The taurine-conjugated acids are strongly bound even at low pH and in the presence of 0.1 *M* chloride. The glycocholic acid binding is strongly pH dependent, and effective binding is only obtained at low pH in the absence of competing chloride ion. However, previous work (9) showed that much more glycocholate is bound by cholestyramine at higher pH (5.4–6.8), even in the presence of 0.15 *M* sodium chloride or sodium carbonate. Under these conditions, the glycocholate is present almost entirely in the dissociated anionic form.

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Tumor-Inhibitory Agent from *Montezuma speciosissima* (Malvaceae)

S. D. JOLAD, R. M. WIEDHOPF, and J. R. COLEˣ

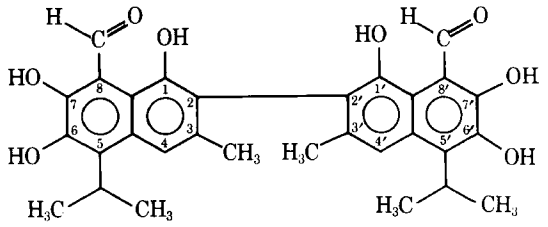
Abstract □ The petroleum ether extract of *Montezuma speciosissima* Sesse and Moc. demonstrated tumor-inhibiting properties in the P-388 lymphocytic leukemia test system (3PS). The constituent responsible for this activity was shown to be a symmetrically substituted 2,2′-dinaphthol, identified as gossypol ($C_{30}H_{30}O_8$).

Keyphrases □ *Montezuma speciosissima*—extract, PMR, UV, IR, and mass spectra, gossypol isolated □ Gossypol—isolated from *Montezuma speciosissima*, PMR, UV, IR, and mass spectra, tumor inhibitory activity □ Tumor inhibition—gossypol, isolated from *Montezuma speciosissima*, PMR, UV, IR, and mass spectra

As a result of the continuing search for plants yielding tumor-inhibiting constituents, it was found that the petroleum ether extract of the woody stems and stem barks of *Montezuma speciosissima* Sesse and Moc. (Malvaceae)[1] showed inhibitory activity toward the P-388 lymphocytic leukemia test system (3PS)[2].

DISCUSSION

The ether-soluble portion of the petroleum ether extract, after mild alkali extraction followed by careful acidification, gave a product which, when subjected to silica gel column chromatography, yielded a brilliant golden-yellow compound. This compound was characterized as 1,1′,6,6′,7,7′-hexahydroxy-5,5′-diisopropyl-3,3′-dimethyl[2,2′-binaphthalene]-8,8′-dicarboxaldehyde, identical to gossypol (I) in all respects. This identity was demonstrated by



I

means of its melting point, mixed melting point, elemental analysis, PMR, mass spectrometry, and comparison of UV and IR spectra with authentic sample spectra. Gossypol, the principal yellow pigment of cottonseed, was isolated first by Marchlewski (1) and then synthesized by Edwards (2). The structure of gossypol has been extensively studied by various workers (3).

The petroleum ether extract demonstrated activity of 131% test/control (T/C) at 50 mg/kg, 136% T/C at 25 mg/kg, and 125% T/C at 16 mg/kg. Gossypol demonstrated activity of 150% T/C at 10 mg/kg and 129% T/C at 5 mg/kg in the 3PS test system. Activity in the 3PS system is defined as an increase in the survival of treated animals over that of controls resulting in a T/C ≥ 125% (4).

EXPERIMENTAL[3]

The woody stems and stem barks (10 kg) of *M. speciosissima* were ground and extracted exhaustively in a Lloyd-type extractor with petroleum ether. After removal of the solvent in air, the residue (69 g) was repeatedly extracted with ether and filtered. The

[1] Identification was confirmed by Dr. Robert E. Perdue, Medicinal Plant Resource Laboratory, Plant Genetics and Germ Plasm Institute, Beltsville, Md. A reference specimen was deposited in that herbarium. The plant was collected in Puerto Rico in February 1972.

[2] Of the Division of Cancer Treatment, National Cancer institute, National Institutes of Health, Bethesda, Md.

[3] Carbon and hydrogen analyses were performed by Chemalytics, Inc., Tempe, Ariz. PMR, IR, and mass spectra were determined using a Varian T-60 spectrometer, a Beckman IR-33, and a Hitachi Perkin-Elmer double-focusing spectrometer (model RMU-6E), respectively. The melting points were determined on a Kofler hot-stage apparatus and are uncorrected.

combined ether solution (about 2.5 liters) was subjected to mild alkali extraction (5% aqueous sodium carbonate). The alkaline phase was carefully neutralized with 10% aqueous hydrochloric acid, extracted with ether, and dried, and the solvent was removed *in vacuo*.

The resulting product (26 g) was then subjected to silica gel (130 g) column chromatography. The column was eluted with hexane–ether (4:1) followed by ether, acetone, and methanol. Fractions from the hexane–ether eluate, which showed two spots on TLC, were combined, and the solvent was removed *in vacuo*. The residue was treated with an excess of acetone, stirred, and filtered. The filtrate, on concentration *in vacuo*, gave a buff-colored precipitate, which was removed by filtration. On further concentration followed by cooling, the filtrate gave tiny yellow needles of gossypol, which were collected by filtration. More crystals were obtained from the mother liquor by repeating this step.

Crystallization from methylene chloride–petroleum ether yielded pure gossypol in long brilliant golden-yellow needles, mp 178–180°; this melting point was not depressed by mixing with an authentic sample[4]. Several melting points have been reported for gossypol: 184° (5), 199° (6), and 214° (7); gossypol exists in three crystalline modifications, each with a characteristic melting point.

All spectra obtained by UV, IR, PMR, and mass spectrometry agreed with literature data (8–10).

[4] The authors are grateful to the Division of Drug Research and Development, National Cancer Institute, Bethesda, Md., for providing an authentic sample.

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COMMUNICATIONS

Isolation of Lysergide (LSD) with Agarose-Bound Antibodies to Lysergic Acid

Keyphrases □ Lysergide—isolation, agarose-bound antibodies to lysergic acid, column chromatography □ Antibodies—lysergide binding, coupled to agarose, isolation of lysergide

To the Editor:

Several research groups (1–3) have obtained antiserums specific to lysergide (D-lysergic acid diethylamide, LSD). These antiserums have been useful in the development of sensitive and specific radioimmunoassay procedures for lysergide in plasma and urine (2, 3).

We have now succeeded in purifying lysergide-binding antibodies from sheep antiserum, utilizing affinity chromatographic (4) columns prepared by covalent binding of D-lysergic acid to a suitable derivative of beaded agarose. Purified specific antibodies are themselves coupled to beaded agarose and subsequently utilized for the selective absorption of lysergide from solution. Elution of lysergide from the column is readily achieved.

D-Lysergic acid (I) was readily coupled to the long chain hydrazide derivative of beaded agarose (II) (5) (Scheme I). To 10 g of II was added 40 mg of D-lysergic acid dissolved in 32 ml of saline–phosphate buffer (0.15 *N* NaCl and 0.01 *M* phosphate, pH 7.4) containing 5% ethanol. Then 50 mg of water-soluble carbodiimide reagent (III) was added, and the pH was adjusted to 5.50. After stirring for 24 hr, the agarose was washed thoroughly with buffer and made up to a final volume of 20 ml. The washings were analyzed for uncoupled D-lysergic acid by UV absorbance at 308 nm and the resultant affinity adsorbent (IV) was calculated to contain 2.5 μmoles (0.67 mg) of D-lysergic acid/g.

To a column of IV (1.5 ml, 0.5 × 15 cm, packed in pH 7.4 buffer) was added 1.0 ml of undiluted lysergide antiserum obtained from sheep after immunization with a lysergic acid–human serum albumin conjugate (3). [This antiserum (10 μl) showed the ability to bind 50% of 1 ng of tritium-labeled lysergide at a dilution of 1:1000 (3).] One hour was allowed for equilibration prior to elution from the column. Both protein absorbance (280 nm) and ability to bind tritiated lysergide in the radioimmunoassay system were monitored.