• *Short Communication*

Gas-Liquid Chromatographic Determination of Gossypol as the Trimethylsilyl Ether Derivative

GOSSYPOL (2,2′-bis(8-formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthyl) has long been recognized as one of the toxic substances present in cottonseed. Current analytical procedures for the determination of free gossypol involve formation of the para-anisidine or aniline derivatives of gossypol and spectrophotometric measurement at 447 or 440 mμ (1,2). These procedures detect the presence not only of free gossypol but also of gossypol-like pigments (3). Application of gas-liquid chromatography (GLC) to the determination of gossypol has been unsuccessful to date because of the low volatility of the compound. Trimethylsilyl (TMS) derivatives of hydroxyl-containing compounds have been widely used to achieve the desired volatility for gas chromatographic analysis although early attempts to prepare TMS-gossypol derivatives by using hexamethyldisilizane and trimethylchlorosilane were unsuccessful. However the use of bis-(trimethylsilyl) acetamide, a recently reported highly reactive silyl donor, permitted the preparation of TMS-gossypol derivatives. This communication describes the quantitative gas chromatographic determination of gossypol as the TMS-derivative.

Five milligrams of pure gossypol, prepared by the procedure of Pons et al. (4), was suspended in one milliliter of carbon disulfide, and 0.1 milliliter of N,0-bis(trimethylsilyl) acetamide (Pierce Chemical Company, Box 117, Rockford, Ill.) was added. The solution was mixed well and allowed to stand at room temperature for 30 minutes before GLC analysis. Analyses were performed on an Aerograph Model 1520-B Gas Chromatograph, equipped with a flame-ionization detector. A 0.91-m × 0.24-cm (ID) stainless steel column, packed with 3% JXR on 80–100 mesh Gaschrom-Q (Applied Science Laboratories, State College, Pa.), was used with an on-column injection system.

A typical chromatogram of the TMS-gossypol derivative is shown in Figure 1. The three component peaks have been labeled a, b, and c for the purpose of discussion. That peaks a and c are not attributable to impurities in the gossypol was determined by the following tests. Gossypol, purified by silicic acid column chromatography to remove any oxidized form of the compound which might be present, produced a similar chromatogram. Purification of the TMS-gossypol derivative by thin-layer chromatography on Silica gel-G (Applied Science Laboratories), by using a solvent system composed of 60 parts hexane and 40 parts diethyl ether, did not remove shoulder peaks a and c. Carrying out the reaction in different solvents (diethyl ether, acetone tetrahydrofuran, and chloroform) produced the same spectrum, but the individual peaks varied in size with solvents. If peaks a and c were caused by impurities, they should have been present in the same relative proportion in all solvents.

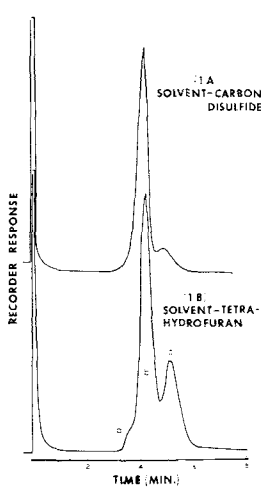

FIG. 1. GLC analysis of gossypol as the TMS derivative. Column temperature 240C, helium flow 100 ml/min.

Figure 2 represents the structure of gossypol as proposed in 1938 and verified by synthesis in 1957. Recent work with NMR spectroscopy indicates that, at least in the solvents deuterochloroform and dioxane, gossypol exists exclusively in the aldehyde form (Figure 2-I). The possibility still exists however that, in other solvents, the other two tautomeric forms may be present in varying amounts. The presence of the three peaks in different solvents in varying proportions supports this point of view. Carbon disulfide was found to be a solvent which reduced the shoulder peaks to a minimum.

Comparison of the infrared spectra of gossypol and the gossypol-TMS derivative shows a greatly diminished absorption at 2.8 and 6.2 microns, which is indicative of the binding of the hydroxyl and carbonyl groups respectively in the TMS derivative. This suggests that both the hydroxyl and carbonyl groups are involved in the formation of the TMS derivative. Thin-layer chromatography of several derivatives of gossypol on Silica gel-G with a nonpolar solvent system (n-hexane-diethyl ether, 60:40, v/v) showed that gossypol-TMS moved with the solvent front whereas gossypol and its para-anisidine and oxime derivatives (which react primarily with the carbonyl groups) remained at or near the origin. This also is indicative of the reaction of the hydroxyl groups in the formation of the TMS derivative.

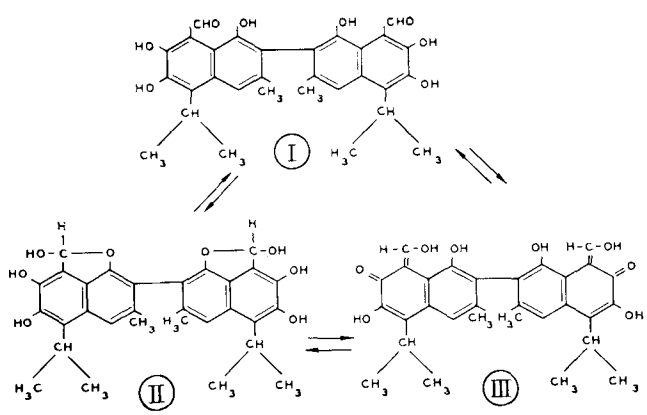

FIG. 2. Structure of gossypol in three tautometric forms.

TABLE I

Gossypol Content[a]

Exp. No.	Weight %[b]	Peak area %[c]	Weight % / Peak area %
1	10.31	13.51	1.310
2	23.97	23.80	.993
3	32.17	31.91	.992
4	36.75	38.46	1.047
5	42.85	43.86	1.024
6	60.59	60.98	1.006
7	70.52	70.09	.994
8	74.76	75.76	1.013

[a] Average of two replicates in each case.

[b] $\text{Weight } \% = \frac{\text{wt. of gossypol}}{\text{wt. of tricaprin} + \text{wt. of gossypol}} \times 100$

[c] $\text{Peak Area } \% = \frac{\text{area of peak b of TMS-gossypol}}{\text{area of tricaprin} + \text{area of peak b of TMS-gossypol}} \times 100$

Application of GLC to the quantitative determination of gossypol requires the use of an internal standard. Among the various compounds tested for this purpose, tricaprin was chosen since it is eluted quantitatively from the column and its retention time under the conditions for the analysis was approximately one-half that of gossypol. Also the compound is available commercially in pure form (Applied Science Laboratories). Standard mixtures with various quantities of gossypol and tricaprin by weight were prepared and analyzed by GLC. The results of these analyses are shown in Table I. The peak areas were calculated by triangulation; only the area of peak b of gossypol was computed. It can be seen that, if one considers all three peaks (a, b, and c), then the area percentage of gossypol will be greater than its weight percentage. This is to be expected in view of the fact that up to eight trimethylsilyl groups may have been added to the molecule. However the relatively constant nature of the factor obtained by dividing the weight percentage of gossypol by its area percentage indicates that this method can be used for quantitative determinations.

As a practical application of this method to pigments in cottonseed products, uncooked cottonseed flakes were extracted by the official method (2) for determining free gossypol. An aliquot of this aqueous acetone extract was reduced to dryness on a flash evaporator at 30C, and carbon disulfide and the silylating reagent were added. It was observed that,

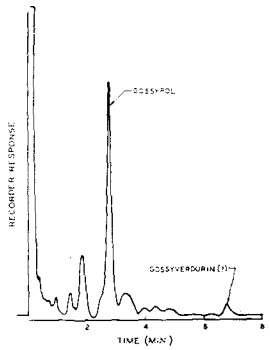

FIG. 3. GLC analysis of the aqueous acetone extract of uncooked, flaked cottonseed meats for gossypol as the TMS derivative. Temperature 240–300C programmed at 4 C/min. Other conditions the same as in Figure 1.

in addition to the normal gossypol peaks, several other smaller peaks were obtained (Figure 3). The last peak in Figure 3 was found to have the same retention time as the TMS derivative of an authentic sample of gossyverdurin. It is known that there are other gossypol-like pigments present in cottonseed which probably account for the other minor peaks. Identification of these peaks and investigation of the extension of this method to pigments in other cottonseed products are in progress.

ACKNOWLEDGMENT

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P. K. RAJU
Department of Biochemistry and Biophysics
CARL M. CATER
Texas Engineering Experiment Station and
Department of Biochemistry and Biophysics
Texas A&M University
College Station, Texas

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• *Letters to the Editor*

Calculation of the Linolenic Selectivity Ratios of Hydrogenation Catalysts

THE CALCULATION of the selectivity ratios or ratios of the reaction rates of the various reactions that occur during the hydrogenation of unsaturated oils may be made by using an analog computer (Butterfield et al., JAOCS *41*, 29, 1964) or may be estimated graphically (Albright, JAOCS *42*, 250, 1965). Since an analog computer is not always available, the graphical method has wider utility. However the present graphical method does not provide for the calculation of the linolenic selectivity ratio, which is the ratio of the reaction rates of the hydrogenation of linolenic to linoleic and linoleic to oleic.

Since the selective reduction of the linolenic in soybean oil is a desirable characteristic (Koritala and Dutton, JAOCS *43*, 556, 1966) of some catalysts, this report describes a method of calculation of the Linolenic SR so catalysts may be classified by this criterion.

Essentially the same method of calculation from the equations described by Albright was used. A GE-265 Time Sharing Computer system was programmed with the first order, nonreversible, kinetic equations of the reaction sequence

$$\text{linolenic} \xrightarrow{K_1} \text{linoleic} \xrightarrow{K_2} \text{oleic}$$

and the compositions were calculated over a range of