Tissue tocopherol content was not affected adversely by the oxidized oil. This may be due in part to the high tocopherol content (10 mg. %) of the diet or due to interference from ubiquinone in the assay for tocopherol. Bacq and Alexander (19) reported no change in tissue tocopherol in rats receiving whole body radiation and noted an increase in tocopherol as the fat reserves were mobilized. Though Alfin-Slater (2) found that tocopherol was helpful in relieving the "toxic" symptoms in rats fed fried fats, Machlin *et al.* (20) reported that tocopherol did not reduce the mortality or pathology of highly-oxidized fats fed to chickens.

It should be pointed out that the oils used in this research were much more severely oxidized than the fats and oils used in human dietaries in the United States. Melnick (21) and Keane *et al.* (1) have shown that commercial food fats that have been used in commercial frying are not toxic.

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[Received June 27, 1960]

Spontaneous Conversion of Gossypol to Anhydrogossypol

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An alkali-fast pigment is produced at room temperature when gossypol is dissolved in ethyl acetate. This pigment has been isolated from ethyl acetate as an orange crystalline material and identified as anhydrogossypol. The identity was established by identity of the infrared spectra with that of authentic anhydrogossypol; by elementary composition; by mixture-melting point behavior with authentic anhydrogossypol; and by the identity of the aniline derivatives produced from the orange crystalline product from ethyl acetate and authentic anhydrogossypol.

GOSSYPOL (2,2'-bi-1,6,7,-trihydroxy-3-methyl-5-isopropyl-8-aldehydronaphthyl) occurs in specialized structures in the seeds of species of the genus *Gossypium* (3). It is present in the seeds of upland cotton, *G. hirsutum,* to the extent of 0.4–1.7% (6). This yellow pigment is extracted along with the oil in the commercial processing of cottonseed and is found in solution in the oil.

There is strong evidence that gossypol so extracted is responsible for the undesirable fixed-red coloration that occurs in about 25% of the domestically-produced cottonseed oil (1). For example, it was shown by Berardi and Frampton that a large part of the gossypol added to either crude or refined and bleached cottonseed oil disappears in a relatively short period of time, and in the case of refined and bleached oil to which gossypol is added there is a concomitant increase in fixed-red coloration with an increasing disappearance of gossypol. Apparently the initial reaction in the fixation of gossypol in refined and bleached cottonseed oil is of the second order with respect to gossypol (5). However none of the gossypol reaction products have been isolated from cottonseed oil.

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Because of the experimental difficulties encountered in studying the series of reactions which gossypol undergoes in solution in glyceride oils, some experimentation has been carried out with simpler systems; one of the observations is that gossypol cannot be recovered quantitatively from ethyl acetate solution if the solution is permitted to age. Several of the colored reaction products that occur in aged ethyl acetate solutions of gossypol can be resolved chromatographically on a cellulose column. One of these products is anhydrogossypol.

Experimental

An ethyl acetate solution of gossypol (0.5 g./100 ml.) was permitted to age in the dark at room temperature (25–27°) for a month. The unreacted gossypol was removed from this solution by scrubbing it with 0.5 N aqueous NaOH which contained a small quantity of dithionite. The ethyl acetate solution was then washed with water, dried over anhydrous Na_2SO_4, and reduced to a volume of about 5 ml. by evaporation on a water bath. A small quantity of powdered cellulose was added to this concentrate, and after the residual ethyl acetate had evaporated, the dried powder was placed on the top of a chromatographic column composed of powdered cellulose. The yellow component eluted when the chromatogram was developed with petroleum ether was rechromatographed on a second cellulose column. Orange crystals, m.p. 224–225°, separated when the eluent from this second column was concentrated on a water bath, yield, 10%. They were recrystallized from toluene, m.p. 225–226°. *Anal.*: C, 74.47; H, 5.50; mol. wt. (ebullioscopic method in ethanol), 443. Calcd. for

anhydrogossypol: C, 74.66; H, 5.46; mol. wt., 482. Mixture melting-point with anhydrogossypol prepared according to the method of Miller and Adams (4), 225–226.5°. Mixture melting-point with anhydrogossypol prepared in accordance with the method of Clark (2), 225.5–226.5°.

An ethyl acetate solution of gossypol (0.5g./100 ml.) was refluxed under N_2 for 3 days. The solvent was removed under reduced pressure, and the residue was taken up in diethyl ether. The unreacted gossypol was removed from the ethereal solution by scrubbing it with 4% aqueous Na_2CO_3 containing a small quantity of dithionite. The ethereal solution was then dried with anhydrous Na_2SO_4 and concentrated on a water bath. Orange crystals separated when the solution was cooled. They were recrystallized from toluene. *Anal.*: C, 74.23; H, 5.56; m.p., 227–228°. Mixture melting-point with anhydrogossypol prepared according to the method of Miller and Adams (4), 226–227°.

Anhydrogossypol from each of the preparations was converted to dianilinogossypol by the method of Clark (2). Each crystalline preparation was dried *in vacuo* at 50°. Melting points of anils prepared from anhydrogossypol derived from ethyl acetate solutions were 275–276° and 274.5–276°, respectively, and of an anil prepared from anhydrogossypol prepared in accordance with the method of Miller and Adams, 267–268°. Mixture melting-point with gossypol anil prepared in accordance with the method of Miller and Adams, 268–269°.

The infrared spectra of all of the anhydrogossypol preparations, as determined in KBr discs and also in cyclohexane solution with a Beckman IR-2T spectrophotometer, were identical.

Discussion

Anhydrogossypol and several other alkali-fast products are formed spontaneously at room temperature when gossypol is dissolved in ethyl acetate whereas gossypol can be recovered quantitatively from benzene, toluene, cyclohexane, diethyl ether, and chloroform solutions of gossypol even when these are boiled under reflux for 3 days. Only 10–25% of the gossypol dissolved in ethyl acetate can be recovered if the solution is boiled under reflux for 3 days.

The fate of the water removed from gosypol on its conversion to anhydrogossypol was not determined. Apparently it was not involved in the hydrolysis of ethyl acetate since very carefully-conducted potentiometric titrations failed to reveal the presence of acetic acid in any of the solutions containing anhydrogossypol.

It is noteworthy that anhydrogossypol is not removed by alkali washing when it is added to refined and bleached cottonseed oil unless the alkaline solution is stronger than 2.5 *N*. Anhydrogossypol has not been separated from or identified in off-colored cottonseed oil.

Kinetic studies of the fixation of gossypol in cottonseed oil indicate that the reactions involved may be relatively simple (5). Comparable studies of the spontaneous conversion of gossypol in ethyl acetate solution to other products, including anhydrogossypol, do not lend themselves to any simple interpretation. Apparently the spontaneous conversion of gossypol to other products is much more extensive in ethyl acetate than in cottonseed oil.

Acknowledgment

The authors express their appreciation to L. E. Brown for the elemental analyses and to R. T. O'Connor for the infrared measurements.

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[Received July 29, 1960]

ABSTRACTS . . . . R. A. REINERS, Editor

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• Fats and Oils

OPTIMUM CONDITIONS FOR SEPARATION IN GAS CHROMATOGRAPHY. J. C. Giddings (Dept. of Chem., Univ. of Utah, Salt Lake City 12, Utah). *Anal. Chem.* 32, 1707–1711 (1960). A procedure is outlined in which the separation function (a quantitative measure of the extent of separation) can be mathematically optimized with respect to the variables that influence the chromatogram. By using a simplified plate height expression for packed columns, optimum column length, flow velocity, temperature, particle diameter, and pressure are either derived or discussed. The optimum values of flow velocity, pressure, tube diameter, temperature, and thickness of the liquid layer are derived for capillary columns. The method may be extended to other variables and techniques. The problem of minimizing the analysis time is discussed, and a method of obtaining the variables for this is presented.

SIMPLE AUTOMATIC VALVE FOR CONSTANT VOLUME COLLECTION IN COLUMN CHROMATOGRAPHY. G. J. Nelson (Donner Lab. of Biophys. and Med. Phys., Univ. of Calif., Berkeley). *Anal. Chem.* 32, 1724 (1960). The valve stem is constructed entirely of Teflon and encloses a soft iron core which opens the valve when the coil is activated by the photocell. The spring is stainless steel and prevents the valve from lifting too high and obstructing the flow through the outlet. The eluent collecting tube is made of a constant bore glass tubing 1 cm. in outside diameter. Thus, the volume collected in each fraction may be continuously varied, simply by raising or lowering the position of the photocell along the tube.

IONIZATION DETECTORS FOR GAS CHROMATOGRAPHY. P. H. Stirling and H. Ho (Canadian Ind., Ltd.). *Ind. Eng. Chem.* 52(11), 61A–64A (1960). Gas chromatography separates complex mixtures into a series of dilute binary mixtures with the carrier gas. The usual differential detectors, such as the thermal conductivity cell or the gas density balance, sense the small changes