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Photodecomposition of Gossypol by Ultraviolet Irradiation

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ABSTRACT

Decomposition of gossypol as a thin film or as a solution by ultraviolet irradiation was studied. The decomposition of gossypol followed monophasic exponential kinetics in which the rate of decomposition varied and depended upon the irradiation condition. The lowest rate of gossypol decomposition was observed as a thin film which showed a half-life of 97 min, while the highest rate was attained as a solution in acetone as indicated by a half-life of 4.5 min. Solutions in methanol and ethanol showed relatively lower rates of decomposition with similar half-lives of approximately 50 min. Acetonitrile and chloroform solutions showed intermediate rates of decomposition for gossypol with half-lives of 15 and 19 min, respectively. Although the degradation products of gossypol were not identified, their HPLC profiles were characteristic of the solvent used. HPLC profiles of gossypol degradation products in methanol, ethanol, acetone and acetonitrile were similar, each exhibiting two peaks with variable ratios depending on the solvent and the time of exposure. The degradation products of gossypol when irradiated as a thin film and as a solution in chloroform were different from those in other solvents. In all cases, when gossypol and/or its degradation products were continuously exposed to ultraviolet radiation, they decomposed to products no longer having an aromatic structure.

INTRODUCTION

Gossypol [1,1′,6,6′,7,7′-hexahydroxy-5,5′-diisopropyl-3,3′-dimethyl (2,2′-binaphthalene)-8,8′-dicarboxaldehyde] is a yellow coloring matter present in various parts of cotton plants (1-3). Gossypol causes many deleterious effects to nonruminant animals, a characteristic which limits the use of cottonseed meal as a source of protein for animals and humans (1-3). It uncouples oxidative phosphorylation of rat liver mitochondria in vitro (4).

Recently, gossypol was found to cause reversible male infertility (5). This was first observed in humans, and later it was found that some animal species are sensitive (e.g. rats and hamsters) while others are not (e.g. mice and rabbits) (5-7). The compound is being tested as a male contraceptive (5) and as a vaginal spermicide (8). It has been suggested that gossypol might be used to lower plasma cholesterol levels (9) and for treatment of Chagas disease, which is caused by *Trypanosoma cruzi* (10). Furthermore, gossypol inactivated influenza, parainfluenza-3, and herpes simplex viruses (11,12) and showed general antifungal (13), antibacterial (14) and antitumor activity (15).

Recent studies from this laboratory showed that gossypol was highly unstable when stored in solution at room temperature or upon refrigeration at 4 C. The instability was highly dependent on both the storage temperature and the solvent used (16). The rate of decomposition of gossypol increased in the following order with respect to the solvent: acetone < acetonitrile < chloroform < ethanol < methanol. This communication reports on the continued investigation of the stability of gossypol upon irradiation by ultraviolet light when the gossypol is a thin film or a solution in any of the five solvents used in the previous investigation.

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EXPERIMENTAL

Reagents

Gossypol acetic acid was purchased from Sigma (St. Louis, Missouri). Its purity was determined by high performance liquid chromatography (HPLC) to be 99.5%. All solvents and reagents used were HPLC grade and obtained from Fisher Scientific (Raleigh, North Carolina).

Ultraviolet Lamp

An Ace-Hanovia medium pressure mercury lamp (approximately 100 watts) was purchased from Ace Glass Inc. (Louisville, Kentucky). The lamp was reported by the manufacturer to emit ultraviolet enery at the following wavelengths: 185, 238, 248, 254, 265, 280, 297, 302, 313 and 366 nm.

Exposure of Gossypol to Ultraviolet Radiation

Gossypol solutions were made freshly at a concentration of 1 mg/ml in methanol, ethanol, chloroform, acetonitrile and acetone. Solutions were placed in a 3 ml quartz spectrophotometric cuvette (Fisher Scientific Products, Raleigh, North Carolina) mounted on a 30 × 10 cm glass plate by pieces of styrofoam and white sticking tape. The glass plate was inserted into a 29 × 25.5 × 9.5 cm glass thin layer chromatography (TLC) jar in which the ultraviolet lamp also was inserted in a position directly facing the cuvette with a constant distance of 1.4 cm between the lamp and the cuvette. Only one side of the cuvette was exposed to the ultraviolet radiation. Water was circulated in the TLC jar (by means of a water tube in the bottom of the jar and a water vacuum pump hose mounted on the internal side of the jar just below the top level of the quartz cuvette) to maintain the temperature below 20 C. The TLC jar was heavily wrapped in aluminum foil, and the experiment was run inside a highly efficient hood. At various time intervals (Fig. 1 and 2), the lamp was turned off, and 100 μl of gossypol solution was taken from the cuvette from which volumes of 1-5 μl were analyzed by HPLC. Because of acetone's high absorbance at 254 nm, this solvent was first evaporated by a gentle stream of dry nitrogen, and the residue was dissolved in 100 μl of methanol prior to HPLC analysis. Six determinations were made from three separately exposed cuvettes for each solvent at each time interval.

Gossypol was spread as a thin film on the surface of 3 × 1 cm glass plates at a concentration of 100 $\mu g/cm^2$. The compound was first dissolved in a mixture of ethanol/ether 1:1 (v/v) at a concentration of 300 μg/10 μl. From the resulting solutions, 10 μl was spread as evenly as possible on the glass plate by a capillary tube, then the solvent

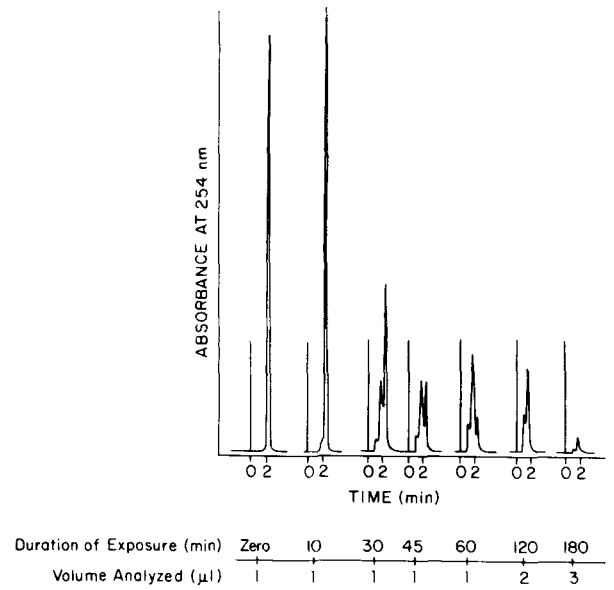

FIG. 1. Typical time course degradation profile of gossypol as solution in either methanol, ethanol, acetone or acetonitrile upon exposure to ultraviolet irradiation. Duration of exposure refers to the time which gossypol was exposed to the ultraviolet irradiation. Volume analyzed refers to the volume of the exposed gossypol solution (originally 1 mg/ml) which was analyzed by HPLC.

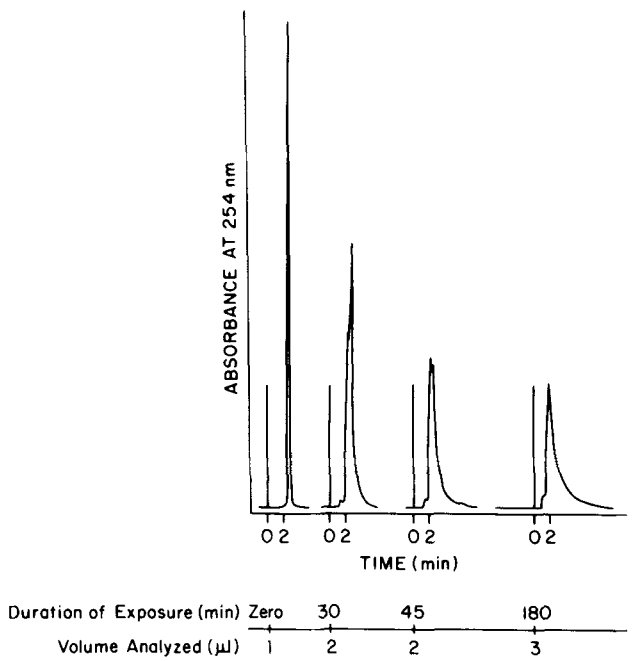

FIG. 2. Time course degradation profile of gossypol as a solution in chloroform upon exposure to ultraviolet irradiation. See Figure 1 for explanation.

was allowed to evaporate. Only the plates with an even layer of gossypol were used. Coated glass plates were inserted into the quartz cuvette with the gossypol layer facing the surface of the cuvette which is exposed to the ultraviolet radiation. At each exposure time (Fig. 3), the glass plate was removed and washed with 3 × 100 µl acetone. Acetone was evaporated with nitrogen, and the residue was dissolved in 300 µl of methanol prior to HPLC analysis. Six determinations were made from three separate exposures for each time point.

The HPLC system and the solvent used to elute gossypol and its degradation products were described previously (16). Gossypol and its degradation products were separated on a 10 µm C_{18} cartridge fitted into an RCM-100 radial compression separation system. Elution was carried out isocratically using 0.1% phosphoric acid in methanol/water 9:1 at a solvent flow rate of 2 ml/min. Detection and quantification of gossypol and its degradation products were carried out by monitoring the ultraviolet absorbance of the column eluates at 254 nm and measuring the peak area.

The half-life of gossypol was calculated by a linear regression of the percentqage of gossypol remaining versus time on a semilog scale using an Apple 2 Plus Personal computer.

RESULTS AND DISCUSSION

Rate of Decomposition

The present investigation was designed to study the degradation of gossypol as a thin film upon exposure to ultraviolet radiation. Also studied was the effect of various solvents on the rate of decomposition of gossypol when irradiated in solution. Gossypol was unstable when exposed to ultraviolet radiation, and its decomposition followed monophasic exponential kinetics. Gossypol showed the lowest rate of decomposition when irradiated as a thin film with a half-life of 97 min. The decomposition rate of gossypol in solution was highly dependent on the solvent used. When gossypol was irradiated in acetone, it demonstrated the highest rate of decomposition with a half-life of 4.5 min. By contrast, the lowest rate was observed in methanol and ethanol with similar half-lives of approximately 50 min. Acetonitrile and chloroform showed intermediate rates of decomposition of gossypol with half-lives of 15 and 19 min, respectively. Gossypol's high rate of decomposition in acetone probably is related to high ultraviolet absorbance of this solvent. Such absorbance may transfer acetone molecule into energetically excited state. As the excited state molecule returns to the ground state, energy is emitted and transferred to the gossypol molecules; this results in the enhancement of its decomposition rate. The fact that acetone enhances the decomposition of some compounds upon exposure to ultraviolet radiation has been reported previously (17).

Decomposition Products

None of the degradation products of gossypol were identified in this study. However, general patterns were observed from the HPLC profile of the products. Gossypol decomposition products in methanol, ethanol, acetone and acetonitrile appeared similar. Two different peaks with retention times of 1.48 and 2.12 min were present in all chromatograms as a result of the decomposition of gossypol in each of the four solvents. Under the same HPLC conditions, gossypol had a retention time of 2.85 min (18). A typical sample of the time course degradation profile of gossypol in these solvents is presented in Figure 1. The ratio of the two peaks at various time intervals and the rate of their appearance and disappearance were dependent on the solvent used.

The degradation pattern of gossypol in chloroform was somewhat different from the previous four solvents (Fig. 2). This was manifested in both the rate of appearance of various degradation products and in the shape of the chromatograms. It is likely that more than two degradation products were formed from gossypol when irradiated in chloroform.

The decomposition pattern of gossypol when irradiated as a thin film did not change as time progressed. The only obvious change was the gradual disappearance of the gossypol peak as the exposure time increased; the amounts of the

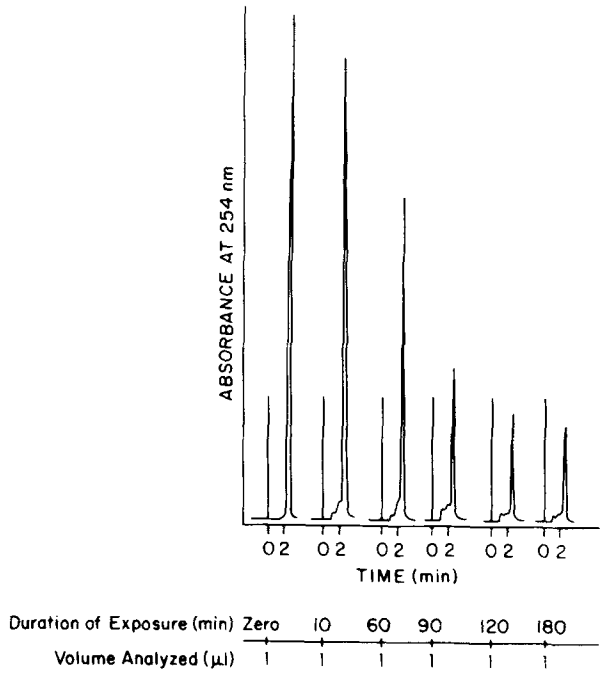

FIG. 3. Time course degradation profile of gossypol as a thin film upon exposure to ultraviolet irradiation. See Figure 1 for explanation.

degradation products (R.T. = 2.17, 2.40 min) remained almost unchanged (Fig. 3).

In all cases, as the exposure time increased, gossypol and its degradation product levels decreased as deduced by the area under each peak. This was attributed to the decomposition of gossypol and its degradation products to compound(s) that did not absorb at 254 nm. This can take place only by the breakdown of the aromatic structure, which in turn means the decomposition of the binaphthalene skeleton of the gossypol molecule.

This study demonstrates that gossypol is sensitive to degradation by ultraviolet light. Therefore, as a potential male contraceptive agent, it should be protected from light when formulated as a drug.

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A Comparison of the Fatty Acids and Sterols of Seeds of Weedy and Vegetable Species of *Amaranthus* spp.

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ABSTRACT

The seeds of weedy and vegetable species of *Amaranthus* were analyzed for sterols and fatty acids. The major sterol was spinasterol, which ranged from 46 to 54% by weight of the total sterol mixture. Δ^{-7} stigmasterol occurred in the next higher amount with lesser amounts of Δ^{-7} ergosterol, stigmasterol and 24-methylene-cycloartenol. There was little difference in the sterol composition of the vegetable species compared to the weedy species. The fatty acid compositions of the species were essentially all the same. Linoleic acid was present in the greatest amount, with lesser amounts of oleic, palmitic, stearic, myristic, linolenic, arachidic and lignoceric acids.

INTRODUCTION

The amaranth plant (*Amaranthus* spp.) has been a major food source for ancient civilizations in the tropical highlands of Central America and Asia. The consumption of amaranths as food was displaced by larger seed grains, such as maize, and as a result amaranths have become relatively unimportant in human diets (1). Amaranths are fast growing plants found in tropical and temperate areas of the world and are considered pioneers of early successional stages of vegetation development. Most of the *Amaranthus* species, including *A. dubius* and *A. spinosus*, are considered weeds (5). Recently some vegetable species of amaranth plants that were used for food by ancient cultures have received attention because of the high levels of protein in their leaves and lysine in their seeds. For example, one of the vegetable amaranths *(A. edulis)* is reported to contain 6.2 g lysine/100 g protein (3), which is of considerable importance since lysine is one of the critical amino acids frequently deficient in plant protein (1). Seeds of amaranths contain 13% protein and 63% starch. The level of starch is equivalent to that of premium priced waxy maize (3,6).

Because of their high protein and lysine content the vegetable species of amaranths are receiving a great deal of attention in developing countries of the world as a means to combat protein malnutrition. Since protein rich foods of animal origin are either unavailable or expensive to purchase,