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The Photo-epimerisation of Gossypol Schiff's Bases

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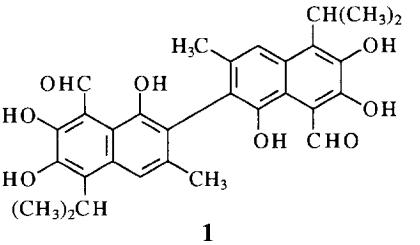
Abstract: ^{1}H NMR studies show that the diastereoisomeric adducts **3** of gossypol **1** and (L)-phenylalanine methyl ester undergo epimerisation, in hexane/ethyl acetate or deuteriochloroform, in sunlight. The individual diastereoisomers **3** can be equilibrated to a 50:50 mixture of diastereoisomers, thus allowing an efficient procedure for the recycling of the biologically-inactive D-gossypol.

Gossypol (1,1',6,6',7,7'-hexahydroxy-5,5'-diisopropyl-3,3'-dimethyl-2,2'-binaphthalene-8,8'-dicarboxaldehyde) **1**, a yellow compound isolated from cotton seed, exhibits atropisomerism as a result of hindered rotation about the 2,2'-bond and, although both enantiomers are found in nature, the (+)-isomer exists in high enantiomeric excess in most plants from which gossypol is isolated.[1] Gossypol does not racemise easily.[2] Gossypol has attracted pharmacological interest particularly with regard to fertility regulation,[3] and as a potential treatment for such diverse diseases as HIV infections,[4,5] diabetic complications,[6] and cancer.[7,8]

The cytotoxic effects of racemic gossypol on different histological types of tumour cells *in vitro* are variable[9,10] and it has been shown that the two enantiomers exhibit different antitumour activities with the L-isomer generally showing greater biological activity.[10] We have recently shown that L-gossypol is cytotoxic to a melanoma cell line but that D-gossypol has little or no effect when equimolar drug concentrations were incubated with cells over a 5 day period in the presence of serum containing media.[11] This differential effect of enantiomers is also observed when both isomers are incubated individually with a melanoma cell line for one hour in serum-free media.



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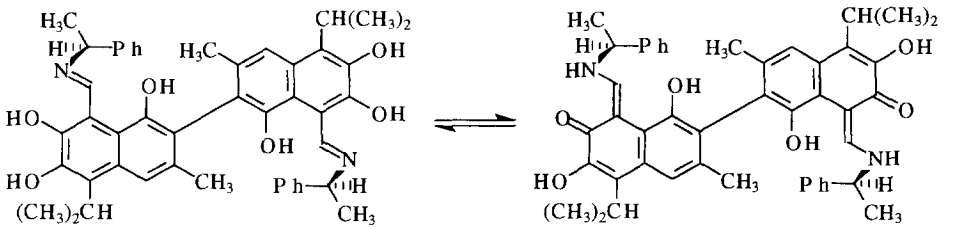
A number of workers[12,13,14] have succeeded in resolving gossypol **1** and the available methodology provides a useful means of obtaining large amounts of either optically pure enantiomer for studies of the toxicity, metabolism and mechanism of

action. Having sufficient quantities of optically pure L-gossypol should also allow a reappraisal of the clinical potential of this compound, particularly with regard to its use in the management of cancer patients.

We wish to report here some observations on the resolution of gossypol **1** using the diastereoisomeric Schiff's base adducts **2** with L-phenylalanine methyl ester. These aducts exist in the enamine **3** rather than the imine **2** tautomer and are readily prepared by reaction at room temperature in a mixture of dichloromethane/propan-2-ol[14]. Once prepared the diastereoisomers are easily separated by column chromatography, in the dark, on silica using hexane/ethyl acetate as the eluent. The individual diastereoisomers can then be readily hydrolysed to the D- or L-gossypol in acetic acid/sulphuric acid/ether at 0°C.



2 **3**

Although this method is simple, rapid and efficient it is wasteful in that 50% of the diastereoisomeric mixture, in our case that containing the unwanted D-isomer, is discarded. We have, however, found that by allowing a solution of any of the four individual diastereoisomers prepared from gossypol and D- or L-phenylalanine methyl ester, in hexane/ethyl acetate or deuteriochloroform, to stand in sunlight, epimerisation occurs to give a 50:50 equilibrium mixture of diastereoisomers. This mixture can then be re-chromatographed to give more of the desired diastereoisomer. We have thus discovered a simple procedure for the recycling of the unwanted, in our case D, enantiomer which employs the solvent system used as column eluent and sunlight.

We have followed the epimerisation of the diastereoisomer prepared from D-gossypol and D-phenyalanine methyl ester, in deuteriochloroform, in diffuse light by ^{1}H NMR. This diastereoisomer gives a doublet at δ9.36 (J=12Hz) for the enamine CH, due to coupling to the NH. In addition, there is a singlet at δ5.35 due to one of the hydroxyls, presumably that at the 1(1') position. Upon standing in diffuse light, a further doublet appears at δ9.24 and a singlet at δ5.3 for the corresponding protons in the L-gossypol, D-phenylalanine diastereoisomer. After 48 hours equilibration to a 50:50 mixture of diastereoisomers is complete. Upon standing for longer than 48 hours the diastereoisomers bein to decompose and other, unidentified, products appear. We have confirmed that epimerisation is taking place at the gossypol 2,2'-bond by separating the diastereoisomeric mixture obtained after 48 hours, hydrolysing the diastereoisomers back to the gossypol enantiomers and reforming the diastereoisomers with L-phenylalanine methyl ester. H.p.l.c. of the mixture showed the presence of the L-gossypol, L-phenylalanine methyl ester and D-gossypol, L-phenylalanine methyl ester diastereoisomers. No equilibration occurs in the absence of sunlight and all of the other individual diastereoisomers undergo identical photo-equilibration to 50:50 mixtures. This epimerisation presumably arises, in part, due to the bathochromic shift of the peak at longest wavelength in the u.v./visible spectrum

of gossypol upon formation of the diastereoisomers (λ_{max} 367nm for gossypol and 392nm for diastereoisomer).

We have studied the epimerisation of the D-gossypol, L-phenyalanine methyl ester (D, L) diastereoisomer at 350nm, in deuteriochloroform, in more detail and the results are shown in Figures 1 and 2 and Table 1. Figure 1 shows a plot of the δ9.0-9.5 region of the ^{1}H NMR spectrum against time. As time increases, the doublet at δ9.24 decreases in size whilst that at δ9.36 [due to the L, L-isomer] increases. The ratios of the integrals for the enamine doublets and hydroxyl singlets are given in Table 1 and the % D, L-diastereoisomer in the mixture (from the average of the ratios) is plotted against time in Figure 2.

Table 1 Ratio of L-gossypol, L-phenyalanine methyl ester diastereoisomer to D, L from irradiation at 350nm from ^{1}H NMR.

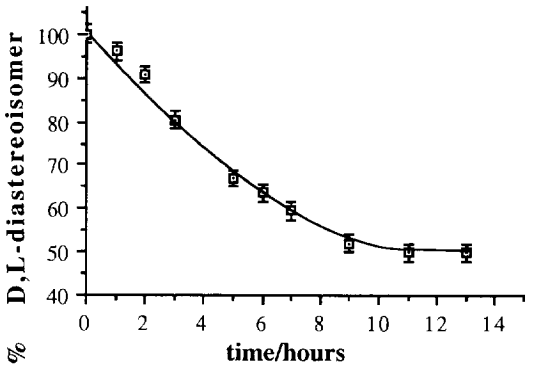
time/hours	ratio of L, L to D, L from CH integral[a]	ratio of L, L to D, L from OH integral[b]	average ratio
0	0:100	0:100	0:100
1	5.2:94.8	2.7:97.3	3.95:96.05
2	9.9:90.1	8.5:91.5	9.2:90.8
3	20.5:79.5	18.7:81.3	19.6:80.4
5	33.5:66.5	32.4:67.6	32.95:67.05
6	34.8:65.2	38.0:62.0	36.4:63.6
7	41.7:58.3	39.1:60.9	40.4:59.6
9	47.9:52.1	*c*	47.9:52.1
11	50:50	*c*	50:50
13	50:50	*c*	50:50

a Measured from integrals of doublets at δ9.36 and 9.24.
b Measured from integrals of singlets at δ5.35 and 5.3.
c Not measureable due to broadening of peaks.

Figure 1 Plot of δ9.0-9.5 region of ^{1}H NMR spectrum of mixture of diastereoisomers against time

The equilibration of partially pure diastereoisomers **4** of gossypol and *S*-1-methylphenethylamine has been briefly reported by Huang *et al*[15] although no mention

was made of the conditions employed except that the rate of equilibration was increased by free radical initiator and light.



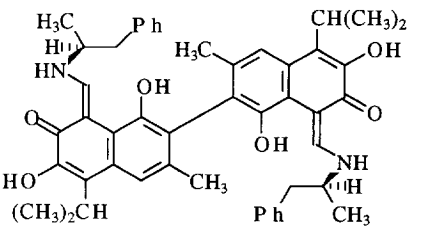


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Figure 2 Plot of % D,L-diastereoisomer against time

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