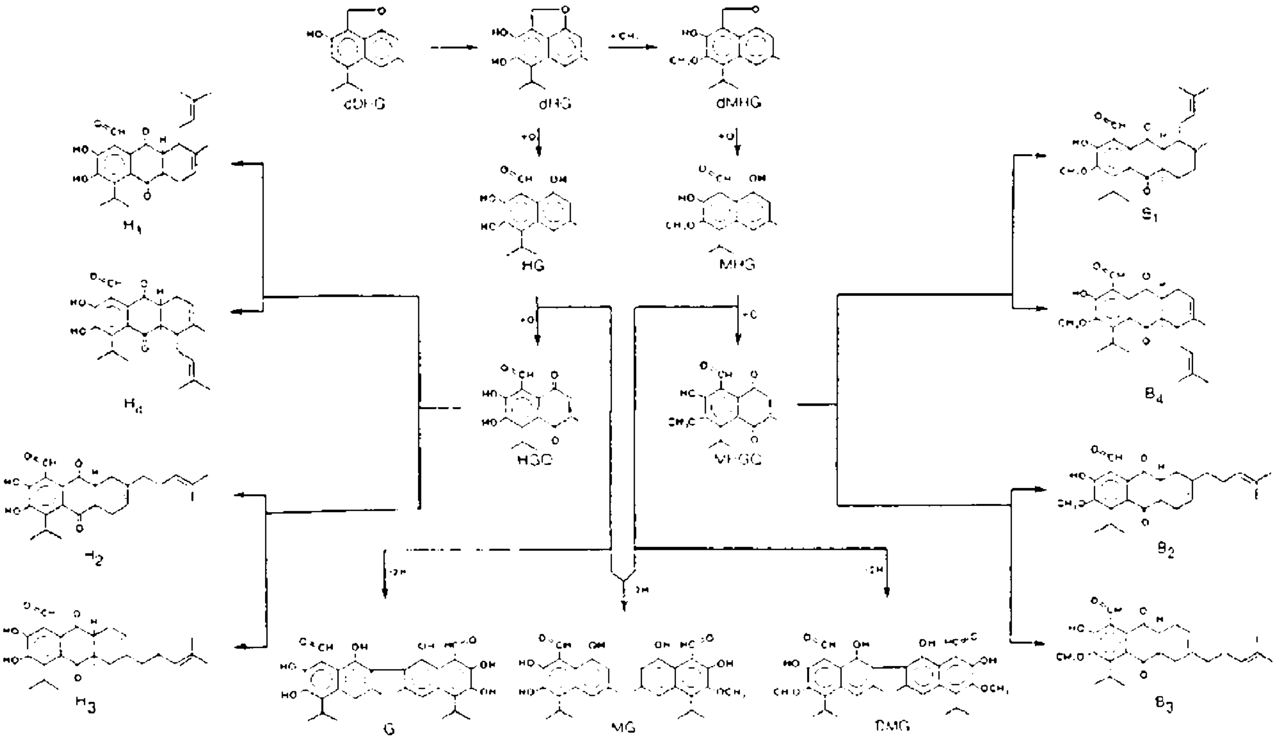

FIG. 2. Proposed pathway for biosynthesis of terpenoid aldehydes in cotton. dDHG=desoxy-6-deoxyhemigossypol; dHG=desoxyhemigossypol; dMHG=desoxy-6-methoxyhemigossypol; HG=hemigossypol; MHG=6-methoxyhemigossypol; HGQ–hemigossypolone; MHGQ–6-methoxyhemigossypolone; G=gossypol; MG=6methoxygossypol; DMG–6,6' dimethoxygossypol; H_1-H_4–heliocides H_1-H_4; B_1-B_4–heliocides B_1-B_4. Adapted from Stipanovic et al. (13).

doses cause circulation failure; subacute doses cause pulmonary edema; and chronic doses cause symptoms of ill health and malnutrition (9).

Because gossypol exhibits this dose response, it allows for the safe use of cottonseed meal in livestock rations. Broilers can safely tolerate 150 ppm of free or unreacted gossypol in their diet while layers should be restricted to 50 ppm free gossypol in the diet to prevent pigment discoloration of egg yolks when stored. Growing swine can tolerate 100 ppm of free gossypol in the diet. The addition of iron salts to the diet in all these cases can increase free gossypol tolerances (11). The practical load limits of free and total gossypol on functional ruminants have not been established and are not encountered under usual feeding conditions.

Any cottonseed protein products intended for human use in the United States must contain no more than 0.045% free gossypol as set by FDA. The Protein Advisory Group of the U.N. had set limits of 0.06% of free gossypol and 1.2% total gossypol for human consumption in their programs.

Gossypol is concentrated in discrete glands within the leaves, stems, roots, and seeds of the cotton plant. The pigment glands in the foliage parts of the plant are located below the epidermis and hypodermis. In the seed embryo, or kernel, these pigment-containing glands are 100 to 400 microns in diameter. Gossypol makes up ca. 20-40% of the weight of these glands and results in levels of gossypol in the whole kernels of 0.4 to 1.7% (12).

The concern about the toxicity of gossypol resulted in a series of events in the past two or three decades that have recently led to a great expansion of knowledge about cotton plant pigments. However, these events have diverted the main emphasis of gossypol research away from the area of its effect in cottonseed protein for feed and food uses.

In the early 1960s, commercial development of genetically glandless varieties of cotton was undertaken on the basis that the value of cottonseed oil and meal could be improved and its usefulness extended if gossypol were not present. That assumption is still valid and has been the basis for continued productive work on glandless varieties. The feasibility of some glandless cottonseed plantings in selected parts of the cotton belt are now being considered. With the advent of the glandless varieties in the 1960s, it was found that many of the insects which did not attack glanded cotton inflicted damage on glandless strains in areas where the insect infestation was heavy (12-14). Such observations then stimulated studies on the importance of pigment glands in host plant resistance. Concern with regard to agricultural chemicals also emphasized the need for natural control measures.

Toxicity of glanded flower buds to some insects has been correlated with gossypol content (12,13,15,16). The traditional method of analysis commonly used employed aniline which is a nonspecific reagent for aromatic aldehydes. It is not known exactly how many aromatic aldehydes exist in glands, and this left the above correlation in doubt. In addition to this, Bell and Stipanovic (12) suspected that some wild types of cotton had more insecticidal activity than could be accounted for by the gossypol concentration alone. This set the stage for the extensive work on terpenoids in cotton glands. Much of this work has been carried out by the USDA (12,13). Initially, the toxic activity was attributed to "X-factors." These X-factors have recently been identified as two sesquiterpenoids and a series of eight derived sesterterpenoids called heliocides. The sesquiterpenoids are illustrated in Figure 2 as HGQ and mHGQ while the sesterterpenoids are heliocides H_1, H_2, H_3, and H_4, and also B_1, B_2, B_3, and B_4.

Although commonly cultivated varieties of cotton are presently almost all glanded, they are by no means insect-free. The common cotton cultivars contain gossypol, hemigossypolone, and the heliocides H_1, H_2, H_3, and H_4

(Fig. 2). Insect resistant wild types of cotton are observed to contain these same compounds, but the concentrations are as much as three times as great (12). This is especially true of heliocides H_3 and H_4. True glandless cotton cultivars do not contain the terpenoid aldehydes (17).

The above research is directed toward insect control and not toward gossypol in seed products. Actually, since the seed is not the site of the heaviest insect attack, it has been somewhat left behind in the most recent gossypol research. One of the long range goals of host plant resistance researchers is to develop cotton lines with optimal terpenoid biosynthesis for pest control while preserving the value of the seed products. Thus, the beneficial terpenoids might be concentrated in the vegetable plant parts, while the seed, which is not the first point of insect attack, could be kept terpenoid-free, and thus be more valuable for food and feed.

An enumeration of the major terpenoid aldehyde components in the glands of different tissues of commonly cultivated cotton as presented by Stipanovic et al. (13) is given in Table II. As indicated, not all glands in all locations of the plant have a similar terpenoid make up. The predominant terpenoid aldehyde of the seed is gossypol, but Bell and his coworkers have shown that it is not the only one (18). Table III indicates the relative terpenoid content of both a representative upland and a pima long staple cotton. In both cultivars, gossypol is by far the most predominant terpenoid aldehyde. The triterpenoids 6-methoxygossypol and 6,6′-dimethoxygossypol are present in much lower relative amounts, while the sesquiterpenoids hemigossypol and 6-methoxyhemigossypol appear in only trace amounts. There is no indication that there may be any heliocides in the seed itself (13). Figure 2 illustrates the structures of gossypol, which is by far the predominant terpenoid aldehyde in cottonseed, as well as 6-methoxygossypol and 6,6′-dimethyoxygossypol which are present in slight but quantifiable amounts. Hemigossypol or 6-methoxyhemigossypol are only present in trace amounts.

Hemigossypol may possibly be a branch point to either the heliocides or gossypol and its related compounds. If chlorophyll is present in a plant tissue, then the path may lead to heliocides, whereas if it is not present, as in the seed, then the path may go to gossypol (13).

Several gossypol-related compounds mentioned in Table I that have not been included in the biochemical schemes already presented should be noted. These gossypol-like pigments of the seed have been mentioned in the older literature and in review articles on gossypol. Recent analytical techniques, however, leave some question as to the actual presence of them in the intact seed since current investigations do not identify these historically named compounds. Two of these gossypol-like pigments that have long been thought to occur in seed are gossypurpurin and gossyfulvin (4). Gossypurpurin has been measured at ca. 1% of the gland contents, while gossyfulvin has been measured at ca. 2% of the gland contents. The structure of gossyfulvin has not been elucidated, but Russian workers have proposed a structure for gossypurpurin (19). As well as these two compounds, several others had been historically identified as occurring in various steps of cottonseed processing, either in the meal or the oil or the soapstock. These other products have been called gossyverdurin, gossycaerulin, and diaminogossypol. Since there has been no positive identification of the structures of these compounds using the most recent laboratory techniques, the possibility exists that these other pigments, long thought to exist in cottonseed, might actually be oxidation or condensation products of gossypol.

FLAVONOIDS

While the most recent investigations on gossypol have focused on the foliage of the plant, other workers have continued to look at other pigments of the seed which affect color and therefore quality of seed protein. In the examination of color factors, the contributions of flavonoids have been found to be important.

Fifty years ago research workers concluded that anthocyanins and other flavonoids were present in the seed (20). Bell and Stipanovic (12), who have concentrated their work on insect control, have reported no evidence fot the occurrence of flavonoids in the glands themselves. Glandless cotton tissues appear to contain the same quality and quantity of flavonoids as do their glanded counterparts. The conclusion has been that the known color properties of the pigment glands can be explained solely by the terpenoid content. The flavonoids are present in the surrounding embryo meats in both glanded and glandless varieties.

USDA workers have been conducting investigations on the flavonoids of cottonseed proteins because of the need for vegetable protein flours to be as color-free as possible (21). This work is part of a program to obtain fundamental information on edible cottonseed protein being carried out by USDA.

In a study of the flavonoids of cottonseed protein, the USDA workers started with both flour from glanded cottonseed that had been processed by the liquid cyclone process (LCP) and also from glandless cottonseed. LCP uses differential centrifugation to remove intact gossypol-containing glands from dehulled meats of currently grown cultivars of cotton (22). Flours from both glandless and glanded seed were ether-extracted to remove lipids, then further extracted with aqueous alcohol to remove flavonoids. This fraction was separated into a nonflavonoid component, a minor flavonoid fraction, and a major flavonoid fraction that was 4-5% of the original aqueous alcohol fraction of the flours. Thin layer chromatography was used on the major flavonoid fraction with the result that six major flavonoids were found in glanded liquid cyclone process flour.

The thin layer procedure was also carried out on the major flavonoid fraction obtained by gel filtration of the

TABLE II

Terpenoid Aldehydes[a] in Glands in Tissues of Cotton (*G. Hirsutum*) (Stipanovic, et al. 13)

Seed embryo	G
Stem cortex	H_2, H_3
Stem phloem	G
Leaf cotyledonary	G
Leaf true	HGQ, H_2, H_3
Leaf petiole	H_2, H_3
Flower bracts and calyx	H_2, H_3
Flower petals and stamens	G
Flower ovary and stigma	HGQ, H_2, H_3
Root cortex	G
Root phloem	

[a] G = Gossypol; HGQ = Hemigossypolone; H_2, H_3 = Heliocides.

TABLE III

Terpenoid Aldehyde Content[a] of Cottonseed (Stipanovic et al. 18)

	HG	MHG	G	MG	DMG
	(unmole/100 g fresh embryo)				
Upland	T[b]		1233	26	6
Pima	T[b]	T[b]	1925	62	8

[a] HG = hemigossypol; MHG = 6-methoxyhemigossypol; G = gossypol; MG = 6-methoxygossypol; DMG = 6,6′-dimethoxygossypol.

[b] T = trace.

TABLE IV

Possible Flavor Contributions of Phenolic Acids (Maga and Lorenz, 24)

Compound	Taste threshold (ppm)	Cottonseed	Peanut	Soy
p-hydroxybenzoic	40	10	14	22
vanillic	30	30	43	35
p-coumaric	40	21	20	16
o-coumaric	25	8	10	8
ferulic	90	41	45	32
syringic	240	45	51	43
Total free phenols (ppm)		233	267	256

extract of glandless flour with the same result being obtained as with the glanded flour. It thus appears that the major flavonoids are the same in both glanded and glandless flours (21). There has also been a tentative identification of several flavonoids in the minor flavonoid fraction. An attempt was made to identify some of the six major flavonoids found in both flours. UV spectral analysis showed that four of the six, in both glanded and glandless flours, were 3-0-glycosides of quercetin. In earlier work, Pratt and Wender (23) had also claimed the presence of at least six flavonoid pigments, two of which were identified as isoquercitrin (quercetin-3-glucoside) and rutin (quercetin-3-rhamno-glucoside).

In a practical examination of the effects of these flavonoids on the color of baked biscuits by Blouin and Cherry (21), biscuits were made from 80% wheat and 20% cottonseed flour and were found to be yellow-brown in color. When flavonoids were removed, the biscuits made from the nonflavonoid fraction were light tan to near white in color. When the flavonoid fraction was used, the biscuits were bright yellow and closely resembled the color of biscuits made with commercially purchased rutin added.

PHENOLIC ACIDS

As with color, flavor is another important factor in the acceptance of a vegetable protein for human food applications. With regard to flavor, cottonseed flours are notably bland compared to other vegetable sources, but there are several free phenolic acid fractions which have been identified and may adversely contribute to their flavor (24). Table IV illustrates the levels of some of the phenolic acids that might contribute to taste in cottonseed flour as well as peanut and soy. Quantitatively, there were differences in all three protein sources, but the predominant free phenolic acids in all three were found to be vanillic, ferulic, and syringic acids. Table IV also shows the compounds which could approach or exceed known taste thresholds for each individual source. The combined totals of all the individual free phenolic acids observed exceeds 200 ppm and, as a group, could possibly contribute significantly to astringency in these protein substances.

The use of cottonseed protein as a food source is in its developmental stage with much research ground to be covered. It is of dubious value to assess the unique qualities of cottonseed or any new protein using quality tests of existing protein sources as a yardstick since the applications of the new source may be in wholly nontraditional areas. The best applications of cottonseed protein products should logically be in those food items that will likely be benefitted most by their specific characteristics.

In order to use cottonseed protein properly, the backlog of information must be increased. Color and flavor as well as protein characteristics are important basic knowledge; work in these three areas should be encouraged.

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