ISOLATION OF (+)-GOSSYPOL FROM *MONTEZUMA SPECIOSISSIMA*

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In previous studies on the male fertility-regulating agent gossypol, we have shown that the in vivo bioactive constituent of the racemate is probably the (−)-isomer (1). Like others (2), we have therefore sought (−)-gossypol from natural sources, as well as through the preparative resolution of the racemic form; some success in this area has recently been described by Chinese workers (3).

It had been reported earlier (4) that gossypol was an antitumor constituent of the Puerto Rican plant *Montezuma speciosissima* Sessé & Moç. ex. DC. (Malvaceae) [syn. *Thespesia grandiflora* DC. and *Maga grandiflora* (DC.) Urban] (5,6). However, the optical activity of the isolate was not reported. This is an important determination inasmuch as other members of the Malvaceae have been reported to contain racemic and (+)-gossypol (7-12). In this communication, we show that *M. speciosissima* produces (+)-gossypol.

EXPERIMENTAL

PLANT MATERIAL.—Bark, stem, and voucher specimens of *M. speciosissima* were collected in Puerto Rico near Manatí, March 23, 1983. The collaboration of Dr. A.H. Liogier, Botanic Garden, University of Puerto Rico, San Juan, Puerto Rico, in the collection of the plant material is gratefully acknowledged. Voucher specimens have been deposited at the herbarium of the Field Museum of Natural History. Chicago, Illinois, and at the Herbarium of the Royal Botanic Gardens, Kew, Surrey, England.

ISOLATION OF (+)-GOSSYPOL.—The dried, powdered bark and wood (72 g) were exhaustively extracted in a Soxhlet with petroleum ether (40-60°) at 40°, changing the solvent every 4 h until the extracts were colorless. The marc was extracted with Et_2O at 35°, with stirring for 24 h, then finally with Me_2CO. The three extracts were concentrated in vacuo at 35°, and comparative tlc performed on silica gel eluting with $CHCl_3$-EtOAc-HCOOH (18:2:1). Standards of (±)- and (+)-gossypol were used, visualizing with acidified phloroglucinol in EtOH. The petroleum ether and Et_2O extracts gave the same Rf's as the gossypol standards and were crystallized and recrystallized from C_6H_6-petroleum ether (4:1) affording yellow crystals (10 and 5 mg, respectively) of gossypol (isolated yield 0.02%).

Both samples gave $[\alpha]^{25}D$ +372° (*c* 0.067, $CHCl_3$), and the spectral data were compared with authentic (+)-gossypol isolated from *Thespesia populnea* in our laboratory and with literature values (11). Since (±)- but not (+)-gossypol forms an insoluble complex with glacial HOAc, the two crystalline samples and their mother liquors were separately dissolved in $CHCl_3$ and treated with glacial HOAc. No yellow precipitate was deposited after 2 h. On reextracting with petroleum ether (40-60°), the $[\alpha]D$ of the crystalline samples remained at +372°, indicating that no stereo conversion to (±)-gossypol occurred or that (±)-gossypol was present in the mother liquor at extremely low levels.

Hplc of the concentrated petroleum ether and Et_2O extracts (before recrystallization) and the Me_2CO extract, performed by solubilizing the extracts in acetonitrile and running on C_{18} μ-Bondapak column with mobile phase CH_3CN-H_2O-HOAc (75:24:1), flow rate 1.5 ml/min and uv detection at wavelength 254 nm, indicated the absence of gossypol in the Me_2CO extracts, but an overall level of 0.04% for the petroleum ether and Et_2O extracts. The gossypol peak gave a k′ of 2.4 for a retention time of 6.6 min. This was identical to the values for racemic and (+)-gossypol. Further identification was afforded by spiking with authentic (+)-gossypol and uv spectral analysis of the suspected gossypol peak.

ACKNOWLEDGMENTS

This work was supported in part by a grant from the Special Programme of Research, Development and Research Training in Human Reproduction, World Health Organization (Project HRP 77918C).

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Received 10 August 1984

CONSTITUENTS OF *RUDBECKIA SEROTINA*

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Rudbeckia serotina Nutt. (Compositae) is a folk medicinal herb (1,2) that has not been chemically investigated. We report here that the CH_2Cl_2 extract of the aerial parts of *R. serotina* contains the flavonols 3,3′,4′,5-tetrahydroxy-6,7-dimethoxyflavone (eupatolitin) (3), 3,4′,5-trihydroxy-6,7-dimethoxyflavone (eupalitin) (3), and 3,4′,5-trihydroxy-3′,6,7-trimethoxyflavone, along with sodium salicylate. 3,4′,5-Trihydroxy-3′,6,7-trimethoxyflavone has been synthesized (4) but, to our knowledge, has not previously been reported from natural sources (5). It is interesting to note that the flavonol constituents of the closely related species *Rudbeckia hirta* (6,7) are not identical (8) to the *R. serotina* flavonols.

A standard work-up of *R. serotina* for alkaloids yielded a trace amount of a basic fraction which was a complex mixture of components according to tlc analysis. This alkaloid fraction remains to be investigated.

EXPERIMENTAL

PLANT MATERIAL.—Collections were made along eastbound I-24 at the Nickajack Dam exit, 20 miles west of Chattanooga, TN. The plant was identified by Dr. Gene S. Van Horn, Department of Biology, UTC, and a voucher specimen (Rs-ND61572-TGW) is filed in the UTC herbarium.

EXTRACTION AND ISOLATION.—Air-dried, powdered aerial parts of *R. serotina* (2.56 kg) were exhaustively extracted with CH_2Cl_2 in a Soxhlet apparatus. The concentrate (56.7 g) was triturated with hot petroleum ether, and the petroleum-ether-insoluble fraction was treated with hot EtOH-H_2O, 1:3. The clarified aqueous solution was repeatedly extracted with CH_2Cl_2 to give the final extract (8.96 g). Chromatography of this material on silica gel gave, in order of elution, 3,4′,5-trihydroxy-3′,6,7-trimethoxyflavone (3 mg) (4), eupalitin (6 mg) (3), and eupatolitin (81 mg) (3). Rechromatography of the eupatolitin mother liquors gave sodium salicylate (3 mg).

Full details of isolation and identification of compounds are available on request from the senior author. All compounds were identified by standard spectral data, derivative preparation, and direct comparison with authentic samples.