Chemical Constituents of Fruit of *Cocculus carolinus* D.C. (Menispermaceae)

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Abstract □ A phytochemical investigation of an ethanolic extract of the fruit of *Cocculus carolinus* resulted in the isolation and characterization of the alkaloids, cocculolidine and cocculine. The cyclitols (+)-quercitol and (−)-viburnitol and the alkaloids carococculine, magnoflorine, and palmatine, previously reported in the stems and leaves of this species, were also isolated and identified.

Keyphrases □ *Cocculus carolinus* fruit—isolation and characterization of alkaloids and cyclitols, IR, UV, NMR, and mass spectra, TLC □ Cyclitols—isolation and characterization from *Cocculus carolinus* fruit □ Alkaloids—isolated and characterized from *Cocculus carolinus* fruit, IR, UV, NMR, and mass spectra, TLC

Cocculus carolinus D.C. (Menispermaceae) is a species native to the southeastern United States. In previous phytochemical investigations (1, 2), the cyclitols (+)-quercitol and (−)-viburnitol, the lactone loliolide, and the alkaloids carocococculine, magnoflorine, palmatine, and sinoacutine were isolated and identified from the stems and leaves of the species. In this paper the chemical constituents of the fruit of this plant are reported.

An ethanolic extract of the fruit was fractionated into nonquaternary alkaloid, quaternary alkaloid, and acid–neutral fractions. Chromatography of the nonquaternary alkaloid fraction resulted in the isolation and identification of the *Erythrina* alkaloids cocculolidine (I) and cocculine (II). The insecticidal alkaloid cocculolidine (I) was first isolated in 1966 (3, 4) from the fresh leaves of *C. trilobus* D.C., while II was first found in 1950 (5–8) in *C. laurifolius* D.C.

EXPERIMENTAL[1]

Plant Material—The fruit of *C. carolinus* D.C. (Menispermaceae) was used[2].

Extraction—Air-dried ground fruits of *C. carolinus* (3.75 kg) were percolated at room temperature with 35 liters of ethanol. The extract was evaporated *in vacuo* at 40° to leave a dark-brown syrup (1.5 kg).

Isolation of Cyclitols—During the concentration of the ethanol extract, a white crystalline material precipitated (5.3 g). TLC on precoated cellulose plates[3], using a solvent system of acetone–water (4:1) and visualizing with potassium permanganate spray reagent (9), indicated the presence of two components, R_f 0.30 and 0.20. Fractional crystallization from ethanol–water afforded pure (+)-quercitol (350 mg), R_f 0.30, mp 229–230°, $[\alpha]_D^{25}$ + 20.8° (c 1.2, water), and (−)-viburnitol (50 mg), R_f 0.20, mp 177–178°, $[\alpha]_D^{25}$ − 55.0° (c 1.0, water). They were identical (melting point, mixed melting point, IR spectra, and $[\alpha]_D$) with authentic samples.

Fractionation—The fractionation of the concentrated ethanol extract was accomplished as previously reported (Scheme I, Ref. 1) and resulted in the following: acid–neutral fraction (Fraction A, 310.2 g), nonquaternary alkaloid fraction (Fraction B, 5.7 g), and quaternary alkaloid chloride fraction (Fraction C, 2.9 g).

Chromatography of Fraction B—The nonquaternary alkaloid fraction (Fraction B, 5.7 g) was chromatographed over silicic acid[4] (300 g) packed in petroleum ether (bp 60–90°). Elution with methanol–chloroform (8–20%) afforded a purified nonquaternary alkaloid fraction (4.3 g). This fraction was dissolved in chloroform (250 ml) and partitioned with 1% hydrochloric acid (3 × 250 ml). The aqueous acidic solution (750 ml) was made basic (pH 8) with concentrated ammonium hydroxide and extracted with chloroform (3 × 750 ml). The chloroform solutions were pooled, dried over anhydrous sodium sulfate, filtered, and evaporated *in vacuo* at 40° to leave a residue (3.0 g).[1]

A partition system of petroleum ether (bp 60–90°)–ethylene dichloride–methanol–water (12:3:2:0.4) was established as described previously (2). The purified fraction (3.0 g) was placed on the column (150 g, 3.0 × 60 cm), and alkaloidal bands were observed and collected accordingly.

Isolation of Carocococculine—After elution with 1 liter of mobile phase, an alkaloid band was collected (250 ml). Crystallization of this fraction from ethanol afforded carocococculine (15 mg), mp 218–220° dec.; $[\alpha]_D^{25}$ − 30° (c 0.5, chloroform); UV: λ_{max} (methanol) 214 (log ϵ 4.27) and 292 (3.68) nm; mass spectrometry: M^+ *m/e* 345 (100%). It was identical (melting point, mixed melting point, $[\alpha]_D$, and IR and mass spectra) with an authentic sample.

Isolation of Cocculolidine (I)—After elution with 250 ml of additional mobile phase, an alkaloidal band was collected (175 ml). Crystallization of this fraction from petroleum ether (bp 60–90°)–acetone afforded cocculolidine (200 mg), mp 140–141°; $[\alpha]_D^{20}$ + 258.0° (c 1.0, chloroform); UV: λ_{max} (methanol) 214 nm (log ϵ 4.15); IR: ν_{max} (potassium bromide) 1740, 1648, 1090, 1020, and 1005 cm^{-1}; NMR: δ 3.33 (3H) (OCH_3) and 5.65 (1H) (olefin); mass spectrometry: M^+ *m/e* 261 (15%), 230 (8), 203 (100), 174 (40), and 158 (13). This alkaloid was found to be identical (melting point, mixed melting point, $[\alpha]_D$, and UV, IR, and mass spectra) with an authentic sample of cocculolidine[5].

Isolation of Cocculine (II)—After elution with 150 ml of additional mobile phase, an alkaloidal band was collected (200 ml). Crystallization from methanol yielded II (35 mg), mp 207–208° [lit. (5) mp 217–218°]; $[\alpha]_D^{20}$ + 251.0° (c 1.0, chloroform) [lit. (5) $[\alpha]_D$ + 271.1°]; UV: λ_{max} (methanol) 211 (log ϵ 3.76), 223 (sh) (3.65), and 286 (3.19) nm; λ_{max} (0.1 *N* methanolic potassium hydroxide): 214 (log ϵ 3.84), 230 (sh) (3.62), 248 (sh) (3.44), 291 (3.18), and 307 (2.94) nm; IR: ν_{max} (potassium bromide) 3500, 2940, 2870, 1580, 1500, 1300, 1280, and 1090 cm^{-1}; NMR: δ 3.19 (3H) (OCH_3), 5.58 (1H) (olefin), and 6.50–7.00 (3H) (Ar); mass spectrometry: M^+ *m/e* 271 (19%), 256 (3), 240 (26), 213 (76), and 212 (100) [lit. (7) M^+ *m/e* 271 (15), 256 (3), 240 (20), 213 (100), and 212 (70)]. Repeated attempts to obtain a reference sample of this material failed.

Methylation of Cocculine (II)—The base (12 mg) was treated with ethereal diazomethane (20 ml) prepared from *p*-tolylsulfonylmethylnitrosamide[6] (2.14 g) (10) for 7 days. The solution was evaporated *in vacuo* to leave a residue which, following chromatogra-

[1] Melting points were determined on a Thomas-Hoover Uni-melt melting-point apparatus and are corrected. IR spectra were run in potassium bromide, using a Perkin-Elmer 257 spectrophotometer. Optical rotations were determined on a Perkin-Elmer 241 polarimeter. UV spectra were run on a Perkin-Elmer 202 spectrophotometer. NMR spectra were obtained in deuterated chloroform on a Hitachi Perkin-Elmer R-24 spectrometer, with tetramethylsilane as the internal standard. Mass spectra were recorded on an LKB-9000 spectrometer.

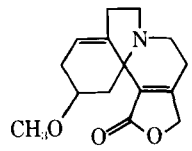
[2] Collected in Oxford, Miss., during the summer of 1973. Voucher specimens are deposited in the herbarium of the School of Pharmacy, University of Mississippi, University, MS 38677

[3] E. Merck, 0.10 mm.

[4] AR, 100 mesh, Mallinckrodt Chemical Works, St. Louis, MO 63160

[5] The authors thank Dr. Kojiro Wada, Nagoya University, Nagoya, Japan, for the reference sample.

[6] Diazald, Aldrich Chemical Co., Milwaukee, Wis.



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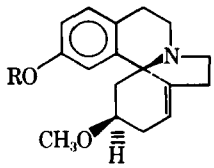

II: R = H
III: R = CH_3

phy over silicic acid[4] (5 g), elution with chloroform, and subsequent crystallization from petroleum ether (bp 60–90°), afforded *O*-methylcocculine (cocculidine) (III) (8 mg), mp 94–96° [lit. (5) mp 84–86°]; $[\alpha]_D^{17}$ + 199.0° (c 0.8, chloroform) [lit. (5) $[\alpha]_D$ + 250.9°]; UV: λ_{max} (methanol) 210 (log ϵ 4.09), 230 (sh) (3.91), 282 (3.37), and 288 (sh) (3.36) nm; IR: ν_{max} (potassium bromide) 2950, 2850, 1600, 1495, 1280, 1230, 1090, and 1040 cm^{-1}; NMR: δ 3.26 (3H) (OCH_3), 3.77 (3H) (OCH_3), 5.60 (1H) (olefin), and 6.65–7.11 (3H) (Ar); mass spectrometry: M^+ *m/e* 285 (10%), 270 (2), 254 (20), 227 (100), 226 (96), and 196 (30) [lit. (7) M^+ *m/e* 285 (15), 270 (3), 254 (17), 227 (100), 226 (55), and 196 (23)].

Chromatography of Quaternary Alkaloids—Fraction C (2.9 g) was chromatographed on silicic acid[4] (75 g) packed in chloroform.

Isolation of Palmatine—Elution with 8% methanol–chloroform afforded a fraction (39 mg) which, following rechromatography on polyamide[7] (25 g) and elution with concentrated ammonium hydroxide–ethanol (1:10), gave a fraction which was passed through a small ion-exchange column[8] (2 g). Evaporation and crystallization from methanol yielded palmatine iodide (5 mg), mp 219–221° dec.; UV: λ_{max} (methanol) 224 (log ϵ 4.65), 267 (4.20), 273 (4.20), 349 (4.22), and 432 (3.55) nm. It was identical (melting point, mixed melting point, and IR and UV spectra) with an authentic sample.

[7] M. Woelm, Waters Associates, Framingham, Mass.
[8] IRA-400 (iodide), Mallinckrodt Chemical Works, St. Louis, MO 63160

Isolation of Magnoflorine—Elution with 16% methanol–chloroform afforded a fraction (400 mg) which was dissolved in 1% hydrochloric acid and filtered. Mayer's (11) reagent was added, and the resulting precipitate was collected, washed with water, and dissolved in acetone–water (1:1) (100 ml). The solution was passed through a small ion-exchange column[8] (10 g). Evaporation and crystallization from methanol afforded magnoflorine iodide (55 mg), mp 251–253° dec.; $[\alpha]_D^{22}$ + 164.0° (c 0.25, methanol); UV: λ_{max} (methanol) 222 (log ϵ 4.67), 272 (4.03), and 311 (3.81) nm; λ_{max} (0.1 *N* methanolic potassium hydroxide) 224 (4.64), 280 (3.88), and 325 (3.91). It was identical (melting point, mixed melting point, and IR and UV spectra) with an authentic sample.

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