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FORMATION OF GOSSYPURPURIN FROM ANHYDROGOSSYPOL

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It has been established that gossypurpurin is formed from anhydrogossypol and not from gossypol itself during column chromatography on Chemapol silica gel. This is explained by the high reactivity of anhydrogossypol and the catalytic role of the silica gel. The results obtained permit it to be considered that gossypurpurin is a compound of molecules of monoanhydrogossypol and of diaminomonoanhydrogossypol through a nitrogen bridge.

Anhydrogossypol, formed by the splitting out of two water molecules from gossypol, is distinguished by high reactivity. It interacts with butadiene, dienic fatty acids, acylglycerols, aniline, and anthranilic acid [1].

Anhydrogossypol is usually obtained by heating crystalline gossypol to 190-200°C or by treating it with pyridine hydrochloride in toluene solution [2]. A hypothesis has been put forward of the dehydration of gossypol on silica gel and in alcoholic solutions [3, 4].

We have established that in methanolic solution gossypol undergoes changes accompanied by a decrease in the proportion of aldehyde groups from 96.3% for gossypol to 10.6% in the final conversion product through the formation mainly of dianhydrogossypol (DAG) and a very small amount of monoanhydrogossypol (MAG) [5].

For the separation of a mixture of anhydroderivatives of gossypol into individual components we made use of column chromatographs (CC) on silica gel. Six colored fractions were obtained. It was established with the aid of analytical TLC that fraction (I) contained mainly MAG (R_f 0.36), a small amount of gossypol (R_f 0.60), and traces of DAG (R_f 0.31); fractions II and III consisted of MAG and traces of DAG; and fractions (IV-VI) contained MAG and a more polar violet-purple substance (R_f 0.27) giving a deep blue coloration with $SbCl_3$.

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A comparison of the chromatographic mobilities of the product obtained and its characteristic absorption maxima in the 530 and 565 nm regions with those for standard gossypurpurin (GP) isolated from the gossypol glands of cotton seeds [6] showed that it was gossypurpurin.

The proportion of GP in the combined substances collected from the column was 22.1%, and the amount of aldehyde groups 55.6%. Consequently, in the products eluted from the column the content of aldehyde groups had increased in comparison with the initial mixture (10.6%), which was due to the conversion of DAG into MAG and, partially, into gossypol.

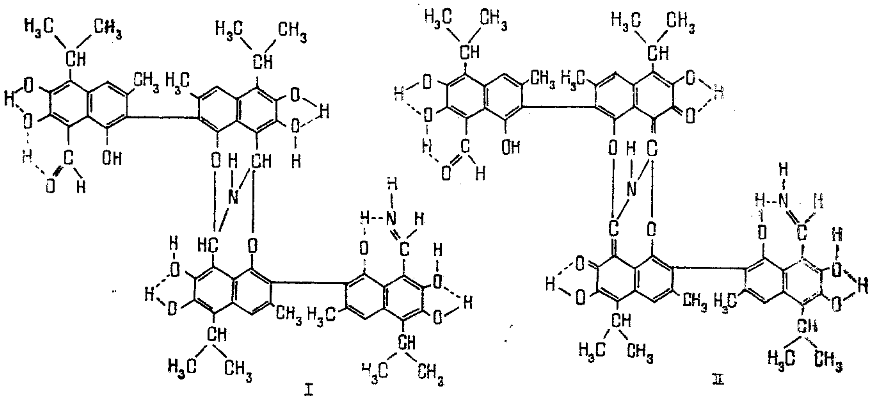
As is known, gossypurpurin is a nitrogen-containing pigment, and therefore its formation from anhydrogossypol under the conditions of CC is possible only when nitrogenous substances are present. And, in fact, nitrogen was detected in the products of the washing of the silica gel with chloroform–methanol (1:1). The yield of these products was 0.14%, and their nitrogen content 7.5%, which, recalculated to silica gel, corresponds to 0.01%.

On the CC of anhydroderivatives of gossypol on silica gel freed from nitrogen compounds no GP was formed. We did not obtain it, either, on passing gossypol through a column of the initial silica gel. It follows from this that the ease of formation of GP from anhydrogossypol as compared with gossypol on CC can be explained by the presence in anhydrogossypol of an unstable oxygen-containing ring possessing a high reactivity.

The mechanical mixing of a benzene solution of anhydro derivatives of gossypol with the products of the washing of the silica gel did not give GP, which indicates the catalytic role of the silica gel.

Gossypurpurin is the second, after gossypol, known pigment of cotton seeds. Information on its structure is contradictory because of the difficulty of obtaining it in the pure form. Some authors consider that it is an monoaminogossypol [6], and others that it consists of a double molecule of monoaminogossypol [7]. According to Adams, GP is obtained as the result of the linkage of a gossypol molecule with a molecule of diaminogossypol through a nitrogen-oxygen bridge (structure I) [8].

T. A. Danilova et al., [9], using the results of UV and IR spectroscopy, showed that the GP molecule contains, in addition to the functional groups characteristic for gossypol, other groups specific to GP alone – quinone, azomethine, and amino groups. These authors showed the formation of intramolecular hydrogen bonds with the participation of aldehyde, quinone, and azomethine groups and between hydroxyls. On the basis of these facts and the molecular weight that they determined (1005), they considered that GP has the structure corresponding to formula (II)



I

II

The GP that we isolated from a column has a red color in solutions in chloroform, benzene, and diethyl ether but in acetone, methanol, and ethanol the color changed in a few minutes and the solutions acquired a yellow tinge, as has been reported by other workers [6 and 8, p. 81), while in the spectrum of alcoholic solutions of gossypurpurin the absorption maximum in the visible region at 530 and 565 nm that is characteristic for it is absent.

T. A. Danilova and A. N. Mironova have established that the purple form of GP corresponds to (II) and the yellow form to (I). In addition, they consider that the transitions from one form to the other, accompanied by a change in the molecular mass of the pigment (increases by 4 hydrogen atoms in the case of structure (I)) are not tautomeric transformations but are caused by photochemical redox processes taking place in various solvents under the action of luminous radiation [9].

The results that we have obtained on the formation of GP during CC on silica gel only from anhydrogossypol and not from gossypol itself, the photochemical transformations of GP, and analysis of the structures given in the literature permit us to consider that the violet-purple product, GP, corresponds to structure (II) and is a combination of monoanhydrogossypol and diaminoanhydrogossypol and not of gossypol and diaminogossypol molecules (structure I) as has hitherto been considered.

Thus, gossypurpurin is formed from anhydrogossypol during CC on silica gel thanks to its lability and the catalyzing role of the silica gel.

EXPERIMENTAL

Anhydroderivatives of gossypol were obtained after storing a methanolic solution of gossypol (C = 5 g/ml) for 10 days at room temperature.

CC was performed on Chemapol silica gel L 100/160. The substances were deposited on the column in the form of solutions in benzene and were eluted with benzene—methanol systems having gradually increasing concentrations of methanol from 0 to 50%. Analytical TLC was conducted on Silufol in the benzene—methanol (4:1) solvent system, the spots being revealed with a 2% solution of $SbCl_3$ in chloroform. UV spectra were taken on a Hitachi instrument.

The numbers of aldehyde groups were determined as described in [10]. The amount of gossypurpurin was calculated from the known optical density of chloroform solutions at 565 nm [11].

SUMMARY

It has been established that gossypurpurin is formed only from anhydrogossypol when a benzene solution of the latter is passed through a column of Chemapol silica gel, and not from gossypol itself.

The results obtained permit it to be considered that gossypurpurin consists of combination of molecules of monoanhydrogossypol and of diaminomonoanhydrogossypol through a nitrogen bridge.

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