9. V. N. Shevts, V. A. Klopova, and N. A. Preobrazhenskii, Khim. Prir. Soedin., 225 (1966).
10. M. A. Flerov and A. G. Vinogradov, Vopr. Med. Khim., 15, No. 2, 197 (1969).
11. S. A. Demopoulos, M. S. Moschidis, and L. M. Kritikou, J. Chromatogr., 256, 378 (1983).

# ENZYMATIC-CHEMICAL ISOLATION OF LIPIDS, PROTEINS, AND GOSSYPOL FROM COTTON SEEDS AND MEAL. I.

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An enzymatic-chemical method of isolating the complex of lipids, gossypol pigments, and water-soluble proteins from cotton seeds and meal has been developed which permits: a) the achievement of the maximum yield of all types of lipids without their degradation; b) a rise in the qualitative and food value of the oil and protein products isolated from the seeds, with an increase in the solubility of the latter; c) the extraction from cotton seeds of more than 75% and from cottonseed meal of 42% of the free gossypol. The method, in combination with Folch's procedure, is suitable for the quantiative estimation of the lipids, strongly bound to the protein of the nucleus.

The seeds of oil crops (cotton, sunflower, rape, etc.) are sources not only of vegetable oils but also of biologically active phospholipids, glycolipids, polyphenols, and a valuable vegetable protein with unique organoleptic and functional properties [1, 2].

In the oils and fats industry, the technology of a two-stage treatment of the seeds first by prepressing and then by extracting the solid residue with a hydrocarbon solvent (gasoline) is used to extract vegetable oils. It presupposes a severe treatment of the raw material, which, while facilitating the extraction of the desired product, simultaneously causes a change and partial breakdown of other valuable substances, the denaturation of the proteins and a deterioration in their nutritional value, the oxidation of the polyphenols, and the binding of part of the phenols and lipids with the protein. For this reason, the industrial oil cake and meal that are treated by the traditional technology as by-products have poor fodder properties. Of the components of the oil seeds that are of interest as independent products, at the present time phosphate concentrates are obtained, together with oil from soybeans and sunflower seeds.

A promising direction in the oils and fats industry is considered to be the development of methods permitting the production, for example, from cotton seeds, of a high-quality food oil, a gossypol-free fodder meal, and gossypol as an independent product for medicinal and technical purposes [3].

The simultaneous isolation of the lipid complex and of protein with no change in their quality is possible by treating an aqueous suspension of the oil-containing raw material with enzymes of microbiological nature (pectolytic enzymes, cellulases, proteinases) breaking down the complexes of polysaccharides, proteins, and lipids [4]. Lipids are isolated from the hydrolysate by extraction with organic solvents, and then the water-soluble and insoluble protein components are obtained from the residue. Under the action of a proteinase, the high-molecular-mass proteins are broken down to low-molecular-mass water-soluble proteins and peptides.

In comparison with the traditional method, the biotechnological method requires a lower consumption of energy, is distinguished by mild conditions of treating the material, and permits an increase in the solubility of the protein components as the result of the cleav-

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age of peptide bonds [5]. Under the actions of enzymes, not only neutral but also polar bioactive lipids and also, possibly, phenolic compounds (in the case of cotton seeds – gossypol) are liberated. However, there is not information in the literature on the behavior of these components during enzymatic hydrolysis.

The aim of the present investigation was to find conditions for the simultaneous enzymatic-chemical isolation of neutral bioactive lipids, native gossypol, and hydrolyzed protein from cotton seeds and cottonseed meal – the product remaining after the industrial extraction of the oil from the seeds.

In the first series of experiments, to evaluate the degree of coextraction of the polyphenolic pigment – gossypol – and polar lipids we used a gossypol-rich fraction of cottonseed flour obtained from a previously defatted flour of cottonseed kernels by the hydrocyclone method [6].

Gossypol-Rich Cottonseed Flour. According to chemical and spectrophotometric analyses, the flour contained 7.6% of residual lipids, including bound and strongly bound lipids, and 2.5% of free gossypol. For the proteolysis of the flour we chose proteinases with their action in the range of from 2 to 8.5, and performed the process at 45-50°C. The lipids that had separated out after proteolysis, which were enriched with polar components, were extracted both from the aqueous buffer phase and from the solid phase. The yield of lipids was evaluated in comparison with the results of the generally adopted chemical method of extracting bound (polar) and strongly bound lipids. It must be mentioned that the chemical method extracts not only the native strongly bound lipids but the products of their alkaline hydrolysis – free fatty acids (FFAs) – from the level of which the amount of this group of lipids in the plant tissues is judged.

The results of the proteolysis of this sample under various conditions are given in Table 1, where the yield of lipids after hydrolysis with papain, which corresponds to the result of the chemical method, is taken as 100%. It can be seen that the contact of the flour with the aqueous buffer medium under the given conditions even in the absence of the enzyme leads to the liberation of ~1/3 of the bound lipids from the lipoprotein complexes. At the same time, with a lowering of the pH the solubility of the polar lipids in the aqueous buffer phase rises. The property of the complexes of breaking down more readily in a more acid medium has been made the basis of known methods for extracting the bound lipids by acidic extractants [7].

Hydrolysis by pepsin led to the liberation of only half the bound lipids, while preparat S and papain led to their practically complete isolation.

The distribution of the lipid products of enzymatic hydrolysis between the phases of the reaction medium also varied. In all the experiments without an enzyme, and also on hydrolysis by pepsin and preparat S, from 63 to 96% of the lipids liberated from the complexes were held weakly in the solid phase, while on hydrolysis by papain, 80% of their amount was present in the aqueous buffer solution (see Table 1).

When aliquots of the lipids were chromatographed by the TLC method we found that they consisted of neutral (system 1) and polar (system 2) components, the main ones of which were triacylglycerols (TAGs), FFAs, monogalactosyldiacylglycerols, digalactosyldiacylglycerols, phosphatidylcholines, and phosphatidylinositols. In the experiments with pepsin and papain, the area of the FFA spot correlated visually with that of the TAGs, while with preparat S it was somewhat greater, and on the basis of this fact we deduced the absence of an appreciable breakdown of the lipids during the proteolysis of the flour in an acid medium and a very slight degree of it at pH 8.5.

According to TLC, the only polyphenols in the lipid extracts from acid proteolysis were gossypol pigments ($R_f$ of gossypol 0.60 and of gossypurpurin 0.27, system 3), while in the products of alkaline proteolysis more highly polar yellow pigments of flavonoid nature were also present [8].

The amount of free gossypol was determined by the spectrophotometric method (Table 2). According to the figures in Table 2, in the experiments without an enzyme the pH of the medium did not appreciably affect either the amount or the interphase distribution of the gossypol. The liberation of gossypol from the plant tissues took place as the result of the breakdown by water of the polysaccharide envelope of the gossypol glands [9], while under the conditions of the control experiment the pigment was sorbed mainly in the solid phase.

TABLE 1. Influence of an Enzyme on the Yield* of the Residual Lipids from a Gossypol-Rich Cottonseed Flour

| Enzyme | pH | Liquid phase | | Solid phase | | Total yield | |
|---|---|---|---|---|---|---|---|
| | | g | % on the weight of the lipids | g | % on the weight of the lipids | g | % on the weight of the residual lipids |
| Pepsin | 2,0 | 0,15 | 37,0 | 0,25 | 63,0 | 0,39 | 51,6 |
| Control | " | 0,09 | 29,9 | 0,21 | 70,0 | 0,29 | 38,2 |
| Papain | 5,0 | 0,61 | 79,9 | 0,15 | 20,1 | 0,76 | 100 |
| Control | " | 0,03 | 16,2 | 0,15 | 83,8 | 0,17 | 22,9 |
| Preparat S | 8,5 | 0,03 | 3,5 | 0,73 | 96,5 | 0,76 | 99,1 |
| Control | " | 0,01 | 5,5 | 0,20 | 94,5 | 0,21 | 27,1 |

*Here and below, the yield of PLs is given as a sum with the polyphenolic pigments.

TABLE 2. Influence of the Nature of the Enzyme on the Yield of Gossypol after the Proteolysis of a Gossypol-Rich Cottonseed Flour

| Enzyme | Amount of gossypol, % by weight | | | | | |
|---|---|---|---|---|---|---|
| | lipids | gossypol | lipids | gossypol | lipids | gossypol |
| | Liquid phase | | Solid phase | | Total amount | |
| Papain | 5,9 | 14,4 | 15,4 | 9,4 | 7,8 | 23,8 |
| Control | 7,0 | 0,8 | 15,0 | 8,8 | 13,7 | 9,6 |
| Preparat S | 0,0 | 0,0 | 30,2 | 88,2 | 29,2 | 88,2 |
| Control | 0,0 | 0,0 | 14,9 | 11,7 | 14,1 | 11,7 |

Hydrolysis by the weakly acid protease increased the yield of gossypol by a factor of 2.4 in comparison with control and led to the passage of the bulk of it into the aqueous buffer phase. The most complete isolation of gossypol and the pigments accompanying it (88%) was achieved under the conditions of alkaline proteolysis, a distinguishing feature of which is the localization of the pigment and lipid products in the solid phase (see Table 1).

The retention of the native nature of the gossypol isolated from the mass of polar lipids by alkaline proteolysis was confirmed by the UV spectrometry of their hexane solutions. Bands were observed in the spectrum which related to the absorption of the chromophoric groups of unchanged gossypol at $\lambda_{max}^{C_2H_5OH}$ 234-236, 289-291, and 376-378 nm. At the same time, the spectrum contained another series of bands of weakened medium intensity at $\lambda_{max}^{C_2H_5OH}$ 228, 245, 260, 303, and 403 nm, which confirmed the presence, together with the gossypol, of other phenolic pigments, of nongossypol nature.

In later experiments, we selected the optimum conditions for the isolation of the bioactive lipids and the complex of phenolic pigments by weakly alkaline proteolysis. As the substrates we used previously defatted cottonseed kernel flour and cottonseed meal, and as the enzyme the domestic industrial preparation protosubtilin G10X, which is employed in the production of synthetic materials for domestic, technical, and medical purposes.

Cottonseed Kernels. The kernels of an industrial sample of cotton seeds had the following indices: moisture content – 7.5% hexane-extractable neutral lipids – 32.72%; residual polar lipids – 4.87% (including 0.5% of neutral, 3.27% of bound, and 1.1% of lipids strongly bound to protein): and 1.29% of total, including 1.19% of free, gossypol.

To increase the efficacy of proteolysis it is necessary to eliminate the neutral fat from the oil-containing substrate [2, 4]. With the aim of extracting it more completely and reinforcing the degree of subsequent fractionation of the components, the kernels were first dried at 85°C for 3 h to a residual moisture content of 3.5% [10]. This drying regime, proposed for the preparation of cottonseed kernels as a source of food flour, causes no appreciable change in the quality of the lipid complex and leads to some increase in the level of salt-soluble and to a fourfold decrease in the level of insoluble proteins. Furthermore, this regime decreases the amount of free gossypol passing into the neutral lipids on their subsequent extraction but it does not lead to its binding with the protein of the kernels. After drying, the kernels were ground, and the neutral lipids were extracted from

TABLE 3. Influence of the Conditions of Hydrolysis by Protosubtilin on the Yield of Polar Lipids from Cottonseed Kernels

| pH of the medium | Amount of enzyme, % on the weight of the sample | Time of proteolysis, h | Yield of lipids, % on their total weight | | Total yield, % on the weight of the sample. |
|---|---|---|---|---|---|
| | | | liquid phase | solid phase | |
| 8,5 | 1,0 | 24 | 21,4 | 78,9 | 4,84 |
| 10,0 | 1,0 | 16 | 20,4 | 79,6 | 4,79 |
| 9,0 | 1,0 | 16 | 8,9 | 91,1 | 4,60 |
| 8,5 | 1,0 | 16 | 16,1 | 83,9 | 4,82 |
| 10,0 | 1,0 | 3 | 11,8 | 88,2 | 3,68 |
| 9,0 | 1,0 | 3 | 17,6 | 82,4 | 4,28 |
| 8,5 | 1,0 | 3 | 6,0 | 94,0 | 4,83 |
| 8,5 | None | 3 | 0,0 | 100 | 2,17 |
| 8,5 | 1,0 | 4 | 6,0 | 94,0 | 4,81 |
| 8,5 | 2,0 | 4 | 6,9 | 93,1 | 4,82 |
| 8,5 | 4,0 | 4 | 7,2 | 92,8 | 4,87 |
| 8,5 | 1,0 | 2 | 8,0 | 92,0 | 2,87 |
| 8,5 | 2,0 | 2 | 4,2 | 95,8 | 3,89 |
| 8,5 | 4,0 | 2 | 5,3 | 94,7 | 4,17 |

TABLE 4. Influence of the Conditions of Hydrolysis by Protosubtilin on the Yield of Protein Hydrolysate from Cottonseed Kernels

| Amount of enzyme, % on the weight of the sample | Time of proteolysis, h | Yield of protein, % on the weight of the protein of the kernels |
|---|---|---|
| Control | 4 | 18,20 |
| 1,0 | 2 | 21,74 |
| 1,0 | 4 | 28,62 |
| 1,0 | 8 | 37,70 |
| 2,0 | 2 | 32,62 |
| 2,0 | 4 | 37,70 |
| 2,0 | 8 | 39,00 |

the flour with hexane in a yield of 33.6%, which was 0.9% greater than that from the flour of the undried kernels.

The neutral lipids had an acid No. of 1.68 mg of KOH and contained 0.08% of free gossypol, which was responsible for their color of 6-7 red units (at 35 yellow units) in a 3.5-cm layer, while they contained no polar lipids (TLC) and sediment. With respect to their color they corresponded to the standard for refined cottonseed oil of the highest grade; with respect to their acid number they did not satisfy the standard, but the level of FAAs in them was 3.5 times less than in the crude oil processed by the traditional technology. In order to obtain oil of good quality the subsequent elimination FAAs was necessary, and in view of their low level it was possible to perform this under comparatively mild conditions of raffination.

After defatting, the flour was subjected to enzymatic hydrolysis with protosubtilin. The experiments were performed by changing one of the parameters of the process at a time: the pH of the medium, the amount of enzyme, and the duration of the treatment. The result of the experiments are reflected in Tables 3 and 4.

It can be seen from Table 3 that the highest yield of polar lipids from the kernels – at pH 8.5 with 1-4% of enzyme and a process time of 3-4 h – corresponded to that determined by the chemical method [11]. In contrast to the hydrolysis of the gossypol-rich fraction of the flour by preparat S (see Table 2), on the hydrolysis of the kernels by protosubtilin the passage of only a small amount of polar lipids into the liquid phase was observed. The experiments also showed that the conditions of the greatest activity of the protosubtilin

TABLE 5. Distribution of the Gossypol in the Products of the Proteolysis of Cottonseed Kernels

| Product | Gossypol, free form, % by weight of the | | Gossypol, bound form, % on the weight of the product |
|---|---|---|---|
| | product | free gossypol | |
| Cottonseed kernels | 1,19 | 100 | 0,1 |
| Neutral lipids | 0,08 | 2,3 | 0,0 |
| Polar lipids | 18,4 | 75,6 | 0,0 |
| Water-soluble protein | 0,02 | 8,5 | 0,03 |
| Solid residue | 0,02–0,05 | 0,4–1,7 | 0,3 |

TABLE 6. Influence of the Conditions of Hydrolysis by Protosubtilin on the Yield of Residual Lipids from Cottonseed Meal

| Amount of enzyme, % on the weight of the sample | Time of proteolysis, h | Yield of lipids, % on their weight | | Total yield, % on the weight of the sample |
|---|---|---|---|---|
| | | liquid-phase | solid-phase | |
| 2,5 | 2 | 1,0 | 99,3 | 6,26 |
| 4,0 | | 1,9 | 98,1 | 6,30 |
| 5,0 | | 2,8 | 97,2 | 6,47 |
| Control | | 2,1 | 97,9 | 2,30 |
| 2,5 | 4 | 1,2 | 98,8 | 6,32 |
| 4,0 | | 1,7 | 98,3 | 6,47 |
| 5,0 | | 4,1 | 95,9 | 6,48 |
| Control | | 1,5 | 98,5 | 4,06 |
| 2,5 | 8 | 2,1 | 97,9 | 6,40 |
| 5,0 | | 3,4 | 96,3 | 6,47 |
| Control | | 0,1 | 99,9 | 4,17 |

G10X (pH 9.5-10, temperature 50-55°C) do not correlate with those that are the optimum for the complete isolation of the polar lipids from the lipoprotein complexes (pH 8.5; temperature 45-50°C).

Table 4 gives the yields of water-soluble protein hydrolysate from the kernels as a function of the conditions of proteolysis. As can be seen, the maximum yield of soluble protein was achieved with hydrolysis for eight hours using a 2% concentration of enzyme.

We determined the amount of free and bound gossypol in all the products obtained after the proteolysis of the kernels under the optimum conditions (Table 5). Almost 76% of the weight of the free gossypol was extracted simultaneously with the polar lipids. Its total amount distributed between the products of enzymatic hydrolysis was 87-88%. Since the level of the bound form of the pigment in the solid residue calculated on the unhydrolyzed kernels did not change, it follows that from 12 to 18% of the free gossypol was bound with the water-soluble protein.

The level of free and bound gossypol in the protein products obtained was low, and in a number of experiments the amount of the free form of the pigment in the solid protein residue (0.02%) corresponded to the requirements of the GOST [State Standard] for fodder cottonseed meal of grades I-II. It must be mentioned that on the use of chemical extraction for the isolation of water-soluble proteins it is impossible to obtain cottonseed proteins with such a high yield at such a low gossypol content [12].

Cottonseed Meal. The amount of residual lipids in the meal according to the results of the chemical method was 6.49%, and the total gossypol 1.2%, including 0.22% of its free form and 0.98% of its bound form.

In the industrial meal the proteins are frequently denatured, and therefore ~5% of enzyme was used for its proteolysis. The yield and distribution of lipids in the products of the proteolysis of the meal are given in Table 6, from which it follows that under the conditions used (4-5% of enzyme, 2-4 h) it is possible to isolate up to 99.8% of residual lipids from the meal.

In comparison with the kernels (Table 3), the proteolysis of the meal was accompanied by the absorption of the lipids mainly on the solid phase, and the proportion of lipids

passing into the liquid phase increased with a rise in the proportion of enzyme by weight. Analysis of the amount of gossypol showed that after the hydrolysis of the meal with 5% of the enzyme for 8 h the solid protein residue contained 0.54% of free and 0.46% of bound gossypol, while 6.05% of free gossypol was found in the polar lipids.

Thus, as the result of the enzymatic treatment, more than half of the bound form of gossypol was hydrolyzed and 42% of the total amount of free gossypol was extracted from the meal together with the polar lipids; the total amount of gossypol in the solid residue had decreased by 20% in comparison with that in the unhydrolyzed sample.

The enzymatic-chemical method in combination with preliminary extraction by Folch's method [13], is suitable for the quantitative estimation of the native strongly bound lipids in the reserve tissues of plants, which makes it possible to investigate their composition and structure and to elucidate the role of this group of lipids in the vital activity of the plant cell. Furthermore, enzymatic hydrolysis permits an improvement in the development of methods for extracting difficultly soluble and partially denatured proteins from processed seeds of oil crops with a rise in their food value.

## EXPERIMENTAL

The UV spectra of the gossypol were taken on a Hitachi instrument in ethanol, TLC was conducted on Silufol plates (Czechoslovakia) in the following systems: 1) hexane–diethyl ether–acetic acid (70:30:1); 2) chloroform–methanol–water (65:25:5); 3) benzene–absolute methanol (20:5).

The polar lipids were identified as described in [13, p. 138], and gossypol and gossypurpurin in comparison with standard samples isolated from cotton seeds [14]. The cottonseed kernels and meal were obtained from the Tashkent Oils and Fats Combine. The samples were ground in a coffee mill and were defatted by steeping at room temperature five times. In each of the experiments ~10 g of sample was used.

The indices of the neutral lipids were determined in accordance with handbook recommendations [15], free gossypol by a spectrophotometric method [16], total gossypol as described in [17], and bound and strongly bound lipids as described in the above-mentioned handbook [15, Vol. 1, Book 1, p. 197]. Enzymatic hydrolysis was performd with: papain (Switzerland), medicinal pepsin, preparat S (All-Union Scientific-Research Institute of Antibiotics of the USSR Ministry of Health), and protosubtilin G10X.

Hydrolysis with papain was carried out in an ammonium acetate buffer at pH 5.0 with an enzyme–substrate ratio of 1:25 in the presence of cysteine at 36°C for 24 h as described in [18].

Hydrolysis with pepsin was carried out in 0.01 N aqueous HCl, pH 2.0, with an enzyme–substrate ratio of 1:50, also at 36°C for 24 h. Hydrolysis with preparat S was carried out in 0.05 M Tris-HCl buffer at pH 8.5 with an enzyme–substrate ratio of 1:20, in the presence of $1 \cdot 10^{-3}$-molar $Ca(CH_3COO)_2$ at 50°C for 8 h, as described in [19].

Hydrolysis with protosubtilin G10X was carried out in 0.05 N Tris-HCl buffer at pH 8.5 with a sample-buffer ratio of 1:20, the enzyme being added in an amount of 1-5% of the weight of the sample. Hydrolysis was continued for 2, 3, 4, and 8 h at 50°C with constant stirring and was stopped by the acidification of the mixture to pH 4.0 with a 10% solution of HCl followed by heating the extract to 90°C for 5 min.

After cooling, the hydrolysate was centrifuged, and the aqueous phase was separated from the solid phase and was extracted three times with diethyl ether to eliminate lipids, after which the residual solution was dialyzed against distilled water to eliminate low-molecular-mass impurities and salts and was then freeze-dried to give the protein hydrolysate.

The solid phase was extracted three times with diethyl ether and then with acetone, which extracts polyphenols most completely. The extracts of lipids were washed with water to neutrality, dried with anhydrous sodium sulfate, and evaporated in a rotary evaporator.

## LITERATURE CITED

1. V. A. Dementii, L. M. Gorshkova, V. I. Tortika, S. P. Andriyanova, V. G. Dianova, and V. B. Tolstoguzov, Maslo-Zhir. Promst., No. 4, 12 (1987).

2. G. Spirad and M. S. Narasinga Rao, J. Agr. Food Chem., 35, No. 6, 962 (1987).
3. A. G. Sergeev, Maslo-zh. Prom., No. 4, 12 (1987).
4. P. D. Fullbrook, J. Am. Oil Chem. Soc., 60, No. 2, 476 (1983).
5. P. P. Rakovskii, V. I. Tortika, and V. N. Krasil'nikov, Maslo-Zhir. Promst., No. 4, 14 (1987).
6. T. V. Chernenko, M. Talipova, A. I. Glushenkova, I. A. Novikova, G. A. Prokopenko, and M. S. Saidov, Maslo-Zhir. Promst., No. 12, 10 (1986).
7. A. Kuksis, in: Chromatography: Fundamentals and Applications of Chromatographic and Electrophoretic Methods, 4th edn., E. Heftmann (ed.), Elsevier Scientific, Amsterdam (1983), Part B, Applications, Chap. 12, p. B75.
8. C. C. Whittern, E. E. Miller, and D. E. Pratt, J. Am. Oil Chem. Soc., 61, No. 6, 1075 (1984).
9. L. Vatsu, T. G. Jacks, H. W. Kircher, and M. A. Godshall, J. Am. Oil Chem. Soc., 63, No. 4, 534 (1986).
10. T. V. Chernenko, M. Talipova, A. I. Glushenkova, and A. U. Umarov, Maslo-Zhir. Promst., No. 4, 15 (1982).
11. S. D. Gusakova, I. P. Nazarova, A. I. Glushenkova, D. A. Khashimov, Kh. G. Alimov, and P. Kh. Yuldashev, USSR Inventors' Certificate No. 1,500,240; Byull. Izobret., No. 30 (1989).
12. É. F. Redina, M. M. Rakhimov, and P. Kh. Yuldashev, Khim. Prir. Soedin., 626 (1974); M. T. Turakhodzhaev, A. A. Pulatov, A. Aripdzhanov, R. I. Shamsutdinov, T. T. Shakirov, P. Kh. Yuldashev, and S. Yu. Yunusov, USSR Inventors' Certificate, No. 427,693; Byull. Izobret., No. 18 (1974).
13. M. Kates, Techniques of Lipidology, American Elsevier, New York (1972).
14. I. P. Nazarova and A. I. Glushenkova, Khim. Prir. Soedin., 664 (1984).
15. Handbook on Methods of Investigation, Technical and Chemical Control, and the Accounting of Production in the Oils and Fats Industry [in Russian], All-Union Scientific-Research Institute of Fats, Leningrad, Vol. 1 (1958), p. 265.
16. I. P. Nazarova, G. A. Nezhinskaya, A. I. Glushenkova, and A. U. Umarov, Khim. Prir. Soedin., 608 (1979).
17. A. L. Markman and V. P. Rzhekhin, Gossypol and Its Derivatives [in Russian], Pishch. Promst., Moscow (1965), p. 218.
18. G. G. Bagirova, V. V. Bagirova, and A. A. Chirkov, Lab. Delo, No. 6, 331 (1985).
19. I. N. Krest'yanova, L. I. Vasil'eva, E. K. Denyakina, L. I. Petrova, G. A. Penzikova, Yu. É. Bartoshevich, and A. D. Neklyudova, Prikl. Biokhim. Mikrobiol., No. 1, 48 (1985).