Resolution of Racemic Gossypol

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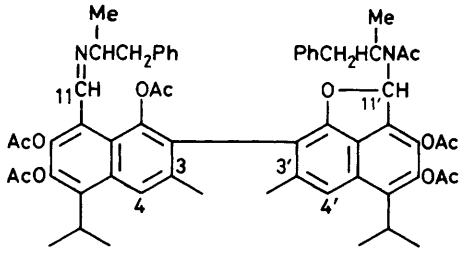
Racemic gossypol, a potential antifertility agent has been resolved through its derivatives with chiral 1-methylphenethylamine into (+)- and (−)-gossypol.

Recently attention has been paid by scientists to the resolution of (±)-gossypol, the potential antifertility agent,[1] for the purpose of comparing the biological action of (−)-gossypol with its naturally occurring antipode.[2] The first successful resolution accomplished by chromatographic separation of the hexa-acetates of the condensation products of (±)-gossypol with (*S*)-1-methylphenethylamine followed by acid hydrolysis of the two main hexa-acetate (**1a**), $[\alpha]_D$ +577.8° (*c* 0.194, $CHCl_3$), and (**1b**) $[\alpha]_D$ −343.7° (*c* 0.135, $CHCl_3$), to give (+)- and (−)-gossypol with identical m.p. (166—167 °C) and $[\alpha]_D$ values: +359° (*c* 0.054, $CHCl_3$) and −353° (*c* 0.056, $CHCl_3$), respectively, was reported in a previous paper.[3]

Treatment of racemic gossypol with either (*R*)- or (*S*)-methylphenethylamine, afforded yellow solids with m.p. 197—200 °C and *m/z* 753 $(M + 1)^+$. The product is a mixture of two diastereoisomers in a 1 : 1 ratio (*RR* and *RS* or *SR* and *SS* depending on the chirality of amine used). Equilibration between these two isomers was observed on two dimensional t.l.c. The specific rotations of the mixture of diastereoisomers obtained from the (*R*) and (*S*) amino derivatives are opposite in sign, −220° ± 3° and +220° ± 3° respectively.

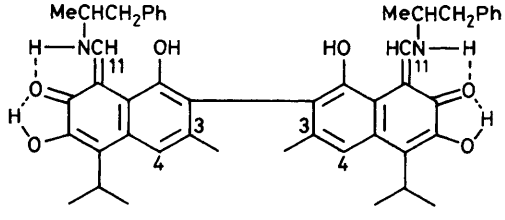
The condensation products exist in a symmetrical keto-enamine structure (**2**) in $CDCl_3$ instead of the imine form as indicated by the n.m.r. spectrum of the diastereoisomeric mixture of the (*S*)-amino derivative of (±)-gossypol. The n.m.r. spectrum of the diastereoisomeric mixture was used instead of the single isomer which equilibrated in solution to the diastereoisomeric mixture. The ^1H n.m.r. spectrum (90 MHz, $CDCl_3$) showed peaks at δ 1.40 (2 × 3H, d, *J* 7 Hz, $-CHCH_3$), 1.53 [4 × 3H, d, *J* 7 Hz, $(CH_3)_2CH$-], 2.09 (2 × 3H, s, CH_3Ar-), 2.93 (2 × 2H, d, *J* 6 Hz, $-CH_2Ar$), 3.71 [4 × 1H, m, 2 $-HC(CH_3)_2$ and 2 -*H*C-N-], 7.18 (2 × 5H, m, Ph), and 7.54 (2 × 1H, s, 4-H). On adding D_2O three of the remaining peaks at δ 5.38 (2H, s), 8.0 (2H, br.), and 13.50 (2H, br.) disappeared and were assigned to the four OH and two NH protons. Also, at δ 9.59 three broad peaks corresponding to 2H were observed. At 200 MHz they appeared as two doublets at δ 9.52 (*J* 12 Hz) and 9.44 (*J* 12 Hz) which changed to two singlets at δ 9.39 (1H) and 9.46 (1H) on addition of D_2O,



(1)

a; (*S*)-Amino-(+)-gossypol

b; (*S*)-Amino-(−)-gossypol



(2)

a; (*S*)-Amino-(+)-gossypol

b; (*R*)-Amino-(−)-gossypol

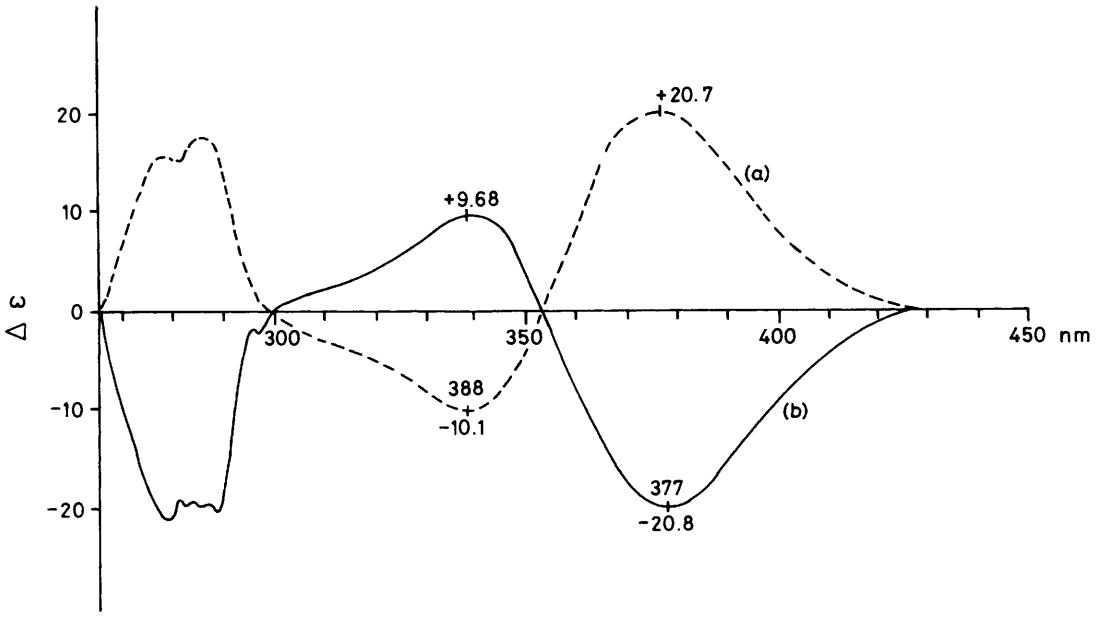


Figure 1. C.d. curves of (a) (+)-gossypol and (b) (−)-gossypol in $CHCl_3$.

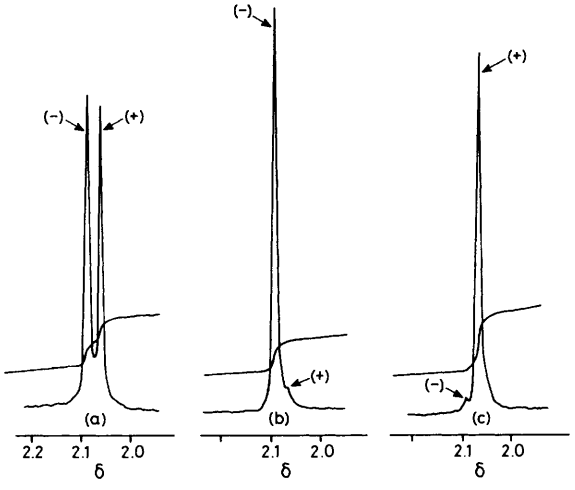


Figure 2. Aromatic methyl resonances of gossypol in the ^1H n.m.r. spectrum in $CDCl_3$ containing (+)-1-methylbenzylamine; (a) (±)-gossypol, (b) (−)-gossypol, and (c) (+)-gossypol.

indicating coupling of the =CH with a neighbouring NH proton. This suggested the presence of a keto-enamine group (=CH–NH) and these peaks were assigned to the amino vinylic protons of two diastereoisomers of ketonic structure. The *J* value found here was in agreement with Brown's[4] suggestion for the protons of the =CH–NH group in Schiff bases of the 2-hydroxynaphthaldehyde series, which has also been studied by Dudek.[5] This structure was further supported by the appearance of signals at δ 172.87 (C=O) and 162.52 (C-11, doublet in the off-resonance ^1H-decoupled spectrum) in the ^{13}C n.m.r. spectrum of compound (**2**), and the keto-enamine structure of certain amine condensation products of gossypol have also been proposed by Biktemirov *et al.*[6]

Because of the labile property of the diastereoisomers, separation by fractional crystallization and centrifugal chromatography was unsuccessful. The complexity of hexaacetylating the products[3] rendered our previous separation impractical. A simplified and more practical method being developed is described below. By quick chromatography of the condensation products of either the (*S*) or (*R*) amine with (±)-gossypol on a silica column with diethyl ether–light petroleum as eluant, pure compound (**2a**) with $[\alpha]_D^{16}$ + 930 ± 20° (*c* 0.127, $CHCl_3$) from the (*S*)-amine derivative and pure (**2b**) with $[\alpha]_D^{21}$ −944 ± 20° (*c* 0.545, $CHCl_3$) from the (*R*)-amine derivative were obtained from the early fractions. In the later eluates less pure diastereoisomers ($[\alpha]_D$ 350—459°) were found. Acid hydrolysis of (**2a**) and (**2b**) yielded respectively (+)- and (−)-gossypol with m.p. 185 ± 3 °C and $[\alpha]_D^{15}$ +376° (*c* 0.115, $CHCl_3$) and $[\alpha]_D^{23}$ −377° (*c* 0.116, $CHCl_3$) after recrystallization (c.d. curves, Figure 1). Hence compound (**2a**) and (**2b**) were assigned as (*S*)-amino-(+)-gossypol and (*R*)-amino-(−)-gossypol respectively. The gossypols thus obtained contained less than 5% of their antipodes as estimated by n.m.r. spectra of their $CDCl_3$ solutions containing (+)-1-methylbenzylamine (Figure 2).

The (±), (+), and (−) isomers of gossypol, although different in their antifertility action, unexpectedly demonstrated the same degree of inhibitory activity against the proposed specific target enzyme, lactate dehydrogenase-X for the antifertility action of (±)-gossypol.[7] Comparison of the antifertility activity of the three isomers in male rats revealed that (−)-gossypol in half of the dose of the (±) isomer showed comparable activity with the latter, while the (+)-gossypol proved to be inactive.

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