[Contribution from the Southern Regional Research Laboratory[1]]

PIGMENTS OF COTTONSEED. V. METHYLATION OF GOSSYPURPURIN

PATRICIA VON DER HAAR and CATHERINE HALL POMINSKI

Received July 20, 1951

The existence and partial characterization of a purple pigment, gossypurpurin, in cottonseed has been previously reported (1, 2). In order to further characterize gossypurpurin, it was methylated and its methyl ether compared with that of gossypol prepared by the same means. Gossypurpurin has been prepared artificially from gossypol and it has been isolated from cottonseed pigment glands (2), but the former can be obtained in a purer form than the latter, hence the artificially prepared product was used in the work reported here. Gossypurpurin and gossypol were methylated with diazomethane in ether solution and with methyl iodide and potassium carbonate.

It is known that gossypurpurin exhibits phenolic properties (1, 2), but the exact number of hydroxyl groups replaceable by methoxyl groups under conditions of complete methylation has hitherto been unknown. It is known that gossypol contains six hydroxyl groups (3) and forms dimethyl, tetramethyl, and hexamethyl ethers (4, 5).

Adams (4) reported having obtained two isomeric forms of a white hexamethyl ether which exhibited different melting points, 221° and 235–237°, depending on the solvent from which they were crystallized, and a red hexamethyl ether, m.p. 158–160° prepared by the use of methyl sulfate. The red and the white tautomeric forms were reported to be interconvertible. Treatment of a solution of the white hexamethyl ether in concentrated sulfuric acid with excess aqueous sodium hydroxide was found by Adams to cause conversion to the red form. Adams also reported that treatment of a methanolic solution of the red ether with aqueous sodium hydrosulfite, followed by treatment of the resulting cream-colored product in cold methanol solution with alcoholic alkali in an atmosphere of nitrogen brings about conversion to the white form. In subsequent articles Adams and co-workers obtained a number of other polymorphic forms of hexamethoxy gossypol with different melting points by a two-stage methylation of gossypol (5, 6).

On the other hand, Murty and Seshadri (7) reported that they had prepared a pale yellow hexamethoxygossypol, m.p. 130°, having specific and constant properties. The same product was obtained repeatedly using three different methylating agents, namely, diazomethane in ether solution, methyl iodide and potassium carbonate, and methyl sulfate. No partially methylated product was obtained when they treated gossypol with less drastic methylating agents such as diazomethane or methyl iodide, and they concluded, therefore, that gossypol offered no resistance to methylation.

---

[1] One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

It was desirable that methylating agents which produced hexamethoxygossypol also be used in the methylation of gossypurpurin. The methyl ether of gossypurpurin could then be compared more strictly with the methyl ether of gossypol obtained under the same conditions. It was believed that conditions which produced hexamethoxygossypol would also produce a completely methylated ether of gossypurpurin, thereby making it possible to estimate the number of hydroxyl groups in the latter which were amenable to methylation.

It was found that the two less drastic methods of methylating gossypol were sufficient to produce a hexamethoxygossypol which agreed in all properties except melting point with that obtained by Murty and Seshadri. Using either diazomethane in ether solution or methyl iodide and potassium carbonate, the same methyl ether of gossypol was obtained. This hexamethoxygossypol, after recrystallization from dilute acetone, was obtained in the form of pale yellow rectangular plates which melted at 160–162°. Elementary analyses, methoxyl value, and molecular weight, determined by the cryoscopic method in benzene, agreed with those calculated for hexamethoxygossypol.

Treatment of gossypurpurin with the same two methylating agents which were used with gossypol produced an ether which, after crystallization from diethyl ether and light petroleum naphtha, was obtained in the form of microcrystals melting with decomposition at 280–285°.

Cryoscopic molecular weight determinations in benzene solution indicated that the molecular weight of the ether was approximately 1200. A tentative empirical formula for gossypurpurin, $C_{30}H_{32}NO_7$, has been previously proposed (2). However, the molecular size of gossypurpurin in relation to that of gossypol has heretofore been unknown. Molecular weight determinations on gossypurpurin itself did not give reliable values because of the instability of the pigment in most of the liquids in which it is soluble. The molecular weight of the methyl ether of gossypurpurin clarifies the relationship of the molecular sizes of gossypurpurin and gossypol, which can now be estimated to be approximately 2 to 1. The molecular formula for gossypurpurin can now be assumed to be $C_{60}H_{64}N_2O_{14}$.

The methoxyl value of the gossypurpurin methyl ether was found to be 14.6%. Calculated on the basis of the above-proposed molecular formula for gossypurpurin, this methoxyl value indicates the presence of four methoxyl groups in the gossypurpurin ether molecule. Consequently, the molecular formula for this ether is assumed to be $C_{60}H_{60}O_{10}N_2(OCH_3)_4$. The values for the elementary analysis of this product agreed consistently with the proposed molecular formula.

Both of the ethers obtained by methylating gossypol and gossypurpurin resisted further methylation, and, in each case, the original ether was recovered unchanged. The two ethers were treated individually with ammonia and with aniline but did not react with either reagent and were recovered unchanged. The gossypol ether gave an orange color with concentrated sulfuric acid, whereas the corresponding ether of gossypurpurin gave a dark yellow color with the same reagent. The gossypol ether may be considered to be completely methylated to the hexamethoxy state.

The similarity of behavior of the gossypurpurin ether would seem to indicate that all hydroxyl groups available for methylation in the gossypurpurin molecule were replaced by methoxyl groups.

EXPERIMENTAL

Methylation of gossypol with diazomethane. Diazomethane was prepared directly from 10 g. of nitrosomethylurea and collected immediately in 100 ml. of diethyl ether. A solution of 1 g. of gossypol in 30 ml. of absolute methanol was treated with an excess of the ethereal solution of diazomethane. The mixture was shaken and allowed to stand in the cold for 24 hours. The diethyl ether and methanol were distilled off, leaving as small a residue as possible. The residue, after being cooled, was treated with additional diazomethane in diethyl ether solution and again allowed to stand in the cold for 24 hours. The solvent was almost completely removed by distillation. Upon the addition of cold distilled water to the residue, a pale yellow, crystalline solid separated. Recrystallization of this product from dilute acetone yielded pale yellow rectangular plates melting at 160–162°; yield, about 0.075 g.

Anal. Calc'd for $C_{30}H_{24}O_2(OCH_3)_6$: C, 71.1; H, 7.0; OCH_3, 30.9; M. W., 602.
Found: C, 71.1; H, 6.9; OCH_3, 29.1; M. W., 605.

Methylation of gossypol with methyl iodide. Gossypol (1 g.) was dissolved in 25 ml. of anhydrous acetone, 5 g. of potassium carbonate and 8 ml. of methyl iodide were added, and the mixture was refluxed gently for 48 hours. At the end of this period, the original orange-colored solution had become a very light yellow. The acetone was then removed by distillation and the residue treated with cold distilled water. A pale yellow microcrystalline solid separated. Recrystallization from dilute acetone gave a product which agreed in melting point (160–162°), crystalline form, and elementary composition with that obtained when methylating gossypol with diazomethane in ether solution. Yield, about 0.05 g.

Anal. Found: C, 71.5; H, 6.9.

Methylation of gossypurpurin with diazomethane. The same diazomethane procedure as that used for gossypol was repeated with gossypurpurin, except that anhydrous acetone instead of methanol was the solvent. When the final residue obtained as described above was treated with cold water, a greenish-yellow amorphous solid separated. Upon recrystallization of the crude product from diethyl ether and light petroleum naphtha, a bright yellow microcrystalline compound was obtained. The purified gossypurpurin ether melted at 280–285° with decomposition; yield, about 0.060 g.

Anal. Calc'd for $C_{60}H_{60}O_{10}N_2(OCH_3)_4$: C, 70.3; H, 6.6; N, 2.6; OCH_3, 15.0; M. W. 1092.
Found: C, 70.0; H, 6.6; N, 2.7; OCH_3, 14.6; M. W. 1235.

Methylation of gossypurpurin with methyl iodide. The exact procedure used in treating gossypol with methyl iodide and potassium carbonate was repeated in the case of gossypurpurin. A product was obtained which was identical in melting point (280–285°) and elementary composition with that obtained by treating gossypurpurin with diazomethane in ether solution. Yield, about 0.05 g.

Anal. Found: C, 70.4; H, 6.5; N, 2.64.

Molecular weight determination. Molecular weights of the methylated gossypurpurin and methylated gossypol were determined by the cryoscopic method in benzene using 5.07° as the molar-freezing-point depression (8).

The freezing-point depressions observed when pellets of methylated gossypol weighing 0.1002 g., 0.0820 g., and 0.0867 g. were successively added to 16.5066 g. benzene were 0.053°, 0.040°, and 0.044°, respectively, corresponding to calculated molecular weights of 581, 630, and 605, respectively; an average of 605.

The freezing-point depressions observed when pellets of methylated gossypurpurin weighing 0.1319 g. and 0.1348 g. were successively added to 17.9485 g. benzene were 0.030° and 0.031° respectively, corresponding to calculated molecular weights of 1242 and 1227; an average of 1235.

Acknowledgment. The authors wish to thank Lawrence E. Brown and Frank C. Magne for the microanalyses and molecular weight determinations reported above.

SUMMARY

Gossypurpurin and gossypol, two polyphenolic pigments found in cottonseed, were individually methylated by treating each pigment with diazomethane in diethyl ether solution and with methyl iodide and potassium carbonate. In the case of gossypol, the completely methylated ether, hexamethoxygossypol, was obtained with either methylating agent. In the case of gossypurpurin, a bright yellow ether derivative which resisted further methylation was obtained with either methylating agent. Molecular weight determination showed that the gossypurpurin methyl ether was approximately twice the molecular size of hexamethoxygossypol. It was concluded, therefore, that the size of the gossypurpurin molecule was approximately twice that of the gossypol molecule, and a tentative molecular formula for gossypurpurin, $C_{60}H_{64}N_2O_{14}$, was proposed which is twice the empirical formula previously proposed for this pigment. A methoxyl determination on the gossypurpurin ether indicated the presence of four methoxyl groups per molecule. From the elementary analysis, methoxyl value, and moecular weight of the methyl ether of gossypurpurin, a tentative molecular formula for this ether has been proposed.

New Orleans, Louisiana

REFERENCES

(1) Boatner, *Oil & Soap*, **21**, 10 (1944).
(2) Pominski, Miller, Von der Haar, O'Connor and Castillon, *J. Am. Oil Chemists' Soc.* **28**, 472 (1951).
(3) Clark, *J. Biol. Chem.*, **75**, 725 (1927).
(4) Morris and Adams, *J. Am. Chem. Soc.*, **59**, 1731 (1937).
(5) Adams and Geissman, *J. Am. Chem. Soc.*, **60**, 2163 (1938).
(6) Adams, Geissman, and Morris, *J. Am. Chem. Soc.*, **60**, 2967-2970 (1938).
(7) Murty and Seshadri, *Proc. Indian Acad. Sci.*, **16a**, 146 (1942).
(8) Landolt-Börnstein, *Physikalisch-Chemische Tabellen*, 5 Aufl., III Ergänzungsbd., t. 3, 2668 (1936).