The Occurrence of (+) Gossypol in *Gossypium* Species[1]

J.M. DECHARY and **PAUL PRADEL**, Southern Regional
Research Laboratory,[2] New Orleans, Louisiana 70119

ABSTRACT

(+) Gossypol, 2,2'-bi[8-formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthyl], has been isolated as the (+) dianilino derivative from cottonseed grown in widely different areas of the cottonbelt of the United States. The optically active derivative obtained had rotations varying from 43% to 54.7% of the value reported for the optically pure enantiomer. Attempts to resolve racemic gossypol by chemical means were not successful, but (+) gossypol was obtained by solvent fractionation (7.2%) and by chromatography on β-lactose (2.2%).

INTRODUCTION

Gossypol is a yellow, unstable phenolic binaphthaldehyde, which occurs in the genus *Gossypium* (subtribe *Hibisceae*, natural order *Malvaceae*) and in certain other members of the natural order. The correct structure for this compound was proposed by R. Adams in 1938 (1) and confirmed by total synthesis in 1957 by J.D. Edwards and J.L. Cashaw (1). Figure 1 shows the structure of gossypol which is formulated as 2,2'-bi[8-formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthyl]. Inspection of Figure 1 shows that the molecule can exhibit optical isomerism due to restricted rotation, "atropisomerism," since any nonplanar form of the molecule is devoid of a plane, center or alternating axis of symmetry. The ortho substituents of gossypol, 2,2'-dihydroxy and 3,3' dimethyl, are bulky enough to exhibit sufficient steric interference and therefore render the molecular nonplanar, as can be seen from an examination of the Godfrey molecular model, Figure 2.

Indeed, Bhakuni et al. (2) demonstrated that thespesin (3), isolated from the tree *Thespesia populnea* Soland, was identical with gossypol and that the optical activity detected, $[\alpha]_D = +457^\circ$ (benzene), was due to atropisomerism, i.e., restricted rotation of the two naphthalene rings about the pivot 2,2'C-C bond. The possibility that the optical activity of gossypol arises from two identical asymmetric carbon atoms of a hemiacetal form of the molecule was discounted when it was shown that acid treatment of (+) gossypol did not alter its optical activity (2).

King and de Silva (4) also isolated (+) gossypol from the bark and flowers of *Thespesia*. These workers were unable to prepare from (+) gossypol the acetic acid complex commonly used to isolate gossypol from cottonseed, but noted that (+) gossypol is much more soluble in organic solvents (except acetone) than the racemate and that (+) dianilinogossypol also displays enhanced solubility in organic solvents.

Datta et al. (5) isolated (+) gossypol from *Thespesia* flowers and reported that the mature pericarp contained the (+) enantiomer in an almost pure state. No optical activity could be detected in gossypol isolated from cottonseeds by these workers who did not specify how the gossypol was isolated and purified.

The specific rotation of the orange dianilino derivative of pure (+) gossypol is high, $[\alpha]^{19}_D$ = 2,740 (4), and the derivative is very insoluble compared to the free aldehyde (1). It was decided therefore to examine cottonseed of various origins for the presence of optically active gossypol by utilizing the formation of the dianilino derivative in solvent extracts of defatted seed. The employment of organic solvents in which the optically active derivate (if present) would be more soluble than the racemate was avoided.

EXPERIMENTAL PROCEDURES

Cottonseeds were decorticated by immersing in liquid nitrogen and removing the seed coat manually. Forty to 50 g of decorticated seed were finely ground at O C in an Omni-mix with pentane and the meal slurry filtered on a Buchner funnel. The extraction with pentane was repeated (three to four times) manually at room temperature until the meal was free of lipid. The lipid-free meal (15 g) was then exhaustively extracted at room temperature by stirring with diethyl ether containing a small amount of water (0.5%) and ethanol (2%); the extract was evaporated to a small volume (3-5 ml) under vacuum at 10 C or less and aniline (0.2-0.3 ml) was immediately added. After standing at room temperature overnight the crystalline dianilino derivative was chilled in an ice bath, collected, washed thoroughly with cold pentane, and dried in vacuum over Drierite; yield, 240-250 mg. The optical activity was measured at 20 C usually at c = 0.40-0.60, in chloroform. The optical rotations were accurate to at least ± 5 millidegrees when measured in the ETN-NPL polarimeter. A sample of (+) gossypol was examined for ease of racemization by heating in various solvents at elevated temperatures.

Attempts were made to isolate (+) gossypol from fresh ethereal cottonseed extracts utilizing solvent fractionation and chromatography on optically active adsorbents such as β-lactose or acetyl cellulose. The resolution of racemic

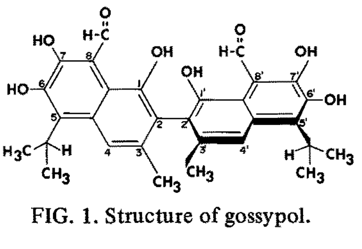

FIG. 1. Structure of gossypol.

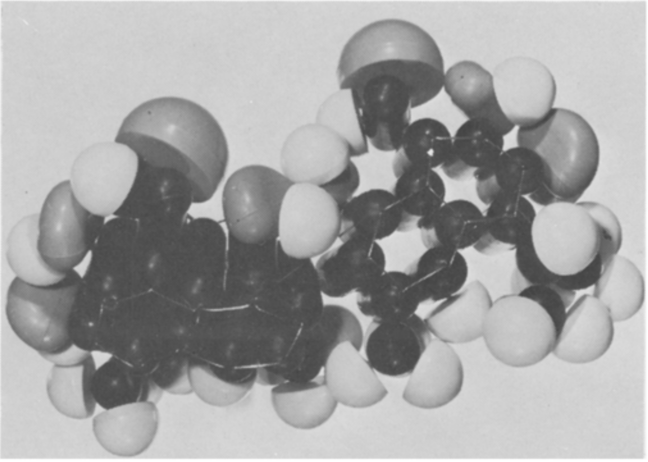

FIG. 2. Godfrey molecular model.

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[2] U.S. Department of Agriculture, Agricultural Research Service, Southern Marketing and Nutrition Research Division, P.O. Box 19687, New Orleans, Louisiana 70179.

TABLE I

Optically Active Dianilinogossypol From Cottonseed

Source	$[\alpha]^{546}_{20}$ ($CHCl_3$)	Optical purity, %
Air classified glanded flour (4.6-5.5% gossypol)	+ 1178°	43.0
Shafter, California Acala SJ-1 (hand harvest)	+ 1581°	57.7
Shafter, California Acala SJ-1 (machine harvest)	+ 1499°	54.7
Leland Mississippi Stoneville 213	+ 1260°	46.0
Brownsville, Texas XG-15 (1.2% gossypol)	+ 1266°	46.2

gossypol utilizing the two weakly acidic phenolic groups of the molecule was examined by employing the optically active bases, morphine, quinine and cinchonine. Resolution of racemic gossypol by the optically active amines *d*- and *l*-α-methylphenethylamine, *l*-α(1-naphthyl)ethylamine, and *l*-2-amino-1-propanol was also attempted.

RESULTS AND DISCUSSION

Cottonseed from California, Texas and Mississippi yielded (+) dianilinogossypol having specific rotations of from 43% to 57.7% of the value reported for pure optically active dianilinogossypol (4) as is shown in Table I.

A sample of (+) gossypol, about 63% optically pure, was refluxed 1 h in toluene, bp 116.9 C, with no loss in optical activity and only a 40% decrease in specific rotation occurred on heating in ethylene glycol (sealed tube) at 190 C for 10 min.

Attempts to resolve racemic gossypol by optically active bases were not successful, and a specific rotation of +33° was obtained for (+) gossypol fractionally precipitated by pentane added to diethyl ether solutions of racemic gossypol. Chromatography of racemic gossypol in diethyl ether on β-lactose yielded (+) gossypol having a specific rotation of +9.7°. Acetyl cellulose failed to effect any resolution whatsoever. The employment of a number of optically active amines did not result in any reaction product which could be worked up satisfactorily to yield optically active gossypol.

One early worker, E.P. Clark (6), did examine polarimetrically a sample of gossypol isolated from cottonseed and purified via the acetic acid complex. No optical activity was detectable. This is understandable since it is now known that the (+) enantiomer does not form an acetic acid complex (4). The (+) gossypol which may have been initially present in cottonseed extract would have thus eluded isolation. Also, the enhanced solubility of the (+) enantiometer would make it much more difficult to isolate by other procedures. The equal solubility of (+) gossypol and the racemate in acetone is probably due to the fact that acetone forms a very stable diacetonate with gossypol (7).

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