# Substituted Arylimino Derivatives of Gossypol[1]

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THE PRODUCTION of cottonseed oils having good refining and bleaching characteristics has become increasingly difficult during the past decade as a result of the rapid changes which have taken place in the techniques of processing cottonseed for oil and meal. It has been estimated that as much as 25 to 30% of the crude cottonseed oil presently received at refineries cannot be bleached readily to a satisfactory color.

The newer solvent extraction and screw-press procedures, which are displacing the older hydraulic press method, permit a larger amount of the cottonseed pigments to escape into the oil, and this circumstance, coupled with the extended storage of the crude oil at elevated temperatures, is mainly responsible for color reversion [3] in crude cottonseed oils (4). During the storage of such oils some of the pigments present are converted into forms which are not readily alkali-soluble and are not removed by the conventional refining and bleaching procedures (2).

Gossypol, a polyphenolic binaphthaldehyde, is the principal pigment present in crude cottonseed oil, and it has been shown that the extent of color reversion of stored, crude screw-pressed oil is directly proportional to the amount of the pigment originally present in the oil (8). This relationship is not strictly obeyed when pre-pressed oils are stored (7), but color reversion in all types of oils can be prevented by treating the freshly prepared oils with p-aminobenzoic acid, which forms an oil-insoluble derivative with gossypol (5).

The present work describes the preparation, characterization, and some of the physical properties of 17 arylimino derivatives of gossypol which were required during a study undertaken to find other aromatic amines which would form oil-insoluble derivatives with gossypol.

## Arylimino Derivatives of Gossypol

The best known representative of this class of gossypol derivatives is dianilinogossypol, or bis-(phenylimino)gossypol, which is formed by the condensation of the two aldehyde groups of gossypol with two molecules of aniline and with the elimination of two molecules of water. This relatively stable, easily purified substance has been used often for the analysis and isolation of gossypol from cottonseed products (3). Other amines which have been shown to react with gossypol in the same manner as aniline are o-phenylenediamine (6, 1) and β-naphthylamine (1). It has been reported that p-chloroaniline, p-anisidine, and the three isomeric toluidines also react with gossypol, but the products do not give the expected percentage composition on analysis (1), an observation which has been confirmed in the present study for the toluidines only.

The general method of preparing the arylimino derivatives listed in Table I was to treat one molar equivalent of gossypol with two molar equivalents of the aromatic amine in refluxing absolute ethyl alcohol for one-half hour. The compounds were obtained in good yield throughout, 75–100%, and were found

### TABLE I
Substituted Arylimino Derivatives of Gossypol

| Compound | % Yield | Recrystallized from | M. P. °C. (dec.) | Abs. Max. in CHCl₃, mμ |
|---|---|---|---|---|
| Bis(α-naphthylimino) | 82 | CHCl₃ | 283–5 | 452, 325 |
| Bis(β-naphthylimino) | 91 | C₆H₅Cl | 302–3 [a] | 472, 447, 312 |
| Bis(o-carboxyphenylimino) | 87 | C₆H₅Cl + 1-ClC₁₀H₇ | >360 | 445, 314 |
| Bis(m-carboxyphenylimino) | 91 | C₆H₅Cl | >360 | (448, 314) [b] |
| Bis(p-carboxyphenylimino) | 93 | ............ | >360 [d] | (457, 322) [c] |
| Bis(o-hydroxyphenylimino) | 91 | Abs. EtOH | 268–9 | 472, 447 |
| Bis(m-hydroxyphenylimino) | 94 | 95% EtOH | 287 | 463, 441, 311 |
| Bis(p-hydroxyphenylimino) | 97 | 95% EtOH | 291 | 445 |
| Bis(p-methoxyphenylimino) | 94 | 95% EtOH | 287–9 | 467, 445 |
| Bis(p-phenylphenylimino) | 88 | C₆H₅Cl | 327–8 | 470, 449, 317 |
| o-Nitrophenylimino | 94 | C₆H₅Cl | 202 | 467, 400, 310 |
| Bis(o-nitrophenylimino) | 97 | 1-ClC₁₀H₇ | 234.5–5.5 | 465, 310 |
| Bis(m-nitrophenylimino) | 90 | 1-ClC₁₀H₇ | 280–2 | 467, 312 |
| Bis(p-nitrophenylimino) | 75 | 1-ClC₁₀H₇ | 305–6 | 467, 323 |
| Bis(p-chlorophenylimino) | 88 | CHCl₃ | 302 | 465, 443, 312 |
| Bis(o-mercaptophenylimino) | 100 | C₆H₆ + CHCl₃ | 227–8 | 450, 315 |
| α-(p-Aminophenyl)-p-tolylimino | 98 | C₆H₅Cl | 322–3 | 454 |

[a] Adams and co-workers (1) report m. p. 310–13°C. (dec.) for this compound.
[b], [c] Determined in anhydrous pyridine.
[d] Previously reported by Dechary and co-workers (5).

to be high melting, brightly colored substances (light yellow to deep red) insoluble, for the most part, in the common organic solvents. It was not possible to recrystallize bis(p-carboxyphenylimino)gossypol from any of the solvents used.

When o-nitroaniline was reacted with gossypol, using the general procedure, a molecule of the amine condensed with one of the aldehyde groups of gossypol, and o-nitrophenyliminogossypol was formed in 94% yield. However, by employing a large excess of the amine (250%) and an extended reaction time (24.5 hrs.), bis(o-nitrophenylimino)gossypol could be obtained in 97% yield. When a 200% excess of the amine was used with only three hours' reaction time, both compounds were formed together, and bis-(o-nitrophenylimino)gossypol could be isolated from the mixture in 29% yield. These results would indicate the existence of steric hindrance of a kind which would make difficult the introduction of a second o-nitrophenylimino group into the gossypol molecule although no such difficulty was observed when a Stuart-Briegleb molecular model of bis (o-nitrophenylimino)gossypol was constructed. It is to be concluded that some other factor, or factors, besides the spatial requirements of the o-nitrophenylimino group, is responsible for this anomalous behavior.

One other amine, p,p′-methylenedianiline, gave a one to one condensation product with gossypol and in excellent yield, 98%. The employing of an excess of the diamine did not alter the course of the reaction.

The visible and ultraviolet absorption spectra of o-nitrophenyliminogossypol and the three isomeric

bis(nitrophenylimino)gossypols are illustrated in Figure 1. These are of interest in that the absorption spectra of the o-nitrophenylimino compound (Figure 1A) is clearly differentiated from that of the others (Figures 1B, C, D) by the presence of an additional absorption maximum at 400 m$\mu$. The positions of the



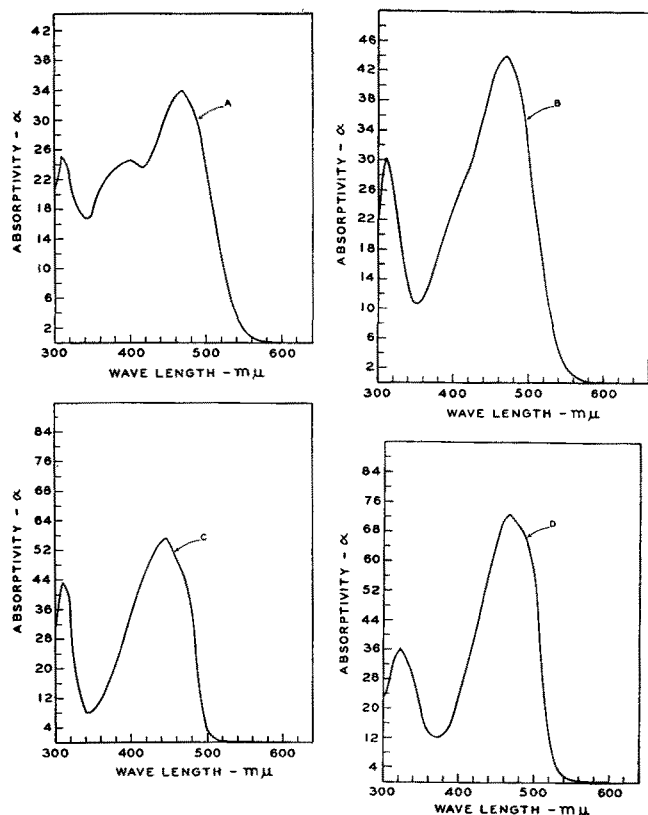Fig. 1A. The absorption spectra of o-nitrophenyliminogossypol in chloroform.

Fig. 1B. The absorption spectra of bis(o-nitrophenylimino)-gossypol in chloroform.

Fig. 1C. The absorption spectra of bis(m-nitrophenylimino)-gossypol in chloroform.

Fig. 1D. The absorption spectra of bis(p-nitrophenylimino)-gossypol in chloroform.

characteristic absorption maxima of all of the gossypol arylimino derivatives are given in Table I.

Twelve of these compounds exhibit almost identical spectra with maxima between 440 and 470 m$\mu$, possessing two cusps, one located at 445 m$\mu$ and a second somewhat less intense cusp located at 466 m$\mu$. The exact positions for these maxima in the spectra of the 12 individually substituted bis(arylimino) derivatives agree with these two average values within ± 5 m$\mu$.

The remaining five compounds exhibit somewhat different spectra. This group includes the three bis-(nitrophenylimino)gossypols and the two aryliminogossypol derivatives obtained from o-nitroaniline and p,p'-methylenedianiline, respectively. All the bis-(nitrophenylimino)gossypols and o-nitrophenyliminogossypol exhibit only a single maximum at 468 ± 2 m$\mu$. a-(p-Aminophenyl)-p-tolyiminogossypol exhibits a spectra consisting of a single band at 452 m$\mu$.

In the small variations noted in the positions of the cusps of the substituted derivatives there does not appear to be any systematic effect of o-, m-, and p-substitution in the phenyl group, nor is any particular effect of polarity of the substituent observed.

Although all of the aromatic amines examined in the present work gave arylimino derivatives when reacted with gossypol in alcohol solution, only p-aminobenzoic acid reacted with the gossypol of crude cottonseed oils to yield an oil-insoluble compound (5). The other amines failed to dissolve sufficiently in the oil to react, or the compound, if formed, remained dissolved in the oil.

## Experimental

*Physical Measurements.* Capillary melting points listed in Table I are uncorrected and were determined in a bath pre-heated to within 3–5° of the melting point of the compound and with a rapid rate of heating, 2° per minute. Reproducible values were obtained only in this manner.

Analyses for the percentage of nitrogen were determined by the micro-Kjeldahl method except for the nitro-substituted compounds which were analyzed by the micro-Dumas method. Elementary analyses of the compounds are given in Table II.

TABLE II

Elementary Analyses of Substituted Arylimino Derivatives of Gossypol

| Calculated For | Requires (%) | | | Found (%) | | |
|---|---|---|---|---|---|---|
| | C | H | N | C | H | N |
| $C_{50}H_{44}N_2O_6$ | 78.10 | 5.77 | 3.64 | 78.02 | 5.88 | 3.65 |
| $C_{50}H_{44}N_2O_6$ | 78.10 | 5.77 | 3.64 | 77.90 | 5.82 | 3.54 |
| $C_{44}H_{40}N_2O_{10}$ | 69.83 | 5.33 | 3.70 | 69.38 | 5.50 | 3.62 |
| $C_{44}H_{40}N_2O_{10}$ | 69.83 | 5.33 | 3.70 | 69.63 | 5.59 | 3.74 |
| $C_{44}H_{40}N_2O_{10}$ | 69.83 | 5.33 | 3.70 | 69.28 | 5.41 | 3.78 |
| $C_{42}H_{40}N_2O_8$ | 71.98 | 5.75 | 4.00 | 71.61 | 5.69 | 3.93 |
| $C_{42}H_{40}N_2O_8$ | 71.98 | 5.75 | 4.00 | 71.25 | 5.81 | 3.91 |
| $C_{42}H_{40}N_2O_8$ | 71.98 | 5.75 | 4.00 | 71.13 | 5.70 | 3.95 |
| $C_{44}H_{44}N_2O_8$ | 72.51 | 6.09 | 3.84 | 72.00 | 6.03 | 3.60 |
| $C_{54}H_{48}N_2O_6$ | 79.00 | 5.89 | 3.41 | 78.79 | 6.00 | 3.26 |
| $C_{36}H_{34}N_2O_8$ | 67.70 | 5.37 | 4.39 | 67.84 | 5.40 | 4.36 |
| $C_{42}H_{38}N_4O_{10}$ | 66.48 | 5.05 | 7.38 | 66.42 | 5.07 | 7.26 |
| $C_{42}H_{38}N_4O_{10}$ | 66.48 | 5.05 | 7.38 | 66.70 | 5.06 | 7.39 |
| $C_{42}H_{38}N_4O_{10}$ | 66.48 | 5.05 | 7.38 | 66.40 | 5.27 | 7.38 |
| $C_{42}H_{38}Cl_2N_2O_6$ | 68.38 | 5.19 | 3.80 | 68.28 | 5.30 | 3.69 |
| | Cl, 9.61 | | | Cl, 9.84 | | |
| $C_{42}H_{40}N_2O_6S_2$ | 68.83 | 5.50 | 3.82 | 68.88 | 5.40 | 4.00 |
| | S, 8.75 | | | S, 8.95 | | |
| $C_{43}H_{42}N_2O_7$ | 73.90 | 6.06 | 4.01 | 74.08 | 6.07 | 3.94 |

Absorption spectra were determined in chloroform solution (unless otherwise noted in Table I), using the Cary spectrophotometer, Model 11 M.

*Reagents.* Solid amines used for the reaction with gossypol were freshly recrystallized samples, agreeing in melting point with the accepted values reported in the literature. Liquid o-aminobenzenethiol, a commercial sample of 95% purity, was used without further purification. The gossypol was of 99% purity or better.

*Preparation of the Gossypol Arylimino Derivatives.* One millimole of gossypol (0.52 g.) and two millimoles of the aromatic amine were dissolved in 40–50 ml. of absolute ethyl alcohol, and the solution was boiled under reflux for one-half hour when the derivative usually crystallized. When required, solvent was removed by evaporation on the steam bath until crystals separated. o-Nitrophenyliminogossypol was isolated by reducing the volume of the reaction mixture by one-third and allowing it to stand at 4–5°C. for one week. On further concentration and chilling of the mother liquors, another crop of crystals was obtained. Repetition of this process gave four crops of crude crystalline material.

The crude compounds were washed three times with 10–15 ml. of cold 95% ethyl alcohol, twice with 10–15 ml. of cold diethyl ether, dried in vacuum over phos-

phorus pentoxide, and recrystallized from the appropriate solvent (see Table I) until the nitrogen content was constant. When high boiling solvents were used, the crystals were given a thorough washing with 95% ethyl alcohol, followed by a few minutes' refluxing in absolute ethyl alcohol.

The crystals were freed of traces of solvent by heating in vacuum for 16 hrs. at 70°C. (4 hrs. at 50°C. for the o-nitrophenyliminogossypol). Because the arylimino compounds tend to decompose markedly on exposure to the combined action of light and air, analytical samples were preserved by storing in evacuated ampules at −15°C.

*Bis(o-nitrophenylimino)gossypol.* A solution of 2.07 g. (four millimoles) of gossypol and 2.76 g. (two centimoles, a 250% excess) of o-nitroaniline in 100 ml. of absolute ethyl alcohol was boiled under gentle reflux for 24.5 hrs. The deep red-brown solution was allowed to stand at 4–5°C. for 16 hrs., and the precipitate which formed was collected, washed twice with 15–20 ml. of cold 95% ethyl alcohol, and dried in vacuum, giving 2.2 g. of deep red micro-prisms, m. p. 229.5–230°C. (dec.). On concentrating the filtrate and washings to 30–40 ml., an additional 0.72 g. of product of the same m. p. was obtained. Further concentration of the mother liquors to 10–15 ml. gave 0.032 g. of impure material, m. p. 225–228°C. (dec.). The total yield of crude product was 2.95 g., 97% of theory. The analytical sample, m. p. 234.5–235.5°C. (dec.), was prepared by recrystallizing a portion of the first fraction four times from boiling 1-chloronaphthalene.

It was also possible to obtain a 96% yield of this compound by treating gossypol with a 1000% excess

of o-nitroaniline (no solvent) for one hour at 80–90° C. and extracting the unchanged amine with boiling absolute ethyl alcohol.

## Summary

Seventeen substituted arylimino derivatives of gossypol have been prepared and characterized. Fifteen of these are new compounds. o-Nitroaniline gave both a mono- and a disubstituted derivative with gossypol while p,p'-methylenedianiline gave only a monosubstituted derivative.

During experiments designed to prevent color reversion in stored, crude cottonseed oils by treatment with various aromatic amines, only p-aminobenzoic acid formed an oil-insoluble derivative with the gossypol in the oils.

## Acknowledgment

REFERENCES

1. Adams, R., Price, C. C., and Dial, W. R., J. Am. Chem. Soc., 60, 2158 (1938).
2. Altschul, A. M., and Thurber, F. H., Cotton Gin and Oil Mill Press, 54(23), 26 (1953).
3. Boatner, C. H., in "Cottonseed and Cottonseed Products," A. E. Bailey, ed., pp. 224 and 302, New York, Interscience Publishers Inc., 1948.
4. Boatner, C. H., Hall, C. M., O'Connor, R. T., Castillon, L. E., and Curet, M. C., J. Am. Oil Chemists' Soc., 24, 97, 276 (1947).
5. Dechary, J. M., Kupperman, R. P., Thurber, F. H., and O'Connor, R. T., J. Am. Oil Chemists' Soc., 31, 420 (1954).
6. Karrer, P., and Tobler, E., Helv. Chim. Acta, 15, 1204 (1932).
7. Pons, W. A. Jr., Thurber, F. H., and Hoffpauir, C. L., J. Am. Oil Chemists' Soc., 32, 98 (1955).
8. Thurber, F. H., Vix, H. L. E., Pons, W. A. Jr., Crovetto, A. J., and Knoepfler, N. B., J. Am. Oil Chemists' Soc., 31, 384 (1954).

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# A Modified Dilatometer for Fast Evaluation of "Solids Content" in Fats

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THE DILATOMETRIC method, noted for its usefulness in phase studies (1), seems to have become increasingly popular in the edible fat industry in recent years. The estimation of "solids content" by this method is made use of in formulation and in hydrogenation control.

The fact that dilatometric methods are time-consuming has been a major drawback in their application in routine analysis of plastic fats. When dilatometry is applied in hydrogenation control, it is of particular importance to cut to a minimum the time required to carry out an analysis. Successful work to this end has been reported in this journal (3), of which one feature was a commendable tempering step introduced in the procedure.

We have made a change in the dilatometer design, which we have found very effective in cutting the time requirements of test operations. Moreover the change was a very convenient and inexpensive one to make since it involves the dilatometer stopper only, and not the bulb itself.

The change resulted in a considerable reduction in the time required to obtain temperature equilibrium throughout the fat sample. This effect results from:

a) Increased dilatometer surface area exposed to the water in the constant temperature water bath.

b) Decreased thickness of the fat body.

## Apparatus

We have been using a dilatometer of the volumetric type, with a bulb volume of ca. 11 ml., and with a capillary graduation in cubic millimeters.[1] The original 14/20 ground glass stopper has now been replaced by a hollow stopper, made by our glassblower. Figure 1 shows the two stoppers separately, and the open-stopper dilatometer assembled.

The hollow stopper was made from Pyrex tubing with standard ground glass joint. The outside diameter of the stopper is well over one-half of that of the dilatometer bulb. Comparing the original ($D_1$) and the modified dilatometer ($D_2$), the ratio of surface area effecting heat transfer is ca. 1:1.5. The sample weights are close to 9 and 6 g., respectively. The 9-g. sample (in $D_1$) has a radius of ca. 9 mm. while the 6-g. sample (in $D_2$) is, roughly, a cylindrical ring of fat, the thickness of which is 3-4 mm.

[1] Manufactured by H. S. Martin, Evanston, Ill.