Phytochemical Investigation of the Flowers of *Cassia reticulata* Willd. (*Leguminosae*)

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A study of the flowers of *Cassia reticulata* has been made. By means of polyamide column chromatography, two anthraquinones, rhein and aloe-emodin, as well as a sterol, β-sitosterol, were isolated and identified.

ALTHOUGH VARIOUS SPECIES of *Cassia* have been investigated extensively as to their phytochemical composition, relatively little is known of the constituents of *Cassia reticulata* Willd. (*Leguminosae*). Cassic acid, a substance possessing antibiotic properties (1), and later identified as the anthraquinone rhein (2), has previously been isolated from the leaves of this plant.

Flavonoids (3, 4), anthraquinones (5), a sterol (5), and alkaloids (6) have been isolated from several species of this genus. A preliminary investigation of a small sample of *C. reticulata* flowers by means of thin-layer chromatography, indicated the presence of flavonoids and anthraquinones. Extraction and fractionation (Fig. 1) of a large sample of the coarsely milled flowers yielded an ethyl acetate fraction which contained the majority of compounds of interest, as evidenced by thin-layer chromatography on Silica Gel G plates. Column chromatography of this fraction on polyamide yielded rhein, aloe-emodin, and β-sitosterol.

EXPERIMENTAL

Materials and Methods—The plant material used in this investigation was collected in the area of Iquitos, Peru during February of 1968, and represented the flowers of *Cassia reticulata* Willd. (*Leguminosae*). Voucher specimens have been deposited at the Economic Herbarium of Oakes Ames, Botanical Museum, Harvard University, Cambridge, Mass.

All melting points were taken on a Thomas-Hoover melting-point apparatus and are uncorrected. IR spectra were taken using a Beckman spectrophotometer, model IR-8, and the UV absorption spectra were measured in 95% ethanol using a Beckman spectrophotometer, model DK-2. Thin-layer chromatography (TLC) was performed in most cases on Silica Gel G plates. Preparative TLC was performed on plates (20 × 40 cm.), coated with a 1-mm. thick layer of Silica Gel G, which were subsequently activated at 100° for 1.5 hr.

Preparation of Fractions—The plant material (1.0 kg.) was milled to a coarse powder and extracted in a continuous extraction apparatus with skellysolve B for 108 hr. Solvent was changed every 24 hr. to prevent excessive heating of the extract. All of the skellysolve B extracts were combined and evaporated *in vacuo* to yield 17.1 g. of Fraction A. The marc was removed from the continuous extraction apparatus, spread out to air dry for 24 hr., and repacked into the continuous extraction apparatus for extraction with 95% ethanol. Extraction was continued for 96 hr., with a fresh change of solvent every 24 hr. The combined ethanol extracts, when dried *in vacuo*, yielded 166.1 g. of a dark brown residue. Redissolving this viscous mass in 1.0 l. of ethanol, and refrigerating for 3 days, produced 16.4 g. of a dark greenish-brown residue, Fraction B. The filtrate was evaporated to dryness to give 133.8 g. of residue, to which was added 800 ml. of distilled water. This solution was then heated on a steam bath with frequent stirring for 30 min., cooled, filtered, and the residue on the filter paper dried (Fraction C, 13.0 g.). The filtrate then was extracted with ethyl acetate six times. After this extract was dried *in vacuo*, it produced 9.4 g. of Fraction D. The aqueous phase remaining after the ethyl acetate extractions were completed was labeled Fraction E (see Fig. 1).

Column Chromatography of Fraction D—Two-hundred grams of MN-polyamide powder[1] was washed with water and then methanol until the methanol filtrate, when taken to dryness using a flash evaporator, yielded no visible residue. The powder was then packed to a constant height (42 cm.) in a 5 × 64-cm. glass column. Fraction D (9.4 g.) was dissolved in a minimum volume of methanol and was added to the top of the polyamide column. One-hundred-milliliter fractions were collected. The fractions were eluted with methanol and grouped according to their TLC patterns using ethyl acetate–methanol–water (100:16:12.5) as the developer and 25% aqueous lead subacetate solution as a spray reagent, followed by drying the chromatograms and visualizing under long-wavelength UV light (366 mμ).

Isolation of β-Sitosterol—Fraction grouping 5–14 from the column on concentration yielded 301.8 mg. of a white amorphous powder which did not react with the spray reagent. A Liebermann-Burchard test (7) subsequently indicated this material to be steroidal. TLC, followed by spraying with 70% aqueous H_2SO_4 solution, followed by heating at 110° for 15 min., showed the isolate to be a mixture of three sterols. Preparative TLC, using the ethyl acetate–methanol–water eluent, followed by elution of the bands with methanol, and crystallization of the material from the highest band from 95% ethanol, yielded 5.8 mg. of β-sitosterol, m.p. 134.5–135.5°, lit. m.p. 139–140° (5). The IR spectrum of the isolate was identical with that of reference β-sitosterol.

Isolation of Rhein—Fraction grouping 30–60 from the column yielded 42.6 mg. of a yellow amorphous powder during slow evaporation, which gave a positive Borntrager's test (8, 9). TLC of the isolate, using the ethyl acetate–methanol–water eluent, and followed by spraying with 2 *N* aqueous potassium hydroxide solution, showed the isolate to be a single entity, m.p. 321–324° (dec.). An IR spectrum of the

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[1] Macherey, Nagel & Co., Düren, Germany.

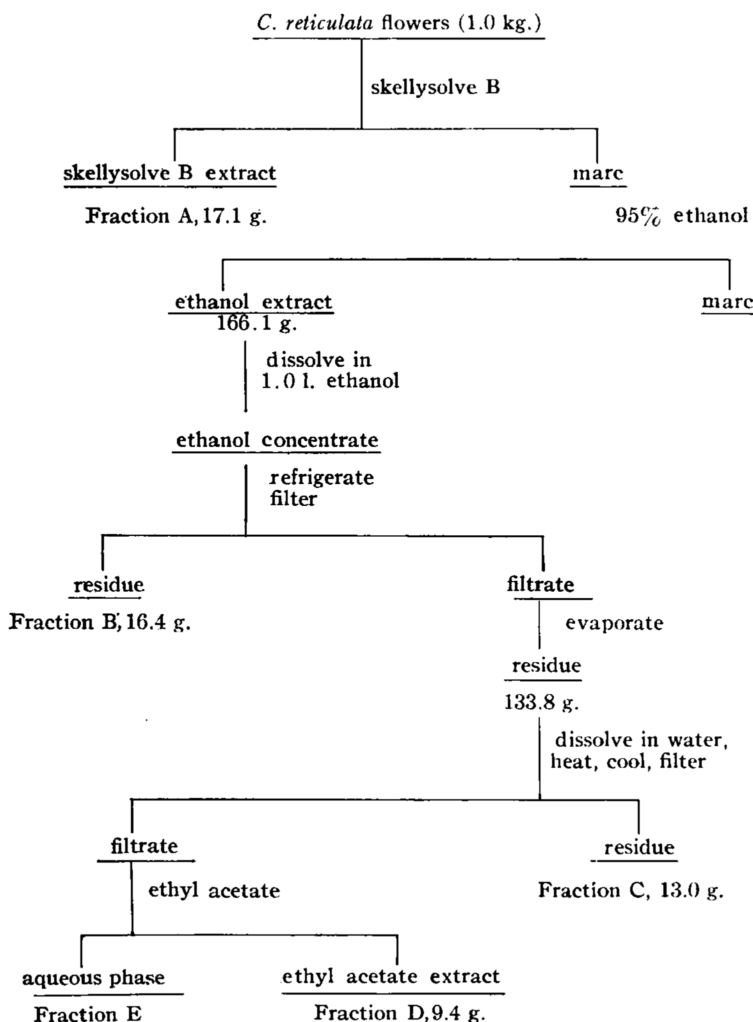

Fig. 1—Extraction and fractionation scheme for C. reticulata *flowers.*

isolate was superimposable with that of reference rhein (4,5-dihydroxyanthraquinone-2-carboxylic acid).[2] UV absorption spectrum, $\lambda_{max.}^{EtOH}$ 229, 258, and 430 mμ (log ϵ 3.8, 3.5, and 3.4), compared favorably with that found in the literature, $\lambda_{max.}^{EtOH}$ 230, 260, and 430 mμ (2). TLC with reference rhein in several different solvent systems showed identical R_f values and chromogenic reactions to the KOH spray reagent. A molecular weight of 284 was confirmed by mass spectrometry.

Fractions 61–110 from the column were combined and slowly evaporated to yield 93.1 mg. of material which was shown by TLC to be a mixture containing mainly rhein, in addition to lesser amounts of a second anthraquinone having a slightly higher R_f value. Column chromatography of this material on a polyamide matrix resulted in an additional crop of pure rhein, but the second anthraquinone could not be separated.

Isolation of Aloe-emodin—The mother liquor from combined Fractions 5–14 from the column, in addition to Fractions 15–29, were combined and taken to dryness to yield 7.4 g. of residue. This was dissolved in distilled water and chromatographed over a column containing 200 g. of MN-polyamide which had been washed several times with distilled water and packed as an aqueous slurry. Elution was initiated with water and 100-ml. fractions were collected. Fractions 1–31 were eluted with water, 32–51 with 10% methanol, 52–67 with 25% methanol, 68–85 with 50% methanol, 86–137 with 75% methanol, 138–212 with methanol, and 213–278 with acetone. The aqueous fractions were frozen and lyophilized, the aqueous methanol fractions were concentrated *in vacuo* to remove methanol, and then were frozen and lyophilized, and the methanol and acetone fractions were taken to dryness *in vacuo*. Each fraction was then dissolved in *ca.* 10 ml. of methanol, followed by TLC to determine those fractions to be combined.

Fractions 113–122 were similar in their chromatographic pattern and were combined. Slow evaporation yielded an orange-yellow product having m.p.

[2] Rhein samples were generously provided by Professor J. W. Fairbairn, University of London, and Professor H. Wagner, Munich, Germany.

220–221°, and weighing 17.9 mg., lit. m.p. for aloe-emodin 223–225° (5). TLC in several different solvent systems showed the isolate to be homogeneous and identical in R_f value with reference aloe-emodin.[3] IR spectra of the isolate and reference aloe-emodin were superimposable, and the UV absorption spectrum for the isolate was $\lambda_{max.}^{EtOH}$ 226, 253, and 287 mμ (log ε 4.2, 3.8, and 3.5).

SUMMARY

A phytochemical investigation of *Cassia reticulata* flowers has resulted in the isolation of rhein, aloe-emodin, and β-sitosterol, which have not been previously reported from the flowers of this plant. At least one additional anthraquinone was observed to be present, in addition to three flavonoids, but these were not isolable using the methods employed in this study.

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[3] A reference sample of aloe-emodin was provided by Professor J. W. Fairbairn.

Keyphrases

Cassia reticulata flowers—phytochemistry
Anthraquinones—isolated, identified
β-Sitosterol—isolated, identified
Column chromatography—separation
TLC—identity
IR spectrophotometry—identity
UV spectrophotometry—identity

Hydroxyindole-*O*-methyltransferase II. Inhibitory Activities of Some *N*-Acyltryptamines

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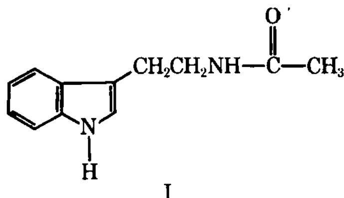
Several *N*-acyltryptamines were found to inhibit hydroxyindole-*O*-methyltransferase. Of these inhibitors the most active was *N*-phenylacetyltryptamine, having an affinity to the enzyme six times greater than the substrate *N*-acetylserotonin. These studies showed that the alkyl and aralkyl groups of the amides were most likely complexed with the enzyme by hydrophobic bonding.

In the previous paper (1), the binding of the substrate *N*-acetylserotonin to the enzyme hydroxyindole-*O*-methyltransferase (HIOMT) was studied. There was a good indication that the CH_3 of the amide I was complexed with the enzyme by



hydrophobic bonding. To explore this hydrophobic region on HIOMT, several substituted *N*-acyltryptamines (II–VIII) were synthesized and their inhibitory activities evaluated (Table I).

The finding that both *N*-cyclobutylcarbonyl-

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TABLE I—INHIBITION OF HIOMT

(Structure: indole with 3-CH_2CH_2—N(R_2)—R_3, R_1 on indole N)

Compound	R_1	R_2	R_3	I_{50},[a] (m*M*)
I	H	H	$COCH_3$	1.40[b]
II	H	H	COC_4H_7	0.88
III	H	H	COC_6H_{11}	0.85
IV	H	H	COC_6H_5	0.37
V	H	H	$COCH_2C_6H_5$	0.18
VI	H	H	$COCH_2CH_2C_6H_5$	0.22
VII	H	CH_3	$COCH_2C_6H_5$	0.16
VIII	CH_3	H	$COCH_2C_6H_5$	0.35
IX	H	H	$SO_2C_6H_5$	0.37

[a] Concentration of an inhibitor giving 50% inhibition of the enzyme. [b] Data from the previous paper (1).