Physical and Chemical Properties of Selected Metal Complexes of Gossypol

H. N. RAMASWAMY[1] and R. T. O'CONNOR,
Southern Regional Research Laboratory,[2] New Orleans, Louisiana 70119

Abstract

In order to understand the chemistry of complexes of gossypol several metal ion-gossypol complexes have been synthesized. They do not melt below 300 C and turn dark in color after drying in a vacuum oven. The stability constants and stoichiometry of CO^{++}, Ni^{++} and Cu^{++} gossypol complexes have been established by several analytical and spectroscopic techniques. The infrared spectra of solid samples show a greatly diminished absorption at 2.8μ and the 6.2μ band is shifted to a lower wavelength indicating binding of the metal through the hydroxyl and aldehyde groups. Electronic absorption spectra also support this conclusion. Bis(o-carboxyphenylimino) gossypol gives a copper(II) complex, where the carboxylic group, but not the phenolic hydroxyl group, of the gossypol is ionized. In this instance copper ion is bound to the carboxy oxygen and nitrogen of the imine.

Introduction

CHRONIC AND ACUTE GOSSYPOL toxicity and adverse physiological effects are barriers to complete utilization of presently available commercial cottonseed meals. Since detoxification of gossypol through complexation has been suggested, it was considered that basic information on the physical and chemical properties, leading to a better understanding of gossypol inactivation via metallic ion complexes, might be profitable. Such an investigation certainly becomes an integral portion of a program of research on improving the nutritional value and utility of cottonseed meal. Previous pertinent work includes reports on gossypol derivatives (1,2) and iron complexes (3,4).

Spectroscopic and electroanalytical studies demonstrate the formation of stable complexes of gossypol with various cations. Elemental analysis, infrared and electronic absorption spectra are presented for copper(II) complexes. The composition and stability constants of cobalt(II), copper(II) and nickel(II) complexes are also reported in this paper. These are, in turn, compared with similar data obtained with gossypol derivatives.

Experimental Procedures

Conductivity data were obtained with an Industrial Instruments, Inc., conductivity bridge, Model RC 16B2. Ultraviolet and visible spectra were obtained on Cary Model 14 instrument and infrared absorption data from a Perkin-Elmer Infrared Spectrometer Model 21.

Gossypol-imino derivatives were prepared according to the method given in the literature (1,2).

TABLE I

Composition of Metal-Gossypol Precipitates[a]

Metallic ion	Color of the precipitate	Metal content calcd. for 1:1 composition	Metal content found
Ca^{+2}	Orange	7.18	6.88
Ba^{+2}	Yellow	20.96	23.50
Mg^{+2}	Yellow-Orange	4.43	4.24
Cd^{+2}	Red	17.83	17.49
Zn^{+2}	Orange	11.21	12.23
Al^{+3}	Yellow	4.95	3.96
Mn^{+2}	Orange	9.59	10.29

[a] These compounds were first identified by Walter A. Pons of this Laboratory.

Copper(II)gossypol complex was prepared by adding 0.4 g of $CuCl_2$ $2H_2O$ in ethanol to 1.2 g of gossypol in acetone. Anhydrous pyridine (~5 ml) and 2–3 ml of 0.01 M NaOH were added with constant stirring. A green precipitate was obtained immediately. It was filtered, washed with distilled water and dried in a vacuum oven at 110 C for 4–6 hr. The infrared spectra and chemical analysis did not show the presence of any pyridine in the sample.

Gossypol-imino copper(II) complex was prepared by refluxing the respective iminogossypol and copper(II) chloride in 1:1 molar ratio in dioxane. When a dark solution was obtained about 5 ml of pyridine was added along with 5–10 ml of distilled water. A pale-green precipitate was obtained. It was filtered, washed with distilled water and dried in a vacuum oven at 110 C.

Metal Complexes of Gossypol

Sodium gossypolate solution was added to metallic salt solution in 1:3 molar ratio. The solution was left in a water bath at 25 C for about an hour, centrifuged, filtered and dried in an vacuum oven at 110 C for 2–4 hr.

Results and Discussion

A number of experimental procedures were employed in which solutions of sodium gossypolate were used as anion sources in reaction with metal chlorides at pH of 7–8.5. Marked color changes occur in the reaction with Ca^{+2}, Ba^{+2}, Mg^{+2}, Cd^{+2}, Zn^{+2}, Al^{+3}, and Mn^{+2} at stoichiometries suggestive of complexation by the gossypolate ion (Table I).

The molar ratio method (5), slope ratio method (6) and conductometric methods were also used to determine the stoichiometry of the reaction product in aqueous solution. The results of these studies, as represented in Fig. 1, 2 and 3 establish that cobalt(II), nickel(II) and copper(II) ions react

TABLE II

Log K Values for 1:1 Complexes

Complex	Log K (±0.50)	ΔF (—K. cal)
Copper(II)gossypol	7.26	10.01
Cobalt(II)gossypol	8.18	11.28
Nickel(II)gossypol	8.02	11.00
Iron(II)gossypol[3]	7.60	10.48
Iron(III)gossypol[4]	6.75	9.20

---

[1] Resident Postdoctoral Research Associate 1967–1968.
[2] So. Utiliz. Res. Dev. Div., ARS, USDA.

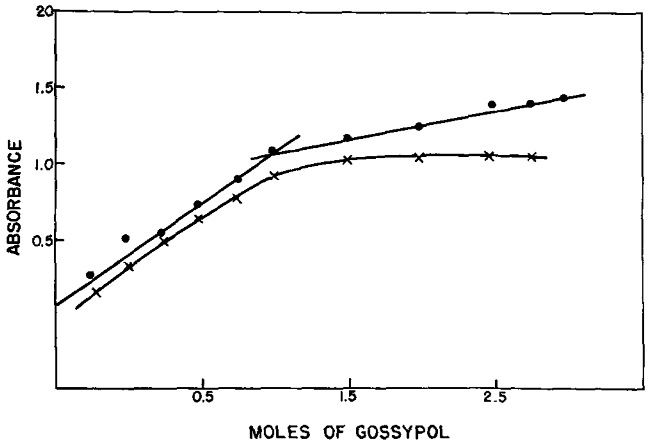

FIG. 1. Molar ratio plots. Moles of gossypol per mole of M^{+2} vs. absorbance at 390 mμ. Final concentration of M^{+2} 10^{-4} M · Ni^{+2}, X Co^{+2}.

with gossypol in the ratio 1:1. The log K values and hence the free-energy values are calculated from continuous variation studies (7) and molar ratio methods (6) (Table II).

Two gossypol-imino derivatives were prepared and their complexes are listed in Table III along with the copper(II) gossypol complex.

All complexes are insoluble in water and in ordinary organic solvents. These samples, on drying in a vacuum oven at 110 C for a few hours, show a fading in color.

Infrared Absorption Spectra

The IR spectrum of gossypol shows no bands in the 5–6μ region, in spite of the chemical evidence for the aldehyde group. But it exhibits an intense band at 6.1μ, which is assigned to C = O stretching, since the carbonyl group conjugated to the naphthalene nucleus produces a bathochromic shift (8). The complexes of gossypol exhibit a strong band at 5.7μ and a weak band at 6.1μ (Fig. 4). The first band can be assigned to normal C = O stretching and the latter to C = C stretching. The disappearance of the 6.1μ band and appearance of the 5.7μ band is a clear indication of disruption of the conjugated carbonyl group. The observed greatly diminished absorption arising from stretching of the hydroxyl group at 2.8μ, can be cited as evidence for chelation with the metal ions. Further examination of the IR spectra reveal the characteristic maxima for certain specific groups of each compound as follows:

Gossypol, (cm^{-}) 3550(OH), 3000(CH), 1625(C=O), 1440($-CH_3$), 1245($\equiv C-O^-$).

Gossypol Complexes, 3400(OH), 3000(CH), 1750 (C = O), 1650(C = C), 1450($-CH_3$), 1275($=C-O^-$). Bis(o-carboxyphenylimino)gossypol and Bis(methylimino)gossypol exhibit an intense band at 6.0–6.2μ and a weak band at 2.7μ assigned here to C = N stretching and imino group respectively. The C = O stretching band is observed at 5.84μ in bis(o-carboxyphenylimino) gossypol and at 5.73μ in its copper(II) complex. The C = N stretching frequency undergoes little change after complexation as expected.

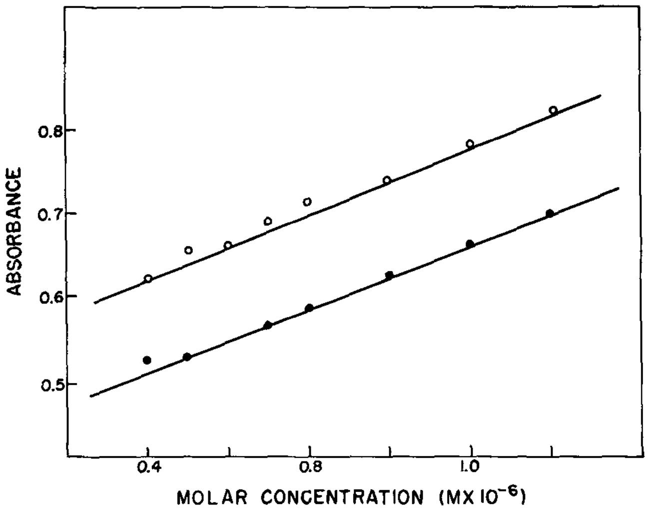

FIG. 2. Slope ratio plots (390 mμ): ● copper varying, O gossypol varying.

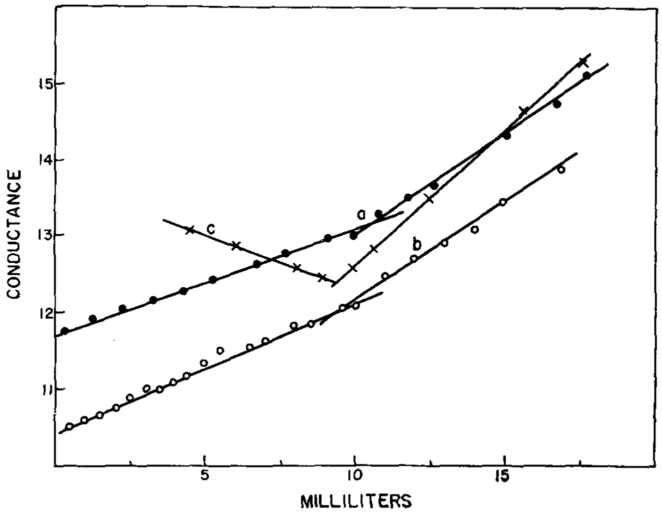

FIG. 3. Conductometric titration of 100 ml 10^{-3} M · Na-gossypolate against 10^{-2} M · (a) Co^{2+} (b) Ni^{2+} (c) control.

Electronic Absorption Spectra

Gossypol and its derivatives exhibit three bands in the UV and visible region (Fig. 5, Table IV). According to Clar (9), the longest wavelength band in the spectra is the α-band, the shorter wavelength band the β-band belongs to the naphthalene nucleus and the intermediate one the p-band. The p-band is generally

TABLE III

Analytical Data[a]

Complex		C	H	N
Bis(methylimino)gossypol	calcd.	70.58	6.62	5.13
	found	70.04	6.84	4.85
Bis(methylimino)gossypol copper(II)	calcd.	63.14	5.92	10.52 (Cu.)
	found	62.82	5.81	10.10 (Cu.)
Bis(o-carboxyphenylimino) gossypol	calcd.	68.83	5.33	3.70
	found	68.36	5.51	3.65
Bis(o-carboxyphenylimino) gossypol copper(II)	calcd.	64.52	4.65	7.61 (Cu.)
	found	64.23	4.69	7.20 (Cu.)
Copper(II)gossypol	calcd.	62.09	4.82	10.71 (Cu.)
	found	61.31	4.66	10.10 (Cu.)

[a] M.P. > 300 C.

TABLE IV

Electronic Absorption Spectra[a]

Complex	max (mμ)		
Gossypol	240	282, 292	370
Bis(methylimino) gossypol	251	302	380 (broad)
Bis(o-carboxyphenylimino) gossypol	243	318	440, 467 (sh)
Copper(II)gossypol[b]	260		390
Bis(o-carboxyphenylimino) gossypol	260	376	440 (sh)

[a] In dioxane.
[b] In LiF.

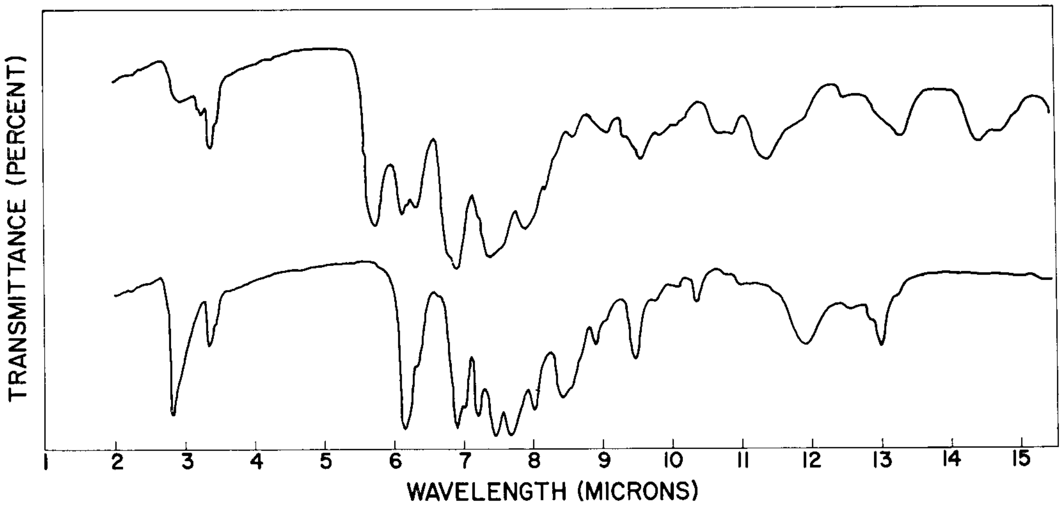

FIG. 4. Infrared spectra: (a) gossypol (b) copper(II) gossypol.

more sensitive to variations in structure and is associated with the transition from the highest occupied to lowest unoccupied molecular orbital. The other two are due to $\pi \rightarrow \pi^-$ transitions (10). In the case of copper(II) complexes the α-band ($>$340 mμ) is shifted towards a longer wavelength and the p-band disappears. These observations indicate that both the hydroxyl and carboxyl groups are involved in binding to metal ions. Similar conclusions were drawn for trimethylsilyl-gossypol derivative from GLC and IR data (11). While structural proof is not as yet available, it would appear highly probable that the orthohydroxy aldehyde complex, forming the stable six-membered ring (Fig. 6a) is more likely than the seven-membered peri-hydroxy aldehyde (Fig. 6b). The isolation of the iron complex of 1,4-binaphthaquinone (12) and formation of stable complexes with various cations and 2-hydroxy-1-naphthaldehyde and other compounds containing oxo group in ortho position to the phenolic hydroxy group (13) is further evidence for this conclusion. Furthermore the observed magnetic moment for the copper(II) gossypol complex (1.80 BM) also supports the formation of a six-membered ring (14). Consequently it may be concluded that reaction with metal ions is occurring at the 1-hydroxyl rather than the 7-hydroxyl position. The possibility of delocalization of negative charge on oxygen is present in formula (a) of Fig. 6.

Bis(o-Carboxyphenylimino)Gossypol Copper (II)

The decrease in position of absorption maximum (~318 mμ) of the bis(*o*-carboxyphenylimino)gossypol

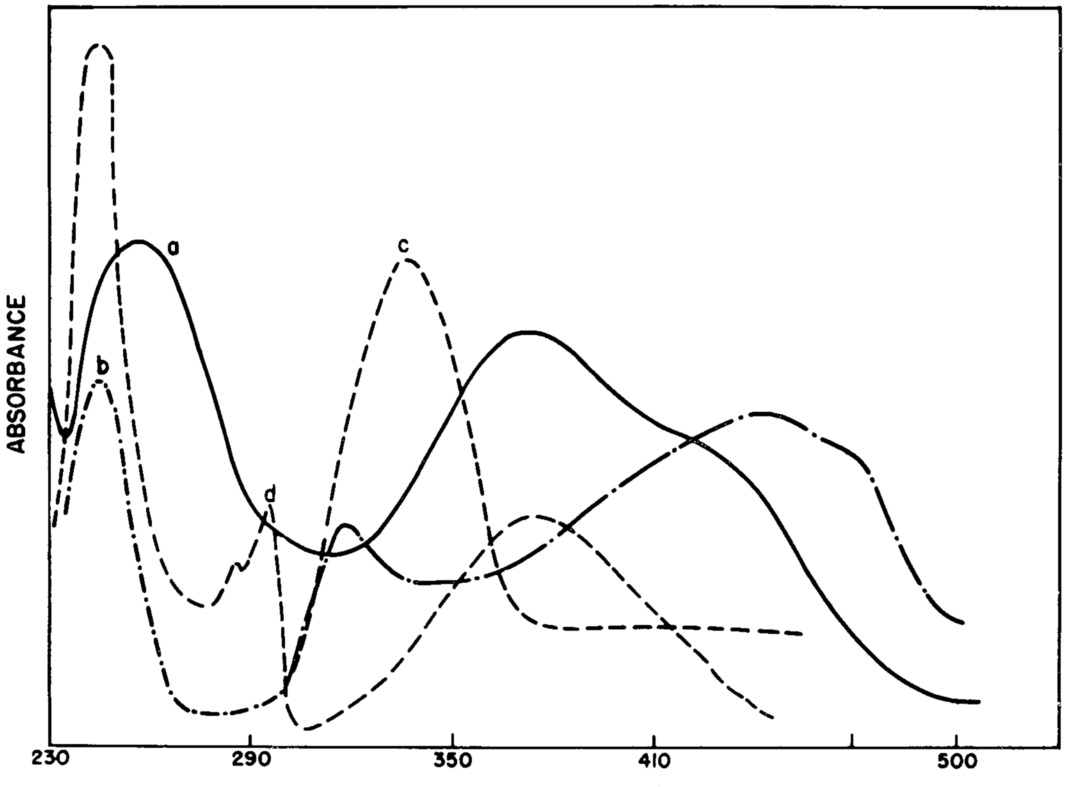

FIG. 5. Absorption spectra: (a) Bis(anthranilato)gossypol copper(II); (b) Bis(anthranilato)gossypol; (c) Anthranilic acid; (d) Gossypol.

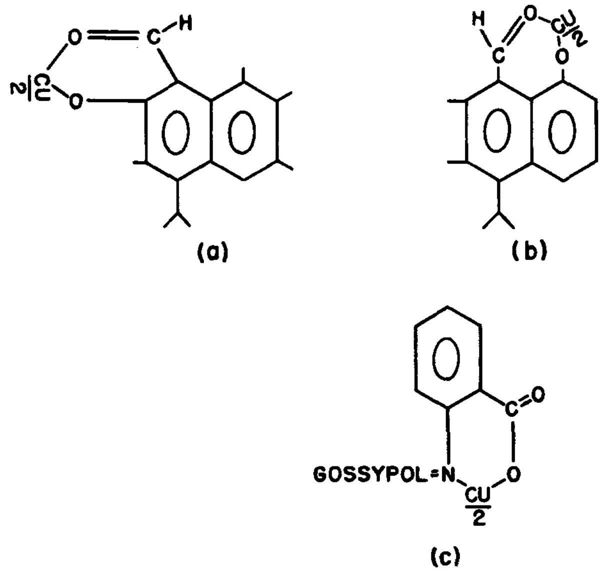

FIG. 6. Structure of gossypol complexes: (a) Six-membered ring copper(II) gossypol complex; (b) seven-membered ring copper(II) gossypol complex; (c) Structure of Bis(o-carboxyphenylimino) copper(II) gossypol.

after complexation is attributed to a combination of ring formation of a N-Cu bond and weaker electron withdrawing character of the COO^- group compared with that of the COOH group. Thus, in the absence of a strong base (see experimental section) only the carboxyl group is ionized. Furthermore, only a slight shift in C = O stretching frequency is observed after complexing with copper(II). This suggests that in the anthranilato derivative COO^-----Cu^{++} electrostatic bonds are made with sp N——Cu bonds (Fig. 6c). Previously it has been observed (15) that relatively strong acids such as picric and acetic acids add to the complex, while very weak acids such as phenols, cresols, etc., do not.

This work provides considerable information on the bonding of metal ions to gossypol and their absorption characteristics. Further work is in progress which when completed should provide basic information needed for the development of treatments to detoxify cottonseed meals and ultimately enable a physiological study by animal feeding.

REFERENCES

1. Dechary, J. M. and L. E. Brown, JAOCS *33*, 76 (1956).
2. Correa, O. G., H. M. Cappi, M. Salem and C. Staffa, Ibid. *43*, 678 (1966).
3. Jonassen, H. B., and R. J. Demint, Ibid. *32*, 424 (1955).
4. Muzaffaruddin, M., and E. R. Saxena, Ibid. *43*, 429 (1966).
5. Yoe, J. H., and A. L. Jones, Ind. Eng. Chem. Anal. Ed. *16*, 111 (1944).
6. Harvey, A. E., Jr., and D. L. Manning, J. Am. Chem. Soc. *72*, 4488 (1950).
7. Job, P., Am. Chim. *9*, 113 (1928).
8. O'Connor, R. T., P. Von der Haar, E. F. Dupre, L. E. Brown and C. H. Pominski, J. Am. Chem. Soc. *76*, 2368 (1954).
9. Streitwieser, A., "Molecular Oribital Theory for Organic Chemists," John Wiley & Sons Publication 1962, p. 215.
10. Heinert, D., and A. E. Martell, J. Am. Chem. Soc. *85*, 183 (1963).
11. Raju, P. K., and C. M. Cater, JAOCS *44*, 465 (1967).
12. Haas, R. H., and D. A. Shirley, J. Org. Chem. *30*, 411 (1965).
13. Ramaswamy, H. N., and R. T. O'Connor, unpublished results.
14. Kishita, M., Y. Muto and M. Kubo, Aust. J. Chem. *11*, 312 (1958).
15. Muto, Y., Bull. Chem. Soc., (Japan) *33*, 604 (1960).

[Received June 27, 1968]