A Rapid Procedure for the Resolution of Racemic Gossypol

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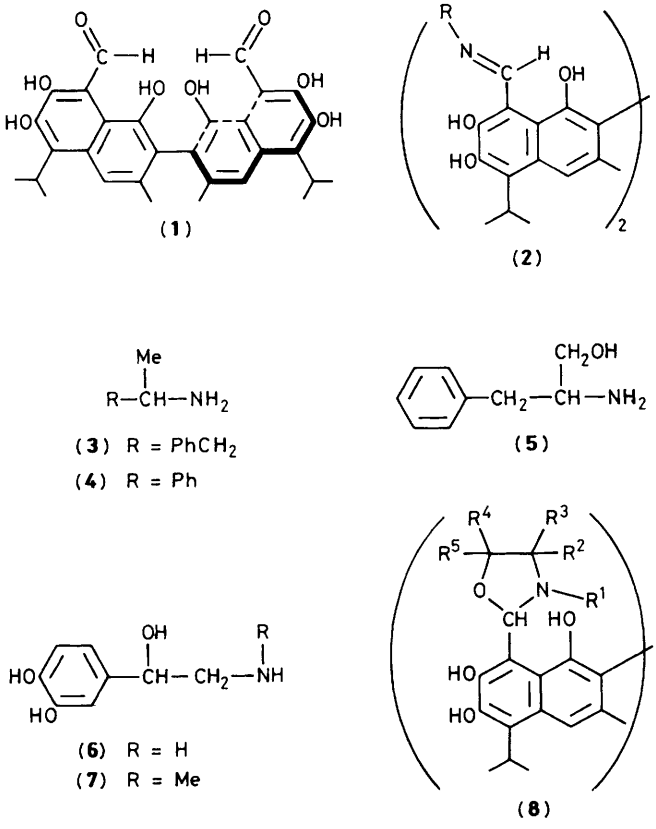
Racemic gossypol has been resolved rapidly and efficiently by h.p.l.c. separation of diastereoisomeric adducts formed with the β-amino alcohol, L-phenylalaninol, on an achiral, reverse-phase C_{18} column.

Gossypol (**1**), a polyphenolic binaphthyl, is a male antifertility agent of considerable current interest.[1] Racemic (±)-gossypol is abundantly available from cotton seed,[2] while optically active (+)-gossypol can be conveniently isolated from *Thespesia populnea*.[3] The (+)-enantiomer has been shown to be inactive as an antifertility agent,[4] suggesting that the male contraceptive properties of gossypol reside in the (−)-enantiomer, which has thus far not been isolated from natural sources.[5] Two recent procedures have been reported for the optical resolution of the racemic pigment.[6,7] The method of Zheng *et al.* involves resolution of a diastereoisomeric mixture of condensation products of gossypol, (**2**), with (*R*)- or (*S*)-methylphenylethylamine, (**3**), on a silica gel column.[6] A symmetrical ketoenamine tautomeric form of the Schiff base has been proposed in solution for the adducts. In this procedure, limited chromatographic resolution requires independent separation of the products derived from the (*R*)- or (*S*)-amine, in order to isolate both gossypol enantiomers. Further, racemization of the chiral amine in the adducts results in equilibration of the diastereoisomers.[6] In the procedure of Matlin *et al.*, separation of the (*R*)-(+)-phenylethylamine (**4**) Schiff base of gossypol is effected on a chiral reverse phase h.p.l.c. column.[7]

We describe a rapid, efficient resolution of gossypol using the readily available chiral β-amino alcohol, L-phenylalaninol (**5**).[8] The diastereoisomeric gossypol adducts derived from (**5**) are readily separated by h.p.l.c.† The adducts are readily prepared by permitting a (±)-gossypol–L-phenylalaninol mixture in methanol (1 : 2 to 1 : 4) to stand at room temperature in the dark for *ca.* 5 h. Quantitative conversion to the adducts is achieved as evidenced by h.p.l.c. (absence of the free gossypol peak at *ca.* 4 min under the conditions described in the footnote) and n.m.r. spectroscopy (disappearance of the aldehyde proton resonance at δ 11.12 and the appearance of two new resonances at δ 9.46 and 9.55, corresponding to the diastereoisomeric species). Baseline resolution is achieved on an achiral reverse phase system. Hydrolysis of the isolated adducts with 2 M HCl, followed by extraction into light petroleum, yielded the individual gossypol enantiomers. The gossypol recovery was *ca.* 50—60%, as estimated from the pure enantiomer fractions. Figure 1 shows the c.d. spectra of the (+)- and (−)-forms of gossypol obtained by this method. $[\alpha]_D$ Values of +373° (*c* 0.468, $CHCl_3$) (lit. $[\alpha]_D$ +376°) and −376° (c 0.376, $CHCl_3$) (lit. $[\alpha]_D$ −377°) were obtained for the (+)- and (−)-forms, respectively. Characterization was also



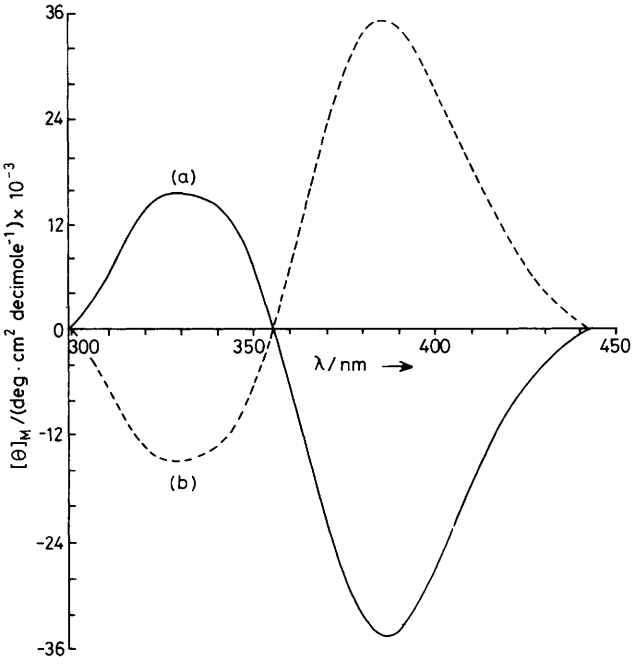


Figure 1. Partial c.d. spectra of (a) (−)-gossypol and (b) (+)-gossypol in methanol.

† Separated using an LKB system with a 4 × 250 mm Lichrosorb RP-18 column (particle size 10 μm). Retention times 9.4 and 12.8 min for the (−)- and (+)-isomers respectively, of the L-phenylalaninol adduct of gossypol, using a 90—95% methanol–water gradient over 15 min. Flow 0.8 ml min^{-1} with detection at 365 nm. Injection volume 20 μl, concentration 50 mg ml^{-1}.

effected by 270 MHz ^{1}H n.m.r. spectroscopy. The excellent chromatographic resolution permits loading of as much as 5 mg of the adduct on an analytical h.p.l.c. column (4 × 250 mm) using an injection volume of 20 μl. Scaling up of this separation may be readily achieved. The present procedure may be compared with loading factors of 120 mg per run (injection volume 1 ml) on a preparative chiral column reported previously.[7]

Resolution of (±)-gossypol has also been effected using (−)-norepinephrine (**6**) and (−)-epinephrine (**7**). However, the gossypol adducts of (**6**) and (**7**) are much less soluble in organic solvents compared with the adduct of (**5**). In the procedures reported previously,[6,7] racemization at the chiral centre on the amine is likely, when the asymmetric atom is adjacent to the aldimine function. Further conjugation to another aromatic group, as in (**4**), may be expected to facilitate configurational inversion. Presumably in procedures using (**4**), separation on a chiral stationary phase[7] is necessary because of rapid diastereoisomer equilibration. In the case of the β-amino alcohols used in this study, Schiff base formation is not expected with secondary amines such as epinephrine (**7**). The observation of stable adducts with (**7**) and the similarity of the spectral (n.m.r., u.v., c.d.) properties of the adducts of (**5**), (**6**), and (**7**) suggest that the species formed may be an oxazolidine[9] [(**8**), where R^1–R^5 refer to substituents appropriate to structures derived from (**5**), (**6**), and (**7**)].

Received, 10th December 1985; Com. 1744

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