GOSSYPOL-LIKE COMPOUNDS OF THE COTTON PLANT.
METHODS OF DETERMINING GOSSYPOL

I. P. Nazarova, A. I. Glushenkova,
and A. U. Umarov

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This review is devoted to the study of gossypol and gossypol-like pigments. It is reported that some of them are phytoalexins with respect to certain fungal pathogens of the cotton plant and possess a high toxicity. Methods of determining free and total gossypol in cottonseed processing products are discussed particularly.

The problem of gossypol and pigments similar to it, methods for their isolation and quantitative determination, and the use of gossypol and its separation from the protein fraction of the seeds has acquired great importance at the present time in view of the possibility that has been found of using defatted cottonseed flour in the food industry and as a raw material for obtaining food protein and phytin in the complex treatment of cotton seeds. In addition, in recent years it has been established that gossypol and gossypol-like pigments play a not unimportant role in the fight against the attack of wilt on the cotton plant.

All this has induced us to attempt to systematize and generalize the material that has been accumulated on this question with the aim of its utilization in the further treatment of the problem of the complex processing of cotton seeds and of methods of combating wilt.

The paper here presented is a supplement to a monograph by A. L. Markman and V. P. Rzhekhin on "Gossypol and its derivatives," which appeared some time ago and in which the majority of the questions which we discuss were not considered.

Gossypol is a triterpene aldehyde found in plants of the class *Gossypium*. It is present in the bark of the roots of the cotton plant in an amount of 0.56-3% of its weight [1] and, in small amounts, in the leaves, the bark of the stems, and the flowers. However, gossypol is concentrated mainly in the seed kernels. Its amount varies within wide limits according to the varieties of cotton plant — from 0.002% [2] to 6.64% in the varity *Gossypium klatzshianium* var. *davidsonii* [3]. The majority of cotton-plant varieties contain from 0.39 to 1.70% of gossypol [4]. In addition, it is found in some plants of the genera *Thespesia* (*Thespesia populnea*) [5-7], *Cienfuegosia*, and *Kokia* [8].

SPECTRAL CHARACTERISTICS AND TAUTOMERISM OF GOSSYPOL

Adams [3] has determined the structure of gossypol, $C_{30}H_{30}O_8$, as 2,2'-bi(8-formyl-1,6,7-trihydroxyl-5-isopropyl-3-methylnaphthyl) (see the legend to the formulas in Fig. 1).

The spectral characteristics of gossypol obtained later had an extremely fundamental influence on the refinement of its structure; they confirmed the results of chemical investigations according to which gossypol is an aromatic compounds with phenolic hydroxy groups and its molecule contains a carbonyl group on the ortho position to a hydroxy group.

The UV absorption of gossypol has maxima at $\lambda_{max}^{ethanol}$ 236, 283 (shoulder), 289, and 376 nm [9, 10]. The IR spectrum contains bands at (cm^{-1}) 3570 (free phenol groups); 3050, 1429 (—CH= groups); 1613 (—C=O); 1351, 1316 ($-CH_3$); 1028, 995, 905, 845 [11, 12]. The mass spectrum is characterised by the peaks of ions with m/e 518 (M^+, 17%); 500 $(M-H_2O)^+$, 100%; 485 $(M-H_2O-CH_3)^+$, 33%; 482 $(M-2H_2O)^+$, 20%; 467 $(M-2H_2O-CH_3)^+$; 457 $(M-CH_2O_2-CH_3)^+$, 40% [10, 13].

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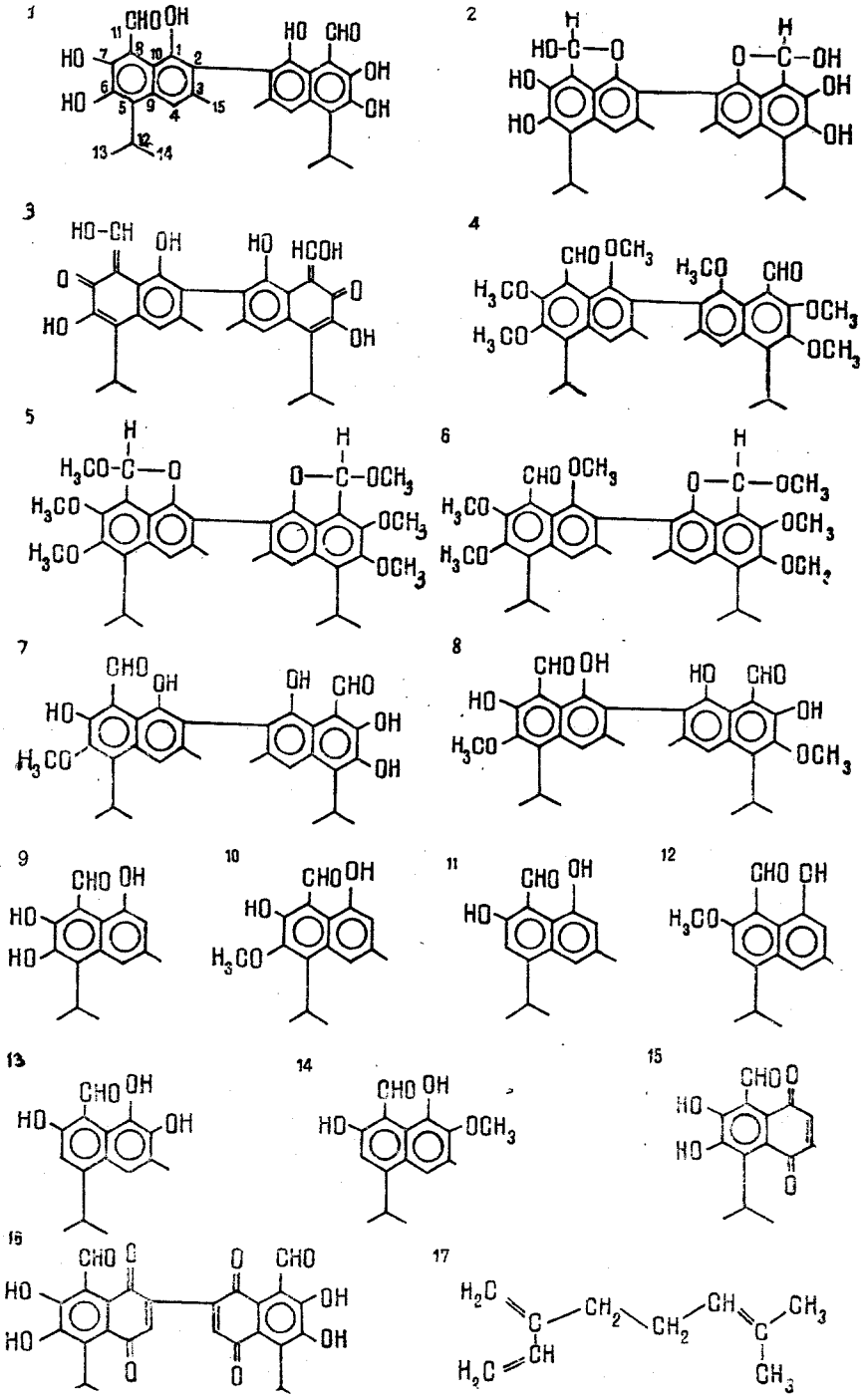

Fig. 1

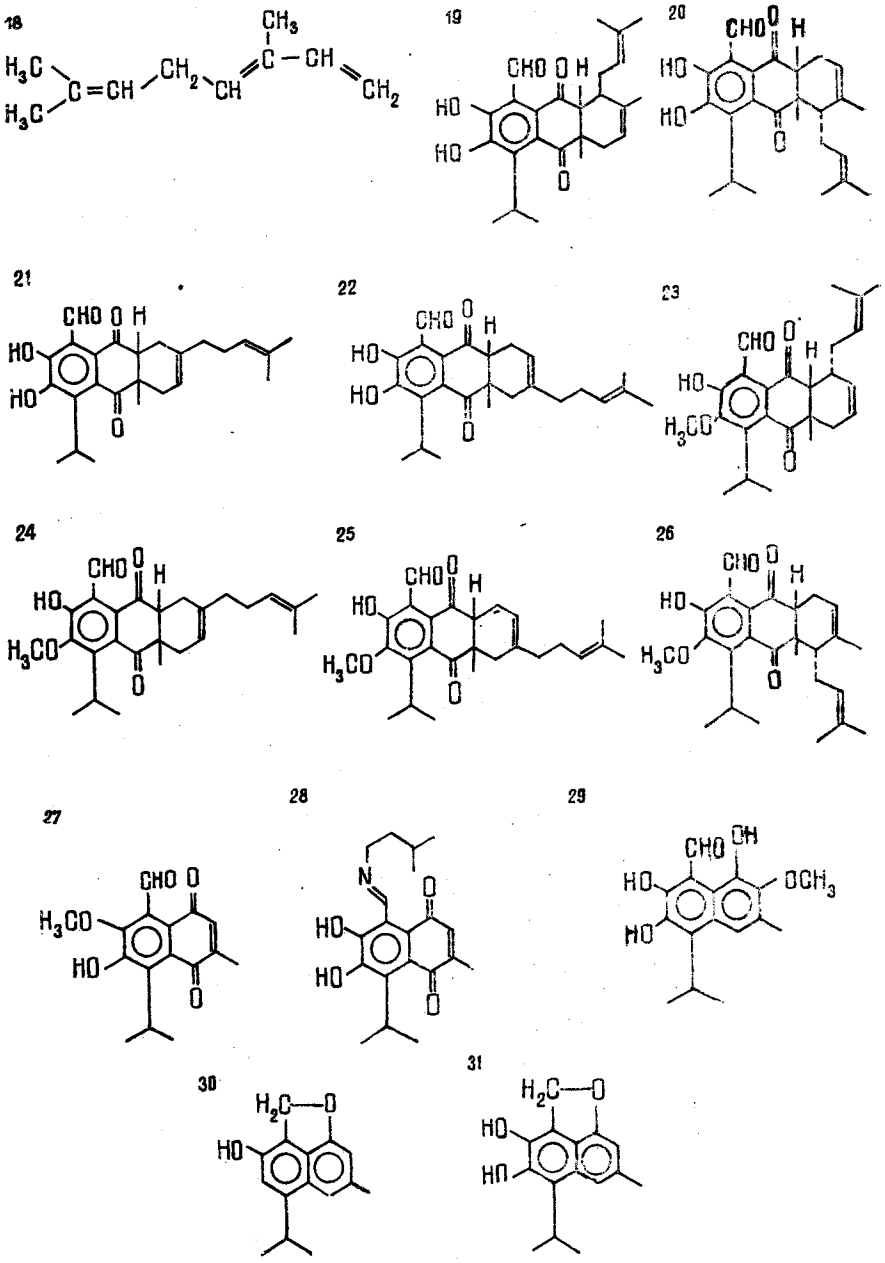

Fig. 1 (Continued). Terpenoids of the cotton plant. 1) Naphthalenealdehyde form of gossypol [4, 5-8]; 2) naphthalene-lactol form of gossypol [3, 14]; 3) naphthalenone-carbinol form of gossypol [3, 14]; 4) dialdehyde form of gossypol hexamethyl ether [15-17]; 5) dilactol form of gossypol hexamethyl ether [15-17]; 6) monolactol-monoaldehyde form of gossypol [15-17]; 7) 6-O-methylgossypol [29-33]; 8) 6,6'-di-O-methylgossypol [29-33]; 9) hemigossypol [29-33]; 10) 6-O-methylhemigossypol [29-33]; 11) 6-deoxyhemigossypol [29-33]; 12) vergosin [42]; 13) isohemigossypol [43]; 14) gossyvertin [45]; 15) hemigossypolone [46]; 16) gossypolone [47]; 17) β-myrcene [51]; 18) β-ocimene [51]; 19) heliocide H_1 [52]; 20) heliocide H_4 [52]; 21) heliocide H_2 [51, 53]; 22) heliocide H_3 [51, 53]; 23) heliocide B_1 [51]; 24) heliocide B_2 [51]; 25) heliocide B_3 [51]; 26) heliocide B_4 [51]; 27) 7-O-methylhemigossypol [54]; 28) gossyrubilone [54]; 29) raimondal [55]; 30) deoxy-6-deoxyhemigossypol [48-56]; 31) deoxyhemigossypol [48-56].

The PMR spectrum in $CDCl_3$ shows the following signals (ppm): d, 1.55, 12 H (CH_3 groups of isopropyls); s, 2.2, 6 H (CH_3 groups at C_3); septet, 3.75, 2 H (isopropyl groups); s, 5.9, 2 H (aromatic OH groups at C_1); s, 6.4, 2 H (aromatic OH groups at C_6); s, 7.8 2 H (aromatic protons at C_4); s, 11.3, 2 H (aldehydic protons); s, 15.2, 2 H (aromatic OH groups at C_7) [10].

To explain the reactions of gossypol with other compounds, three tautomeric forms of it have been put forward: 1) naphthalene-aldehyde; 2) naphthalene-lactol; 3) naphthalenone-carbinol.

The preparation of the corresponding derivatives has confirmed the presence of each of the suggested tautomeric forms [3]. The lactol and carbinol forms of gossypol itself have been detected only recently with the aid of physical methods of investigation. The PMR spectra of various samples of gossypol [14] have shown that in feebly polar solvents it exists mainly in the aldehyde form. However, the use of more polar solvents leads to the appearance of the other tautomeric forms. Thus, in a dimethyl sulfoxide solution of gossypol lactol forms have been detected in equilibrium with aldehyde forms.

Methylation under various conditions permits the existence of the tautomeric forms of gossypol to be revealed most clearly [15-17]. This process gives a mixture of their hexamethyl ethers from the which the dialdehyde (4), dilactol (5), and monolacton-monoaldehyde (6) forms have been isolated.

The structural features of the hexamethyl ethers of gossypol were established with the aid of IR, UV, PMR, and mass spectrometry.

However, the true quantitative ratio of the tautomeric forms of gossypol has been found only recently on the basis of the results of a study of the M^+ DADI spectra of a native mixture of gossypol hexamethyl ethers. The amounts of forms of 4, 5, and 6 in the mixture of hexamethyl ethers obtained by the methylation of gossypol with dimethyl sulfate in the presence of alkali are 27.09, 20.81, and 52.10%, respectively [17].

STEREOCHEMISTRY OF GOSSYPOL

The gossypol molecule is usually shown as coplanar, i.e., with the naphthalene nuclei located in one plane. The presence of substituting groups in the ortho positions with respect to the 2,2'-carbon atoms make free rotation of these two halves of the gossypol molecule impossible [18, 19].

As a result of measurements performed on Stuart-Briegleb models [17] it has been established that rotation of the naphthalene nuclei of gossypol around the connecting axis is prevented by steric hinderance. Only a limited rotational-vibrational about this axis within an angle of 120° is possible. With such a structure of the molecule the existence of two atropo isomers of gossypol becomes probable.

In the main, the genus *Gossypium* contains inactive (±)-gossypol, but (+)-gossypol has also been isolated from cotton seeds grown in a number of American states [19].

In *Thespesia populnea* optically active (+)-gossypol with a rotation $[\alpha]_D^{17}$ +475° predominates [5, 7].

One of the reasons for the optical activity of (+)-gossypol is the presence of the asymmetric carbon atom of the lactol ring. However, the formation of an acid does not change the optical rotation of the gossypol from *Thespesia populnea* [5]. This shows that the optical activity here is due to atropo isomerism and not to the presence of the asymmetric hemiacetal carbon atom.

(±)-Gossypol differs from (+)-gossypol by a lower solubility. The two stereoisomers have the same melting point of 184°C (from benzene), but their methyl ethers and acetates differ appreciably both in melting points and in solubilities.

The methylation of both (±)-gossypol and of (+)-gossypol gives a mixture of three optically inactive products, 4, 5, and 6.

GOSSYPOL-LIKE COMPOUNDS OF THE COTTON PLANT

It was reported long ago that cotton seeds contained, in addition to gossypol, a number of other pigments genetically related to it — gossypurpurin, gossyfulvin, gossycaerulin, and

gossyverdurin. However, no painstaking investigations of these compounds have been carried out at the modern level, and therefore there is as yet no accurate information on their compositions and structures of their molecules.

When cotton seeds are stored during their processing, the amount of these pigments rises [20]; gossypurpurin to 0.055% of the weight of the kernel, while gossyfulvin has been detected in low concentrations in cotton seeds stored at a high moisture content and gossycaerulin has been found only in worked seeds [21].

Gossypol is a yellow, gossypurpurin a red, gossyfulvin an orange, gossycaerulin a blue, and gossyverdurin a green pigment of the cotton plant [20].

The first studies of gossypurpurin were made by Boatner [22]. Gossypurpurin, present in cotton glands together with gossypol, is separated from the latter by repeated recrystallization from a mixture of petroleum ether and chloroform followed by extraction of the residual gossypol with ethyl acetate.

The structure of gossypurpurin has not been accurately determined. It is considered that it consists of a molecule of gossypol and a molecule of diaminogossypol linked by oxygen bridges.

C. H. Pominski et al. [23] obtained gossypurpurin from a diaminogossypol by heating it to 160°C for an hour. They put forward an empirical formula for gossypurpurin ($C_{30}H_{32}O_7N_2$).

E. F. Manevich et al. [24] developed a method of isolating gossypurpurin from the glands of the seeds and the bark of the root of the cotton plant. They determined a molecular weight for gossypurpurin varying from 427 to 608. On this basis, these authors assume that the gossypurpurin molecule includes not two, as considered previously [23, 25], but one molecule of gossypol, and in their opinion gossypurpurin has the empirical formula $C_{30}H_{32}O_7N$.

Gossyfulvin was isolated by Boatner et al. from an ethereal extract of cotton seeds [26]. On treatment with acids, it is converted fairly readily into gossypol. Gossyfulvin is sparingly soluble in ethanol and ether, somewhat more soluble in chloroform and hot aniline, and very readily soluble in dioxane [27]. In its chemical properties, it differs strongly from gossypol (it does not dissolve in caustic alkali, it does not react with aniline, and is not reduced by Fehling's solution). Boatner et al. proposed the formula $C_{34}H_{34}O_8N_2$ for it on the basis of elementary analysis and the yield (82-86%) of gossypol obtained by the acid hydrolysis of gossyfulvin. However, certain analytical results agree better with the formula $C_{35}H_{34}O_8N_2$.

The absorption spectra of chloroform solutions of gossyfulvin in the UV region (maxima at 261 and 312 nm) and the visible region (439-440 nm) show that the structure of gossyfulvin is very similar to that of dianilinogossypol. The detection of gossyfulvin only in cotton seeds stored at a high moisture content confirms that it is a product of the interaction of gossypol with the seed protein.

Gossycaerulin has been detected in cottonseed oil soapstocks from the appearance of a dark blue cloration on heating with sulfuric acid. Boatner has established that gossycaerulin is not a component part of cotton seeds but is formed during their processing [22, 26].

Gossycaerulin changes color according to the pH of the medium: deep blue in acid solution and yellow in neutral and alkaline media. The two forms (blue-acid and yellow-neutral) have different solubilities in organic solvents.

In concentrated sulfuric acid, gossycaerulin gives a purple coloration, and when the solution is diluted a finely disperse blue precipitate deposits. Gossypol gives a bright red coloration with concentrated sulfuric acid, but on dilution with water it precipitates in unchanged form. This indicates that gossycaerulin is more sensitive to oxidation than gossypol.

The empirical formula of gossycaerulin $C_{30}H_{30}O_8$ shows that it is an isomer of gossypol. It is assumed that gossycaerulin is a quinone.

The reaction of gossycaerulin with boracetic acid and ferric chloride gives the purple cororation that is characteristic for compounds containing adjacent carbonyl and hydroxy groups.

Gossyverdurin is a green pigment which has been isolated from gossypol glands [28]. Its yield amounted to 2% of the weight of the glands. Gossyverdurin dissolves readily in the majority of polar organic solvents and is completely insoluble in petroleum ether. Like gossypol, it gives positive qualitative reactions with Fehling's solution and ferric chloride. With concentrated sulfuric acid, gossyverdurin give a stable green coloration, in contrast to the dark red coloration formed in the reaction with gossypol.

The absorption spectrum of a chloroform solution of gossyverdurin has maxima at 250, 320, and 560 nm.

Gossyverdurin has proved to the most toxic of the substances isolated from cotton seeds [20].

FUNGICIDAL COMPOUNDS

Comparatively recently, Bell et al [29] and Mace et al. [30] have found another five terpenoid aldehydes in *Gossypium* roots, in addition to gossypol: 6-O-methylgossypol (7), 6,6'-di-O-methylgossypol (8), hemigossypol (9), 6-O-methylhemigossypol (10), and 6-deoxyhemigossypol (11).

Hunter et al. have isolated the same five aldehydes from hypocotyls of healthy cotton seeds and have found that their concentration increases with ripening or after infection with *Rhizoctonia solani* [31, 32].

The structures of the compound were established chemically and with the aid of physical methods of investigations (IR, UV, PMR, and mass spectrometry) [10, 33].

6-O-Methylgossypol (7) crystallizes from a mixture of benzene and hexane (1:1) in the form of yellow crystals with mp 146-149°C. The UV spectrum shows an absorption maximum at $\lambda_{max}^{ethanol}$ 235, 288, 369 nm; IR spectrum 1615 cm^{-1}. The mass spectrum gave a molecular peak M^+ at m/e 532 (3%). Fragmentation led to the formation of ions with m/e 514 $(M-H_2O)^+$, 20%; 499 $(M-H_2O-CH_3)^+$, 9%; 496 $(M-2H_2O)^+$, 89% and 481 $(M-2H_2O-CH_3)^+$, 100%. The main signals in the PMR spectra of 6-O-methylgossypol coincides with those in the spectra of gossypol, and the only difference between the spectra is the presence of an additional signal of a methoxy group at 3.95 ppm [29].

6,6'-Di-O-methylgossypol forms yellow crystals with mp 181-184°C (from benzene and hexane). UV spectrum: $\lambda_{max}^{ethanol}$ 231, 253, 287, 360, 390 nm. λ_{max}^{KBr} 1612 cm^{-1}. The mass spectrum of 6,6'-di-O-methylgossypol shows M^+ with m/e 546 (12%), and ions with m/e 528 $(M-H_2O)^+$, 82%; 513 $(M-H_2O-CH_3)^+$, 29%, 510 $(M-2H_2O)^+$, 100% and 495 $(M-2H_2O-CH_3)^+$, 85%.

The PMR spectrum of 6,6'-di-O-methylgossypol differs from that of gossypol only by the signal of the methoxy groups present in equivalent positions in the dimeric molecule. These groups appear in the form of a single sharp singlet at δ 3.94 ppm (6H) [9].

Hemigossypol (9) forms bright yellow crystals with mp 159-163°C (from chloroform). The UV spectrum of hemigossypol, $\lambda_{max}^{ethanol}$ 229, 297, 286 (shoulder), 298, 374, is similar to that of gossypol, but the absorption coefficient is only 1/2 of the value for gossypol. The small differences in the absorption maxima are explained by the presence of a hydrogen bond between the hydroxyls at C_1 and C_1'.

The IR spectrum of hemigossypol has bands at 3530, 3350, and 1615 cm^{-1}. The high-resolution mass spectrum showed a molecular composition of $C_{15}H_{16}O_4$ [7]. The main ionic fragments observed had m/e 245 $(M-CH_3)^+$, 32%; 242 $(M-H_2O)^+$, 24% and 227 $(M-H_2O-CH_3)^+$, 48%.

The PMR spectrum of hemigossypol, in the main, coincides with that of gossypol: An aromatic isopropyl group appears in the form of a six-proton doublet at 1.48 ppm and a methine septet at 3.81 ppm. An aromatic methyl group resonants at 2.39 ppm (3 H, singlet). Two phenolic hydroxylic protons appear in the form of a broad signal at 6.00 ppm. Aromatic protons appear at 6.60 ppm (1 H, singlet) and 7.45 ppm (1 H, singlet). The presence of an aldehydic proton is confirmed by the existence of a signal at 11.11 ppm and that of a phenolic proton by a signal at 14.75 ppm [33].

6-O-Methylhemigossypol (10) is obtained in the form of yellow crystals with mp 156-160°C (from benzene). In the UV spectrum there are bands at 225, 268, 281 (shoulder), 352, and 388 nm. A comparison of its spectrum with that of hemigossypol shows a characteristic

displacement of the maxima due to the methoxyl at C_6. IR spectrum: 3300, 1610, 1200 cm^{-1}.

On the basis of the results of high-resolution mass spectrometry this compound is assigned the formula $C_{16}H_{18}O_4$. Important fragments have m/e 259 $(M-CH_3)^+$, 24%; 256 $(M-H_2O)^+$, 43% and 241 $(M-H_2O-CH_3)^+$, 95%. A comparison of the PMR spectra of 6-O-methylgossypol (10) and of hemigossypol (9) shows a great similarity between them. The only important difference in the PMR spectra consists in the appearance of a three-proton singlet at 3.89 ppm, which is characteristic for an aromatic methoxy group.

6-Deoxyhemigossypol (11), on recrystallization from benzene, is also obtained in the form of yellow crystals, with mp 174-178.5°C. UV spectrum: 222, 259, 281 (shoulder), 336, 389 nm.

IR spectrum: 3260, 1615 cm^{-1}. On mass spectrometry, ions are formed with m/e 244 $(M^+$, 100%); 243 $(M-H)^+$, 23%); 229 $(M-CH_3)^+$, 12%; 226 $(M-H_2O)^+$, 65%; 211 $(M-H_2O-CH_3)^+$, 24%; 198 (18%), 185 (15%), 183 (23%), 155 (14%), 153 (10%), 152 (12%): 131 (15%): 128 (17%), 127 (12%), 119 (12%) and 115 (18%). The PMR spectrum of this compound contains the following signals (ppm): d, 1.36 (6 H); s, 2.42 (3 H); septet, 3.56 (1 H); s, 6.72 (1 H); s, 7.39 (1 H); s, 11.10 (1 H).

Thus, it has been established that compounds 7 and 8 are methoxylated triterpene analogs of gossypol closely related to it in structure. Compounds 9, 10, and 11 are sesquiterpene aldehydes with structures consideranly differing from that of gossypol. They have previously been isolated from the stems of *G. barbadense* infected with *Verticillium* and identified [33].

All these compounds consists of yellow crystalline substances and, like gossypol, form pink-red derivatives with phloroglucinol, orange-red colorations with antimony trichloride, and dark orange colorations with 2,4-dinitrophenylhydrazine.

The methoxylated triterpene aldehydes 7 and 8 frequently amount to a considerable percentage of the sum of the terpenoid aldehydes in the bark of the stems and the bark of the roots of *G. barbadense*, but in diseased or bruised tissues, the sesquiterpene aldehydes 9, 10, and 11, and not triterpene derivatives, predominate. In the mature healthy plant, hemigossypol [33] is present only in trace amounts and methoxylated triterpenoid analogs of gossypol in amounts of from 0.003 to 0.01%, while the amount of gossypol is 0.4-0.6% [4].

In considering the numerous other gossypol-like compounds, it is necessary in the first place to show the role of gossypol itself in the resistance of the cotton plant to fungal pathogens [34] and insects [35].

The causative agents of the main disease of the cotton plant — wilt — are the soil fungi *Verticillium* and *Fusarium*. Resistance to pathogenic fungi is determined by the capacity of the plant for forming special antibiotic substances, so-called phytoalexins, in response to infection. The more resistant the variety, the faster and the more copiously are phytoalexins formed when the plant is infected [36]. In their chemical structure, the phytoalexins are complex cyclic compounds of various classes. They also include polyphenols, which are found in minor amounts. In response to an infection, in resistant varieties the polyphenols are oxidized more rapidly than in nonresistant ones, forming from their oxidation products, as it were, a chemical barrier preventing the penetration of the phytopathogens.

The resistance of the cotton plant to the causative agents of verticilliaceous wilt largely depends on the phenolic compounds and, in the first place, on gossypol — the specific phenol of the cotton plant. It possesses antioxidant properties and apparently can protect lipids from oxidation [37]. It is known that gossypol participates in the protective reactions of the cotton plant against attack by wilt [38, 39].

When the plant is infected, in the resistant varieties the amount of gossypol in the stems and roots rises, while in the nonresistant varieties it remains at the same level or decreases [39].

According to our results, the oil from the seeds of the cotton plant of the variety Tashkent-1 infected with verticilliaceous wilt, differs from the seed oil of the healthy plant by a fall in the amount of total gossypol from 0.57 to 0.28% [40].

In resistant varieties, in response to infection new compounds also appear which are not present in the healthy plant. As examples we can give the hemigossypol (9), 6-O-methoxyhemigossypol (10) and 6-deoxyhemigossypol (11) described above, which are found in the roots of the infected cotton plant.

There is much information in the literature on the phytoalexins of the cotton plant infected with verticilliaceous wilt.

Bell [38, 41] called the main fungicidal compound gossypol. Zaki and Keen [42] gave the name phytoalexins to two compounds of phenolic nature which they isolated from the stems of the cotton plant *G. barbadense* infected with *Verticillium albo-atrum*, and which they called hemigossypol (9) and vergosin (12). They showed that vergosin and hemigossypol are more active fungicides than gossipol in relation to *Verticillium albo-atrum*.

Vergosin (12) was identified by Zaki as 1-hydroxy-5-isopropyl-7-methoxy-3-methyl-8-naphthaldehyde.

UV spectrum of vergosin: 225, 248, 258 (shoulder), 293, 306, 323, and 338 nm. The IR spectrum has bands at (cm^{-1}): 3450 (hydroxy groups) — very weak in comparison with gossypol and hemigossypol; 1600; 1478; 1111.

The main spectrum of verogsin has ions with m/e 258 (M^+, 94%); 243 $(M-CH_3)^+$, 100%; 229 $(M-CHO)^+$, 17%; 228 $(M-2CH_3)^+$, 42%; 227 $(M-OCH_3)^+$, 13%; 213 $(M-3CH_3)^+$, 14%. In the PMR spectrum the following signals are observed (ppm): d, 1.55, 6 H (CH_3 groups of isopropyl); s, 2.4, 3 H (CH_3 group at C_3); s, 3.5, 1 H (aromatic —OH); septet, 3.7, 1 H (methine proton of an isopropyl group); s, 3.9, 3 H (methoxy protons); s, 5.8, 2 H (aromatic protons); s, 6.5 1 H (aromatic proton); s, 7.3, 1 H (aldehydic proton). The signal at 7.3 ppm, the usual region of aldehydic protons, is shifted downfield (11.3 ppm) in the case of gossypol through a hydrogen bond in the carbonyl group and the hydroxyl at C_1.

A. S. Sadykov et al. [43] isolated from the stems and roots of the cotton plant infected with *Verticillium dahliae* Kleb. isohemigossypol (13) (1,2,7-trihydroxy-5-isopropyl-3-methyl-8-naphthaldehyde), which is also a phytoalexin of the cotton plant.

Isohemigossypol forms yellow crystals with a green tinge having mp 124-144°C. UV spectrum of the substance in chloroform: 267, 365 nm. The mass spectrum has the intense peak of the $(M-1)^+$ ion corresponding to the loss of a hydrogen atom and peaks of ions with m/e 245 $(M-CH_3)^+$ 243 $(M-OH)^+$, 242 $(M-H_2O)^+$, and 214 $(M-CH_2O_2)^+$. The PMR spectrum of isohemigossypol is similar to that of hemigossypol, giving singlets of the aromatic hydroxyl protons at 5.55, 6.23, and 14.97 ppm.

Isohemigossypol (13) is absent from the uninfected cotton plant in the absence of the causative agent of wilt. When the disease exists in wilt-resistant varieties, the compound is found in larger amounts than in wilt-susceptible varieties. However, Bell et al. consider, on the basis of double-resonance results, that the phytoalexin obtained is not isohemigossypol but hemigossypol [44].

In addition to this compound, several other polyphenols that are absent from healthy plants are found in the stems of a diseased plant. One of them is gossyvertin (14) — a crystalline substance with mp 147-149°C. UV spectrum in ethanol: 223, 266, 350, 385 nm. The mass spectrum gives an intense peak of the molecular ion with m/e 274 $(M)^+$ and of ions with m/e 259 $(M-CH_3)^+$, 257 $(M-OH)^+$, 256 $(M-H_2O)^+$, and 213 $(M-CH_2O_2)^+$.

The PMR spectrum of gossivertin is similar to that of gossypol, but differs from it by the presence of the signals of one more aromatic proton (6.66 ppm) and of a methoxy group (3.90 ppm). The methoxy group is located on either the first or the second carbon atom [45].

A crystalline substance with mp 160-162°C has been isolated from a chloroform extract of the stems of a diseased cotton plant [46]. UV spectrum in chloroform: 242, 270, 313, 408 nm. The mass spectrum has an intense peak of the molecular ion with m/e 274 (M^+ 83%), and also the peaks of ions with m/e 259 $(M-CH_3)^+$, 100%, and 231 $(M-CH_3-CO)^+$, 21%.

The PMR spectrum of the substance shows the following signals: a six-proton doublet of the methyl groups of an isopropyl radical at 1.36 ppm, the three-proton doublet of a methyl group at 2.07 ppm, the septet of a methine proton of an isopropyl radical at 4.1 ppm, two broad signals of OH groups at 6.46 and 12.9 ppm, and a signal at 10.66 ppm assigned to the proton of an aldehyde. Also characteristic for this compound is the presence of one-proton quartet at 6.78 ppm.

On the basis of these results, the conclusion was arrived at that the most probable formula for the substance isolated — hemigossypolone (15) — corresponds to 8-formyl-6,7-dihydroxy-5-isopropyl-3-methylnaphthoquinone [46]. It is known that oxidized polyphenols possess a higher fungitoxicity than their reduced forms [39]. Consequently, it may be assumed that when the plants become diseased the polyphenols undergo oxidation with the formation of highly toxic substances more strongly suppressing the development of the fungus. Since hemigossypolone is assigned to the quinones, it should possess a high fungitoxicity. A comparative study of the fungitoxicities of gossypol and other gossypol-like compounds isolated from the stems of the wilt-infected cotton plant showed that hemigossipolone possesses the highest fungitoxicity in relation to the causative agent of verticilliaceous wilt of the cotton plant.

A similarly high fungitoxicity with respect to *V. dahliae* Kleb. is possessed by gossypolone (16) — the p-quinone of gossypol — and its derivatives [47].

GOSSYPOL-LIKE COMPOUNDS AS INSECTICIDES

In considering the influence of gossypol and compounds similar to it on resistance to insects, it must be born in mind that with the isolation of gossypol-free varieties of the cotton plant in 1960 it was found that many insects that did not damage gossypol-containing varieties of the cotton plant did damage its gossypol-free varieties [48]. Such observations have stimulated the study of the influence of gossypol on the degree of resistance of the plant to insects. It has been found that the pigment glands of upland cotton (*Gossypium hirsutum* L.) contained compounds suppressing the development of insects (*Heliothis zea* and *Heliothis verecens*, respectively) in the seed vessels and buds of tobacco [49]. One of them is hemigossypol (15) [50]. In addition, another four compounds structurally connected with hemigossypol have been isolated from *G. hirsutum*. They are toxic to *Heliothis*, and therefore have been called heliocides H_1, H_2, H_3, and H_4 ($C_{25}H_{30}O_5$). Four similar compounds from *G. barbadense* have been called heliocides B_1, B_2, B_3, and B_4 [51]. Heliocides H_1, H_2, H_3, and H_4 — new cotton-plant terpenoids of the C_{25} series — are found only in the pigment glands of young leaves and buds. Hemigossypolone is the predominating terpenoid of young leaves of *G. hirsutum*, and as the leaves age it is replaced by the heliocide. Reactions performed under laboratory conditions have shown that this replacement takes place through the interaction of hemigossypolone with two of the main monoterpenes of the subepidermal cotton glands — myrcene (17) and ocimene (18).

Heliocides H_1 (19) and H_4 (20) have been synthesized by the Diels—Alder reaction from hemigossypolone (15) and trans-β-ocimene (18) [52]. The main product of the reaction (66%) is heliocide H_1; H_4 is a minor component and according to IR, UV, PMR, and mass spectroscopy, its structure agrees with the heliocide H_4 found in nature. The stereochemical configurations of these compounds has been established from the results of ^{13}C NMR spectroscopy. Heliocide H_1 has an isopentyl side chain at C_{19}, while in heliocide H_4 it is at C_{16}.

Heliocides H_2 (21) and H_3 (22) are formed in the reaction of hemigossypolone with myrcene [52, 53]. Just as heliocide H_1 predominates in the reaction of hemigossypolone with ocimene, in the reaction with myrcene the main product is heliocide H_2. A comparison of the ^{13}C NMR spectra of heliocides H_2 and H_3 showed that they are isomers. The side chain in heliocide H_2 is located at C_{18} and in H_3 at C_{17}.

It has been established that H_1 is four or five times more toxic than hemigossypolone or H_2 in relation to the insect *Heliothis veresens*, which is explained by the structure of the monoterpene diene involved in its synthesis and by the stereochemistry of the product (H_1 and H_4).

Different varieties of the cotton plant differ in the relative concentrations of heliocides in the mixture. This shows that the synthesis of the heliocides in the plant is subject to enzymatic control. Consequently, it becomes possible to isolate cotton-plant varieties containing effective insecticidal mixtures of heliocides.

The heliocides of group B (23, 24, 25, and 26) are formed in the reaction of 6-O-methylhemigossypolone with ocimene (heliocides B_1 and B_4) and with myrcene (heliocides B_2 and B_3). Later, Bell et al. [54] isolated a new quinone, 7-O-methylhemogossypolone (27), from the glands of young green tissues of *G. barbadense* and the dark red pigment gossyrubilone (28) from the glands of young leaves of *G. barbadense* and *G. hirsutum*. Gossy-

rubilone is an isopentylimine derivative of hemigossypol. A new sesquiterpinoid which the authors concerned called raimondal (29) has been detected in the glands of the leaves and unripe stems of *Gossypium raimondii* [55].

BIOSYNTHESIS OF THE TERPENOID ALDEHYDES OF THE COTTON PLANT

A hypothetical pathway of the biosynthesis of terpenoid aldehydes in cotton has been given by Bell [48, 56]. The biosynthetic precursor of gossypol is hemigossypol (9) and that of O-methyl derivatives of gossypol (7) and (8) is 6-O-methylhemigossypol (10).

Deoxy-6-deoxyhemigossypol (30) and deoxyhemigossypol (31) form the source of hemigossypol.

The oxidation of deoxyhemigossypol forms hemigossypol. In the seeds, the bark of the roots, the flowers, and other achlorophyllous tissues of the cotton plant, further biosynthesis leads to the formation of gossypol. The dimerization of hemigossypol is catalyzed enzymatically by peroxidase [54].

The reaction of one molecule each of hemigossypol and of O-methylhemigossypol forms 6-O-methylgossypol, while two molecules of O-methylgossypol form 6,6'-di-O-methylgossypol.

In plant tissues containing chlorophyll (leaves, young seed capsules, flower buds), however, there are enzymes which catalyze the formation of quinones. In them hemigossypol undergoes further oxidation of hemigossypolone. The reaction of the latter with myrcene gives heliocides H_1 and H_4, and that with ocimene gives heliocides H_2 and H_3.

METHODS OF DETERMINING GOSSYPOL

Gossypol shows a harmful physiological action on ruminants and therefore its determination is necessary from the point of view of the possibility of using oil cakes and pellets as feedstuffs and meals as feedstuffs and the oil as a food product. This is also important because at the present time the problem exists of obtaining an edible flour and edible protein from cottonseed meal. The toxicity of cotton seed oil cakes and meals is due to the presence of free gossypol in them. By free gossypol is understood gossypol soluble in diethyl ether or 70% aqueous acetone [57, 58]. Up to 0.02% of free gossypol in oil cakes and meals is harmless for animals, from 0.02 and 0.05% has a weak action, and at 0.15-0.20% it may cause severe poisoning [59]. According to Pons's recent results [4], the amount of free gossypol in cottonseed flour intended for poultry must not exceed 0.045%.

In the moist-hot treatment of the seeds that is necessary for extracting the oil, part of the gossypol changes, losing its capacity for dissolving in diethyl ether and it is liberated only by hot aniline or oxalic acid [60]. This gossypol is called bound, and is not a definite substance but a series of compounds of gossypol through the aldehyde group with proteins, amino acids, phosphatides, and other substances having various compositions and properties. The total gossypol is that which is extracted from material previously heated with oxalic acid.

It was considered for a long time that bound gossypol is not toxic. Consequently, one of the industrial methods for detoxifing oil cakes and meals was the "binding" of the gossypol by a special moist-heat treatment of the mass before the extraction of the oil from the seeds. However, Eagle has shown that gossypol bound with amino acids is also toxic, although at a far higher level in the oil cake than for free gossypol [61].

QUALITATIVE REACTIONS FOR GOSSYPOL

Qualitatively, gossypol can be detected by using its property of giving color reactions with a whole series of substances [62]; with concentrated sulfuric acid — scarlet; with ferric chloride — dark olive green; with nickel acetate — violet; with stannic chloride — purple-red. Under the action of antimony trichloride, a methanolic solution of gossypol acquires a purple color. With phloroglucinol a bordeaux-red derivative is formed, and with 2,4-dinitrophenylhydrazine an orange color. All these reactions are specific for free gossypol. However, there are reactions, such as that of Markman and Zalesov [63] which detect changed gossypol together with the native compounds. The reaction that they have proposed is based on the coupling of gossypol with diazotized p-nitroaniline to form a red-orange precipitate of p-nitrophenylazogossypol. The reaction takes place at C_4 or C_4, where there are unsubstituted atoms in the aromatic nuclei [64].

METHODS FOR THE QUANTITATIVE DETERMINATION OF FREE GOSSYPOL

The naphthalenealdehyde form of gossypol is that which is responsible for the majority of the reactions used in analytical methods of determining free gossypol. Many methods exist for its quantitative determination [65], but they all depend largely on the nature of the solvents used for extraction [4]. Carruth used the percolation of ether through a sample for 3 hours to extract the gossypol. Halverson and Smith found that the addition of water and ethanol to the ether gave more reproducible results. Boatner suggested the 24-hour extraction of gossypol with chloroform. Later, Hall shortened the time of extraction to 30 min by using a mixture of ethanol and water (60:40) as solvent. Smith used ethanol—water—ether (57:24:17) and high-speed stirring to achieve the complete extraction of gossypol in 5 min. Pons [66] carried out extraction with a mixture of acetone and water (70:30) for an hour with vigorous shaking. This solvent extracts the oil to the minimum extent but dissolves the gossypol satisfactorily.

The first methods for the quantitative determination of gossypol were based on its gravimetric precipitation from the sample being analyzed in the form of the dianiline derivative, which is insoluble in the majority of organic solvents [60].

The gravimetric method, very laborious and requiring a long time for the complete deposition of the precipitate, has been displaced by spectrophotometric methods. One of them, based on the reaction of gossypol with aniline was proposed by Lyman and was improved by Smith [4]. Pons's spectrophotometric method [67] is based on the capacity of gossypol for forming with p-anisidine a yellow compound analogous to dianilinogossypol. This method of determination has been adopted as officinal [60]. In other methods, antimony trichloride [68] or phloroglucinol [69] is used. Below, we give the absorption maxima of the gossypol derivatives formed in the methods mentioned (nm):

Gossypol	360
Gossypol with aniline	440
" " p-anisidine	447
" " antimony trichloride	520
" " phloroglucinol	550

The reaction with phloroglucinol, taking place in a strongly acid solution, is the most sensitive, and the reactions with p-anisidine and aniline are somewhat less sensitive (87% and 76%, respectively, relative to the reaction with phloroglucinol taken at 100%). Antimony trichloride gives an insensitive reaction (7%) [4].

Sharma [70] recommends a rapid semimicro method of determining free gossypol in cotton seeds which is based on the quantitative reduction of ferric chloride by gossypol and on the formation by the bivalent iron with α,α'-bipyridyl of a ruby-red product giving an absorption maximum at 510 nm. This reaction is not specific for gossypol alone, since the same complex is formed by tocopherols. Consequently, the preparation of the sample for analysis is very important. To eliminate the tocopherols, the ground seeds are previously treated with petroleum ether.

Colorimetric and spectrophotometric methods are usually employed with the use of various stable derivatives of gossypol, since gossypol itself undergoes profound changes with time. We have shown that the fairly long storage of alcoholic solutions of gossypol (at 5-10°C in the dark) does not change the nature of the spectrum in the 300-400 nm region and does not lead to appreciable quantitative changes. On this basis, an accelerated spectrophotometric method of determining free gossypol as such and not in the form of its derivatives has been suggested [71].

Among other methods of quantitative determination we must mention a method based on the reduction of Fehling's solution [72]. This makes use of the capacity of gossypol, as an aldehyde, for reducing copper(II) to copper(I), the amount of which is found by titration with permanganate.

A. L. Markman et al. have developed polarographic [73] and luminescence [74] methods of determining gossypol in an oil, but because of their laboriousness and the difficulty of their performance they have not come into wide use.

S. P. Yun et al., using as an example seeds of the cotton plant of the variety Tashkent-1, have developed an optical-microscope method of determining gossypol in the kernels of cotton seeds and have shown that the absolute "...probable error of determinations of the volume proportion of gossypol-like secretions..." in the use of this method is 0.07% [75].

Trace amount of gossypol are determined by Scharm and Benedict's paper-chromatographic method [76]. It consists of the operations of selective extraction of the gossypol with dimethylformamide—water (2:1) followed by the quantitative paper chromatography of the extract. The mobile solvent used is heptane—chloroform—acetic acid (80:10:5), and the spots are revealed with antimony trichloride or phloroglucinol. The limit of detection with antimony trichloride is 2 μg of gossypol, and that with phloroglucinol is about 0.5 μg. The use of phloroglucinol is preferable, since it gives a more stable coloration.

For a long time it was impossible to use gas—liquid chromatography for determining the gossypol, because of its low volatility. Wide use is made of trimethylsilyl derivatives of hydroxy-containing compounds to achieve the desired volatility. However, it proved impossible to obtain such derivatives for gossypol with the aid of hexamethyldisilazane and chlorotrimethylsilane, and only by the use of bistrimethylsilylacetamide has it been possible to prepare trimethylsilyl derivatives of gossypol [77].

Gas—liquid chromatography permits the determination in the form of individual peaks both of gossypol itself and of gossypol-like pigments.

A COMPARISON OF THE METHODS OF DETERMINING FREE GOSSYPOL

The methods usually used for determining readily soluble free gossypol (Pons's anisidine method and Smith's aniline method) agree well with one another in the analysis of products of the processing of cotton seeds [4].

When using as solvent a mixture of butan-2-one, aniline, and water (90:10:0.5) (with phloroglucinol as the chromogenic reagent — method of Storherr and Holley), results for gossypol content agreeing with those obtained by Pons's method are achieved [69].

The results of the determination of the amount of free gossypol in samples of cotton seeds, pulps, and oils obtained by the p-anisidine method [60] and the method of spectrophotometry using an aqueous acetone solution of gossypol itself are fairly close, but in simplicity and time the method of spectrophotometry using solutions of gossypol itself and not its derivatives has undoubted advantages [71].

Below we give figures showing the sensitivity and limits of detection of gossypol spectrophotometrically, and by paper and gas—liquid chromatography [4]:

Method	Sensitivity, mg	Limits of detection, parts/million
Spectrophotometry	10	50—100
Paper chromatography	0,5	10
Gas—liquid chromatography	0,2	1—2

The spectrophotometric method has a limit of detection of 50-100 parts per million (0.005-0.01%), while that for paper chromatography is 5-10 times and for gas—liquid chromatography 50-100 times smaller.

In the methods mentioned, both gossypol itself and other gossypol-like pigments are determined. Consequently, the value found is frequently greater than the actual gossypol content. All terpenoid aldehydes present in the cotton plant (hemigossypol, isohemigossypol, gossivertin, methyl ethers of gossypol and hemigossypol, and a whole series of others) give, like gossypol, colored derivatives with aniline, p-anisidine, antimony trichloride, phloroglucinol, and 2,4-dinitrophenylhydrazine. Hence, the figures from the determination of gossypol by the methods described above largely reflect the amount of total terpenoid aldehydes and not of gossypol itself.

Thus, Pons [78], investigating the resins obtained in the form of a byproduct in the water-washing of crude cottonseed oils, established that they contain considerable amounts of gossypol and phosphatides. In addition, a large parct of the gossypol in the resins is present in the bound form, predominantly as chemical compounds with phosphatides; this is also responsible for those physical properties that permit it to be assigned to the "soluble-bound" gossypol. The same compounds with phosphatides are present in cottonseed pulp. Gossypol-(amino acid) complexes have also been isolated from crude cottonseed oil [79]. Mattson and Martin [72], using paper chromatography, separated this "soluble-bound" gossypol from cottonseed flakes into three fractions: gossypol, phospholipid-bound gossypol, and a fraction of more hydrophilic nature. The third fraction — hydrophilic gossypol — is apparently a gossypol-(amino acid) or -peptide complex.

TABLE 1. Fractionation of Soluble Gossypol Derivatives

Cotton-plant product	Free gossypol determined by Pon's Method, %	Soluble classes of gossypol determined by papter chromatography, %		
		gossypol	phospholipid-bound gossypol	hydrophilic gossypol
Gossypol-free seeds	0,011	0,002	0,002	0,007
Seeds containing gossypol	0,63	0,40	0,16	0,07
Steamed seeds	0,11	0,01	0,03	0,06
Expeller pulp	0,05	Not det.	0,02	0,03

The results of a study of the fractions of soluble gossypol derivatives from cotton flakes are given in Table 1 [4].

The Table shows that the p-anisidine method of estimating free gossypol determines not only gossypol itself but also gossypol bound with phospholipids and the hydrophilic gossypol complex. In ordinary cotton seeds 63.5% of the gossypol determined by Pons's method is gossypol proper, and the remainder is in the form of phospholipid-gossypol (25.4%) and hydrophilic gossypol (11.1%). After heating and pressing, gossypol itself is not detected in the cottonseed pulp by paper chromatography and the 0.5% of free gossypol found consists to the extent of 40% of phospholipid-gossypol and 60% of hydrophilic gossypol.

Martin has established that the phospholipid gossypol is similar in its physical activity to gossypol, since it causes the decoloration of poultry egg yolks when this fraction of gossypol is added to poultry feed [4]. Hydrophilic gossypol has a smaller physiological activity. On this basis, Martin concludes that the methods used for determining free gossypol do in fact measure its physiologically active derivatives.

DETERMINATION OF TOTAL GOSSYPOL

Total gossypol consists of the sum of the free and bound gossypols.

The known methods of determining total gossypol are based on its extraction with hot aniline from cottonseed pulp previously treated with diethyl ether to eliminate free gossypol. The extract is concentrated and is left for several days for the deposition of a precipitate followed by the gravimetric determination of gossypol in the form of dianilinogossypol [60]. Smith has modified this method, suggesting the extraction of the free and bound gossypol with a mixture of hot ethanol and aniline [81]. Subsequently, these gravimetric methods of determining total gossypol, like those for determining free gossypol, were displaced by spectrophotometric methods.

Pons [82] was the first to propose a spectrophotometric method, by which the bound gossypol is first hydrolyzed with oxalic acid in the azeotrope of butan-2-one with water with heating for 6 h. Then the liberated gossypol is determined spectrophotometrically in the form of the p-anisidine derivative. This method has been adopted by the American Oil Chemists' Association as the official method for the determination of total gossypol. In Smith's method [81], the sample is treated with hot 72% aqueous ethanol and it is then heated with aniline. The dianilinogossypol formed is extracted with chloroform for an hour and its amount is determined spectrophotometrically. A comparison of the results of the determination of the amounts of free gossypol in a number of samples of cottonseed pulp by Smith's method and Pons's method has shown that the former gives values 5-9% higher than the latter [83].

This may be due to an increase in absorption through nongossypol pigments, while in Pons's method the difference in absorption between samples of an aliquot with p-anisidine and without it permits errors due to nongossypol pigments to be eliminated.

Later, to determine total gossypol in cottonseed pulp, crude oils, and soap stocks, Pons proposed yet another method, based on the rapid (30-minute) extraction of gossypol with a mixture of 3-aminopropan-1-ol and dimethylformamide with the formation of a stable complex followed by the colorimetry of an aliquot of the extract after its reaction with aniline. The difference in the absorptions of portions of the extract before and after the reaction with aniline serves as a measure of the amount of total gossypol present. The determination can be completed in two hours instead of the seven on hydrolysis with oxalic acid. In crude

oils, total gossypol is found by dissolving the oil in a mixture of hexane and isopropanol, heating with p-anisidine, and subjecting the di-p-anisidinogossypol to spectrophotometry [84].

A comparison made by Pons of the method of determining total gossypol proposed by him with the use of hydrolysis by oxalic acid and aminopropanol showed that the latter method gives higher results for a number of samples of pulps and seeds (on an average, by 3-5%) [4]. This is ascribed to the oxidation of part of the gossypol during the six-hour hydrolysis with oxalic acid. In addition, Pons has shown that both methods are fairly accurate but the aminopropanol hydrolysis proves to be more correct. Consequently, a further study of this method is proposed for its possible adoption as an official method.

Thus, as can be seen from the material presented, in spite of a large number of investigations by Soviet and foreign workers the question of the structure of the gossypol-like pigments accumulating in cotton seeds during their storage and processing has not yet been resolved. Nevertheless, some of them (gossyvertin, etc.) possess a toxicity higher than that of gossypol, which prevents the wide use of cottonseed processing products.

Furthermore, investigations of the chemical nature of the phytoalexins of the cotton plant have shown that the main fungitoxic substance with respect to certain fungal pathogens for it are gossypol and pigments related to it (verdosin, hemigossypol, etc.), and therefore a further study is of great interest from the point of view of the fight against the attack of the cotton plant by wilt. So far as concerns the determination of the amount of free gossypol present, it must be observed that the methods existing at the present time measure both gossypol itself and pigments similar to it (hemigossypol, isohemigossypol, O-methyl derivatives of gossypol and of hemigossypol, etc.), and therefore the development of special methods for the separate determination of gossypol and its analogs is necessary.

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THE SEARCH FOR PROSTAGLANDINS AND PROSTAGLANDIN-LIKE COMPOUNDS IN PLANTS

A. G. Panosyan

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A review is given of the literature devoted to the question of the presence and distribution of prostaglandins and analogous products of the oxidative biotransformation of polyunsaturated fatty acids in plants. A comparison is made of the routes of oxidation of polyenic acids and the biological activity of the products formed in animal tissues and plants.

It was considered for a long time that the prostaglandins (PGs) are characteristic exclusively of mammals, where they are present in practically all tissues [1]. Then they were sought and rapidly found in many lower organisms [2]. PGs from the coral *Plexaura homomalla* [3] have even served as a raw material for chemical modifications [4] and for semi-industrial production, so high is their concentration in these organisms. There are reports of the formation and secretion of PGs and PG-like compounds by bacteria [5, 6]. As early as the beginning of the seventies, a search for PGs in plants had been begun but even today it is hardly possible reliably to state that the classical PGs are widely distributed in the vegetable kingdom.

A. L. Mndzhoyan Institute of Fine Organic Chemistry, Academy of Sciences of the Armenian SSR, Erevan. Translated from Khimiya Prirodnykh Soedinenii, No. 2, pp. 139-146, March-April, 1981. Original article submitted July 17, 1980.

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