may exist between terphenylquinones and diphenyl-substituted tetronic acid. Although Read *et al.* (9) suggested that hydroxylation of the phenylpropanoid compound occurs prior to the formation of the terphenyl skeleton, it appears reasonable that hydroxylation could occur after dimerization. If atromentin is a precursor to thelephoric acid, this reaction must take place after terphenyl formation. It appears that Compounds I, II, and III are plausible biosynthetic intermediates in the conversion of atromentin to thelephoric acid. Tracer studies are indicated to establish the biogenetic relationship between atromentin and thelephoric acid as they exist in cultures of *C. subilludens.*

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Isolation and Identification of Constituents from *Cudrania javanensis*

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Abstract ☐ Osajaxanthone, vanillic acid, monomethyl fumarate, *p*-hydroxybenzoic acid, and (−)-(*S*)-stachydrine were isolated from extracts of the bark of the tropical shrub *Cudrania javanensis* Trécul (Moraceae).

Keyphrases ☐ *Cudrania javanensis*—isolation and identification of osajaxanthone, vanillic acid, monomethyl fumarate, *p*-hydroxybenzoic acid, (−)-(*S*)-stachydrine ☐ Column chromatography—separation of *Cudrania javanensis* constituents

Cudrania javanensis Trécul (Moraceae) is a climbing shrub or small tree of tropical Asia, Australia, and Polynesia (1). Extracts of the plant have been used to dye cotton yellow (2). The plant is commonly called cockspur thorn in Australia, where it has been suspected of poisoning stock (3). The absence of knowledge of the constituents of *Cudrania* species prompted us to undertake a phytochemical investigation of this plant. The isolation and identification of osajaxanthone (I), vanillic acid, monomethyl fumarate, *p*-hydroxybenzoic acid, and (−)-(*S*)-stachydrine (II) are reported here.

The plant material was moistened with dilute ammonium hydroxide and extracted by percolation to exhaustion with ethanol. The extract residue was partitioned between dilute hydrochloric acid and ether. The ether solution was extracted with dilute potassium hydroxide, washed with water, and set aside. The alkaline solution was acidified and reextracted with ether. Chromatography of this ether extract over silicic acid–Celite (4:1) afforded osajaxanthone (I), vanillic acid, monomethyl fumarate, and *p*-hydroxybenzoic acid. Osajaxanthone was first isolated from the root bark of the Osage Orange [*Maclura pomifera* (Moraceae)] (4) and subsequently from the trunk wood of Brazilian *Kielmeyera corymbosa* (Guttiferae) (5) and the heartwood of Malaysian *Calophyllum scriblitifolium* (Guttiferae) (6). Closely related xanthones from *Maclura* species have been reported to be toxic to goldfish and mosquito larvae (4). The presence of this xanthone may account for the use of *Cudrania* extracts as yellow dyes.

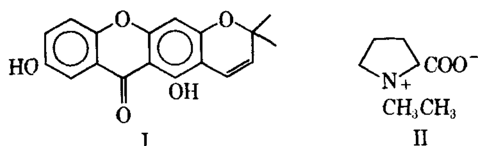
The dilute hydrochloric acid extract was basified with ammonium hydroxide and extracted successively with ether and chloroform. The organic extracts were combined and set aside. The remaining alkaline solution was acidified, and a quaternary alkaloid fraction was collected by precipitation with ammonium reineckate. Treatment of the reineckate complex with an anion-exchange resin in the chloride form afforded a quaternary alkaloid chloride fraction which, upon chromatography over neutral alumina, yielded (−)-(*S*)-stachydrine (II), characterized as the hydrochloride salt.

EXPERIMENTAL[1]

Plant Material—*C. javanensis* (Moraceae) bark[2] was received from Australia and identified at the point of collection. The bark was air-dried and ground to a No. 4 mesh powder.

[1] Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. The UV spectra were obtained on a Perkin-Elmer model 202 recording spectrophotometer, and the IR spectra were determined on a Perkin-Elmer model 257 spectrometer or a Beckman model IR-8 spectrometer in KBr pellets. NMR spectra were recorded in $CDCl_3$ solution on a Bruker Scientific, Inc., model B-90 instrument equipped with a time-averaging computer, with $(CH_3)_4Si$ as the internal standard and chemical shifts reported in δ (p.p.m.) units. Mass spectra were taken with a LKB-9000 mass spectrometer. Optical rotations were measured in a Rudolph polarimeter. All reagents were analytical reagent grade, unless otherwise noted.

[2] Obtained from the Meer Corp., New York, N. Y. (Lot. No. MS 1871).



Extraction and Initial Fractionation—The dried ground bark (3.25 kg.) was moistened with ammonium hydroxide–water (1:10) and extracted by percolation to exhaustion with ethanol. The dark syrupy residue (335 g.) remaining after removal of the alcohol under reduced pressure at 40° was suspended in 2 l. of ether and extracted initially with 3 × 1-l. portions of 5% hydrochloric acid and subsequently with 3 × 1-l. portions of 1% potassium hydroxide. The combined potassium hydroxide fraction was made acidic to pH 2–3 with hydrochloric acid while cooling and was extracted with 3 × 3-l. portions of ether. The resulting dried (anhydrous sodium sulfate) ether fraction, on evaporation under reduced pressure, yielded an oily residue (46.9 g.) of crude acidic substances. The ether layer remaining after extraction with potassium hydroxide solution was washed with water and dried (anhydrous sodium sulfate), and the solvent was removed under reduced pressure to afford a dark oily residue (76.7 g.) of neutral substances.

The combined hydrochloric acid extract was made alkaline (pH 8–9) with ammonium hydroxide and extracted successively with 3 × 2-l. portions of ether and 3 × 2-l. portions of chloroform. The ether and chloroform extracts were combined and dried (anhydrous sodium sulfate), and the solvent was removed under reduced pressure to yield a crude alkaloidal oil (4.2 g.).

The alkaline solution was reacidified to pH 3 with hydrochloric acid and treated with a 2% ammonium reineckate solution until precipitation was complete. The precipitate was filtered by suction, washed with ether, and air-dried to afford a crude quaternary alkaloid reineckate fraction (6.54 g.).

Isolation of (−)-(S)-Stachydrine (II)—The quaternary alkaloid reineckate fraction was suspended in 150 ml. of 50% aqueous acetone and stirred for 5 hr. with 20 ml. of Dowex-1-X4 (Cl) resin[3]. The resin was removed by filtration and washed with 50 ml. of 50% aqueous acetone. The filtrate and the washing were combined and evaporated to dryness to afford a crude quaternary alkaloid chloride residue (6.12 g.). Chromatography of this residue over neutral alumina[4] with methanol as an eluent gave a dark syrup (1.29 g.); on treatment with several milliliters of methanol and enough concentrated hydrochloric acid to afford a distinctly acidic solution, it yielded white plates of (−)-(S)-stachydrine hydrochloride (II) (120 mg.), m.p. 227–229° dec. [lit. (17) 222° dec.], $[\alpha]_D^{28}$ −25.0 (c 0.515, H_2O) {lit. (7) $[\alpha]_D^{22}$ −27.5 [c 2, H_2O]}, identified by comparison (IR, mixed melting point) with an authentic sample. Stachydrine was prepared by treatment of an aqueous solution of (−)-(S)-proline[5] with silver oxide, methanol, and methyl iodide as described by Patchett and Witkop (8) for betonicine [(−)-4-hydroxystachydrine]. Stachydrine hydrochloride, m.p. 228–228.5° dec., $[\alpha]_D^{28}$ −27.0 (c 1.0, H_2O), was prepared by treating an ethereal methanolic solution of the synthetic stachydrine with dry hydrogen chloride gas.

Chromatography of Acidic Fraction—A silicic acid[6]–Celite[7] (4:1) column was packed with 230 g. of the adsorbent and poured as a slurry in benzene. An 18-g. aliquot of the acidic fraction was dissolved in benzene and applied to the column; elution was begun with 1-l. portions of benzene.

Isolation of Osajaxanthone (I)—Evaporation of the fifth benzene fraction and recrystallization of the residue (489 mg.) from hexane–chloroform afforded bright-yellow needles of osajaxanthone (I) (18.6 mg.), m.p. 255–257° dec.; $\lambda_{max.}^{MeOH}$ 206 nm. (log ε 4.04), 232 (4.10), 2.39 (4.10), 248 (4.08), 284 (4.46), 338 (3.73), and 378 (3.49), with a bathochromic shift in methanolic potassium hydroxide and aluminum chloride solutions but no change in methanolic sodium acetate; $\nu_{max.}$ 1645 cm.$^{-1}$ (xanthone C=O). The NMR spectrum showed the characteristic signals of a 2,2-dimethylchrom-3-ene system (9), with a sharp singlet at δ 1.57 for the gem-dimethyl group and a pair of doublets centered at δ 5.61 and 6.69 (J = 10.8 Hz.) for the two *cis*-olefinic protons. The mass spectrum showed a molecular ion peak at m/e 310 for $C_{18}H_{14}O_5$ and a base peak at m/e 295, corresponding to the loss of methyl group from the dimethylpyran ring, leaving a resonance-stabilized pyrilium cation (6). Identification was by comparison of the UV, IR, and mass spectra with an authentic sample and by a mixed melting-point determination.

Isolation of Vanillic Acid—Evaporation of the seventh benzene fraction and recrystallization of the residue (828 mg.) from hexane–chloroform afforded white needles of vanillic acid (14.6 mg.), m.p. 204–206°; $\lambda_{max.}^{MeOH}$ 206 nm. (log ε 4.24), 216 (4.25), 257 (3.98), and 291 (3.67); UV, IR, and mixed melting-point data were identical with an authentic sample[8].

Isolation of Monomethyl Fumarate—Evaporation of the eighth column fraction and recrystallization of the residue (311 mg.) from hexane–chloroform afforded white needles of monomethyl fumarate (30 mg.), m.p. 144–145°; $\lambda_{max.}^{MeOH}$ 208 nm. (log ε 4.28); $\nu_{max.}$ 1718 (C=O, α,β-unsaturated ester), 1685 (C=O, α,β-unsaturated acid), and 1630 cm.$^{-1}$ (C=C, α,β-unsaturated system); UV, IR, mass spectra, and mixed melting-point data were identical with an authentic sample prepared by partial hydrolysis of dimethyl fumarate according to Erlenmeyer and Schoenauer (10).

Isolation of p-Hydroxybenzoic Acid—After further elution of the column with benzene–chloroform mixtures and chloroform, elution with 2% methanol in chloroform yielded a crystalline residue (2.17 g.), which was recrystallized from chloroform–methanol to afford white needles of *p*-hydroxybenzoic acid (310 mg.), m.p. 295–296° dec.; $\lambda_{max.}^{MeOH}$ 208 nm. (log ε 4.21) and 255 (4.09); UV, IR, and mixed melting-point data were identical with an authentic sample[8].

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[4] Woelm, activity grade V, Alupharm Chemicals, New Orleans, La.
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[8] Eastman Organic Chemicals, Rochester, N. Y.