Isolation of Gossypol from Tissue of Porcine Livers

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Abstract

Gossypol has been isolated in crystalline form from the livers of pigs fed cottonseed meal having a relatively high free-gossypol content. The crystalline product was identified as dianilinogossypol by its ultraviolet (248–500 mμ) and infrared light absorption spectra.

Introduction

ALTHOUGH the toxic effects of gossypol have been known for many years (5) and some of the affected organs have been recognized (4), this compound has not been isolated from body tissues. In a recent study, a yellow pigment was extracted by a procedure for total gossypol (3) from the liver tissue of swine that had received dietary gossypol (1). This pigment was tentatively identified as dianilinogossypol by its absorption spectrum. These observations led to an attempt to isolate the gossypol in a crystalline form from the liver tissue in sufficient amounts for positive identification by ultraviolet and infrared absorption spectra.

Experimental

Extraction. Samples of liver (140–230 g) from four pigs fed cottonseed meal high in free gossypol were blended with 300 ml of 95% ethanol in a Waring blendor. The homogenates were transferred to 800-ml beakers which were placed in a boiling water bath for 15 min. The hot homogenates were filtered under vacuum through a paper in a Buchner funnel, and the residues were washed first with 95% ethanol and then with diethyl ether. All the extracts and washings were combined and had a total volume of 5000 ml.

The gossypol content of the combined extracts was determined by treating 5-ml aliquots with aniline as previously described in a method for the estimation of free gossypol (2). The results indicated that the 5000 ml of extract contained 53 mg of gossypol. However, after treating the total volume of extract with aniline and concentrating it on a flash evaporator, no crystals of dianilinogossypol were obtained. (The large amounts of extraneous materials present apparently prevented the precipitation of the dianilinogossypol.)

After the ether extraction, the residues were returned from the Buchners to the respective beakers for further treatment. Fifty ml of 82% ethanol (by wt) and 15 ml of aniline were added to each. After mixing the contents thoroughly, the beakers were placed on the steam bath for 3.5 hr. During the heating the residues gradually turned yellow. The contents of each beaker were transferred to a Waring blendor jar and homogenized for 3 min with 300 ml of chloroform. The mixture was filtered on a Buchner funnel and the residue was washed with chloroform. The residue was extracted a second time with 200 ml of chloroform, filtered, and the residues washed as before. This process was repeated using 200 ml of chloroform. The combined extracts and washings had a golden color suggesting the presence of gossypol as the dianilino derivative. Spectrophotometric measurements subsequently indicated that the combined chloroform extracts from the four pigs contained 84.4 mg of gossypol.

Isolation and Absorption Spectra. The chloroform was removed by distillation, the residue was transferred to a 250-ml balloon flask, and the residual aniline was removed on a flash evaporator using a dry-ice trap. Before all of the aniline was removed, the dianilinogossypol began to crystallize. The walls of the flask were washed down with a few ml of ethyl ether, a few ml of ethanol were added, and the dianilinogossypol was allowed to crystallize overnight. The precipitate was filtered on a paper disk in a tared gooch crucible, washed with 50% aqueous ethanol and then with hexane. The crude precipitate, 88.8 mg, was dissolved in chloroform and was slowly crystallized on a warm steam bath after adding 8 ml of 95% alcohol and 10 ml of ether. The recrystallized product was in the form of needles and weighed 56.6 mg.

Ultraviolet and infrared spectra, Figures 1 and 2, respectively, of the aniline derivative of the purified product and of pure dianilinogossypol were determined.

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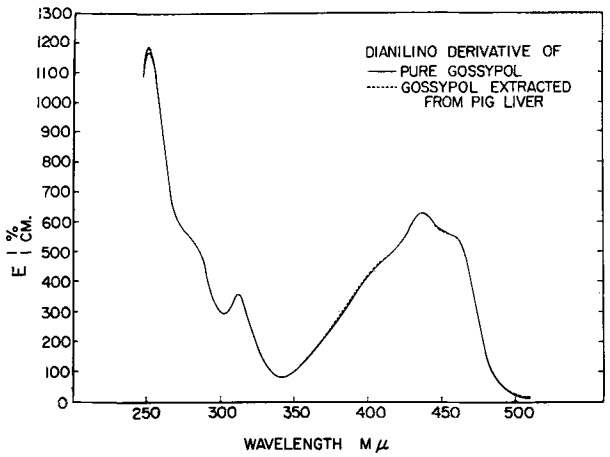

FIG. 1. Absorption spectra of the dianilino derivative of pure gossypol and of gossypol extracted from pig livers.

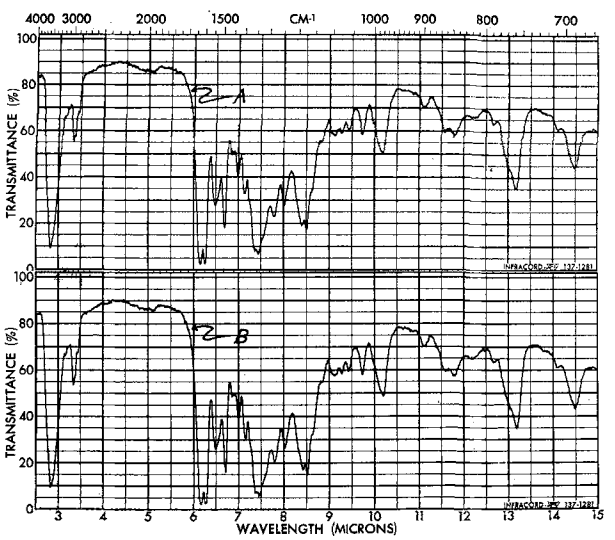

FIG. 2. Infrared absorption spectra of: A. Dianilinogossypol derivative of gossypol extracted from pig livers. B. Pure dianilinogossypol.

Results and Discussion

The light absorption spectrum of the purified product was determined on a Beckman DU spectrophotometer, using chloroform as a solvent, and compared with a similar spectrum determined on pure dianilinogossypol. Both curves, Figure 1, practically coincide except between 249 and 253 mμ. From these spectra, it is concluded that the material extracted from pig liver and crystallized as an aniline derivative was the aniline derivative of gossypol. Substantiating data were provided by comparison of the infrared spectrum of the crystalline material with that of pure dianilinogossypol. Almost identical curves, Figure 2, indicate positively that the crystalline product obtained from pig liver is pure dianilinogossypol.

The isolation of gossypol from animal tissue opens the way for further study on the metabolic fate of gossypol in the animal organism and the mode of action of this compound in damaging tissue.

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Quantitative Analysis of Short Chain Fatty Acids Using Gas Liquid Chromatography[1]

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Abstract

The short-chain fatty acids, propionic to pelargonic, have been analyzed by gas liquid chromatography, as their butyl, phenacyl, and decyl esters to overcome losses due to their volatility. The three methods are compared and the decyl ester procedure offers the most advantages.

Introduction

THE GAS LIQUID chromatographic analysis (GLC) of short-chain fatty acids and their methyl esters has been described by James and Martin (10), Van de Kamer et al. (18), McInnes et al. (7,12), Brandenberger and Müller (2), Hunter et al. (9), Vorbeck et al. (22), Böttcher et al. (1), and Gehrke and Lamkin (6). The main problem in quantitative analyses of these materials is the possibility of losses due to the volatility of the acids or their methyl esters during extraction, removal of solvents and transfer to the GLC unit. Unique techniques have been developed to overcome these problems in using the free acids (8,6). Ostle and Ahrens (15) made use of 2-chloroethanol esters to reduce losses due to volatility in order to obtain quantitative results.

This laboratory has been particularly interested in the quantitative analysis of the acids resulting from oxidation of unsaturated acids with reagents such as permanganate-periodate (21). This publication describes the analysis of the acids as their butyl, phenacyl, and decyl esters, procedures which largely overcome losses due to volatility.

Materials and Methods

Commercial samples of the short chain fatty acids were purified by distilling the methyl esters in a Podbielniak Heli-Grid column. The esters were converted to free fatty acids and the purity of each acid was checked by GLC analysis of the methyl esters prepared from the free acids with diazomethane.

The gas liquid chromatographic unit was of conventional design using thermal conductivity cells for detection. A 4 ft by ¼ in. OD stainless steel column packed with 60–80 mesh, acid washed Celite 545 coated with silicone grease prepared according to the procedure of Cropper and Heywood (4,5) in the ratio of 6:1 w/w was used to separate the phenacyl esters. The unit was operated at 200C, bridge current 200 ma, injector temperature 235C, and helium flow rate 60 ml per minute. The butyl esters of the C_3 to C_9 fatty acids were separated on a 6 ft by ¼ in. stainless steel OD column similarly packed with silicone on Celite and operated at 180C. The butyl esters of the dicarboxylic and long chain monocarboxylic acids were analyzed on the same column at 225C. The decyl esters were separated on an 8 ft by 3/16 in. OD copper column with the same packing and operated at 185–200C.

N-n-butyl-N-nitroso-N′-guanidine, synthesized according to the procedure of McKay (13), was used to prepare diazobutane (14) in ethereal solution. The butyl esters were formed by adding an ethereal solution of diazobutane to an ethereal solution of the fatty acids. The excess diazobutane and ether were carefully removed by evaporation using an air cooled reflux condenser.

The α-bromoacetophenone was prepared as outlined by Vogel (19) and was stored in a brown glass stoppered bottle. Phenacyl esters of the pure acids were made according to the technique outlined in Vogel (20) and were recrystallized from a mixture of ethyl ether and petroleum ether (Skellysolve "F") using low temperature crystallization where necessary.

The phenacyl derivatives of mixtures of fatty acids or soaps were prepared as follows: A mixture of fatty acids (30–100 mg) in 5–10 ml of methanol was saponified with potassium hydroxide. Two drops of bromthymol blue indicator were added and the solu-

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