# Chemical Constituents of *Curatella americana* (Dilleniaceae)

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**Abstract** □ A phytochemical investigation of an ethanolic extract of the leaves of *Curatella americana* Linn. (Dilleniaceae) resulted in the isolation and identification of the flavonol glycoside avicularin and gallic acid.

**Keyphrases** □ *Curatella americana*—chemical constituents, isolation and identification □ Medicinal plants—*Curatella americana*, chemical constituents, isolation and identification □ Avicularin—isolation from *Curatella americana*, identification □ Gallic acid—isolation from *Curatella americana*, identification

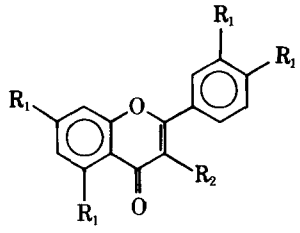
*Curatella americana* Linn. (Dilleniaceae) (1) is a shrub indigenous to Australasia and the tropical Americas (2). The plant has been used in Mexico medicinally for unidentified conditions (3). This use, along with reports of the medicinal and/or toxic potential among other members of the Dilleniaceae (3), and the scarcity of in-depth phytochemical studies on the plant prompted this study.

## DISCUSSION

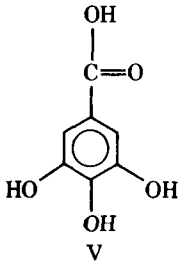
The plant material was extracted by percolation with ethanol to exhaustion. The ethanolic extract was fractionated by successive solvent partitioning and precipitation to yield a fraction that was separated further by chromatography over a polyamide column. Elution of this column with methanol–water afforded avicularin (II) and gallic acid (V).

Compound II was identified by consideration of its physical properties as well as those of derivatives and hydrolysis products. Hydrolysis of II afforded quercetin (I) and L-arabinose. Methylation of II with diazomethane followed by acid hydrolysis afforded 5,7,3′,4′-*O*-methylquercetin (III) and L-arabinose. The identity of III was deduced largely from its mass spectrum, which readily differentiates it from the other tetra-*O*-methylquercetins (4). Peracetylation showed that II was a monoarabinoside.

The NMR spectrum of the peracetate (IV) clearly indicated the presence of phenolic and aliphatic acetate groups in a 4:3 ratio. The mass spectrum of IV showed a weak, but discernible, molecular ion at *m/e* 728, confirming the presence of a single arabinose residue.



I: $R_1 = R_2 = OH$
II: $R_1 = OH$, $R_2$ = L-arabinose
III: $R_1 = OCH_3$, $R_2 = OH$
IV: $R_1 = CH_3COO$, $R_2 = CH_3COO$—L-arabinose



V

The identity of gallic acid (V) was established by comparison of its physical properties with those of an authentic sample.

The presence of a quercetin glycoside in *C. americana* was not unexpected since quercetin (I) was detected chromatographically in *Curatella* species previously (5). Avicularin (II), although not widely distributed in nature, was reported previously in *Polygonum aviculare* var. *buxifolium* (Polygonaceae), *Psidium guaijava* (Myrtaceae), and *Vaccinium myrtillus* (Ericaceae) (6–8). Compound II possesses antibiotic properties (6), which may account for some of the reputed medicinal activity of *C. americana*.

## EXPERIMENTAL[1]

**Plant Material**—The leaves of *C. Americana* (Dilleniaceae) were used[2].

**Extraction**—The air-dried ground plant material (1.56 kg) was extracted by percolation with ethanol (20 liters). The extract was evaporated *in vacuo* at 40° to yield a syrupy extract (508 g).

**Fractionation**—The extract was partitioned between chloroform (2 liters) and water (2 liters). The aqueous layer was partitioned further with ethyl acetate (2 liters), and the solvents were removed from each layer to afford Fractions A (chloroform) (175 g), B (water) (268 g), and C (ethyl acetate) (59 g). Fraction C was dissolved in 100 ml of methanol, and 200 ml of chloroform was added. The resulting suspension was filtered to remove the insoluble matter (8.0 g), and the filtrate was evaporated to a residue. This residue was dissolved in 100 ml of ethyl acetate, and 1 liter of petroleum ether was added to the solution. The resulting precipitate (Fraction D) (9.22 g) was recovered by filtration, and the filtrate was set aside.

**Chromatography**—The precipitated Fraction D was dissolved in methanol (100 ml) and was adsorbed onto 20 g of polyamide[3] by evaporation of the solvent. The dry powder was packed on top of a column (5 × 23 cm, 100 g) of polyamide packed in water. The column was rinsed with 6 liters of water, and elution was carried out first with methanol–water and then with methanol. One-liter fractions were collected.

**Isolation of II**—Elution of the column with methanol–water (1:1) afforded a fraction (2.86 g) from which II (307 mg) was obtained by crystallization from methanol. The compound had a melting point of 224° [lit. (6) mp 217° (dilute ethanol) and 222° (dehydrated)]; $[\alpha]_D^{30}$ −60° (c 0.2, water); UV ($CH_3OH$): $\lambda_{max}$ 215 (log $\epsilon$ 4.36), 258 (4.31), 296 (sh) (3.88), and 357 (4.27) nm; IR (KBr): $\nu_{max}$ 3420–3200 (br), 1650, 1600, 1500, 1450, 1120, 1050, and 1015 $cm^{-1}$; NMR (dimethyl sulfoxide): $\delta$ 5.35 (1H, d, $J$ = 5 Hz), 6.20 (1H, d, $J$ = 2 Hz), 6.40 (1H, d, $J$ = 2 Hz), 6.85 (1H, d, $J$ = 8 Hz), 7.52 (1H, s), and 7.60 (1H, d, $J$ = 8 Hz).

The mass spectrum did not show a molecular ion. However, characteristic of flavonol glycosides (4), the spectrum was that of the aglycone, showing peaks at *m/e* 302 (100%), 286 (10), 237 (7), 153 (6), 151 (3), 137 (9), and 109 (5). Since no physical data on II other than the melting point have been reported and since a reference sample was unavailable, comparison of physical constants was not possible.

[1] Melting points were taken on a Thomas-Hoover Uni-Melt capillary apparatus and are corrected. IR spectra were determined on a Perkin-Elmer model 257 spectrometer in potassium bromide pellets. UV spectra were obtained on a Perkin-Elmer model 202 spectrometer. Optical rotations were measured on a Perkin-Elmer 241 automatic polarimeter. Mass spectra were taken with an LKB-9000 mass spectrometer. NMR spectra were obtained on a Perkin-Elmer R-24 spectrometer in the solvents specified with tetramethylsilane as the internal standard. Chemical shifts are reported as $\delta$ units (parts per million). GLC analysis was performed on a Perkin-Elmer model 3920B instrument operated isothermally at 150°. The column (2 × 0.0004 m, glass) was packed with 3% OV-17 on 80–100-mesh Supelcoport. Nitrogen was the carrier gas at a flow rate of 30 ml/min.

[2] Collected in Brazil and identified by Dr. Basset McGuire of the New York Botanical Gardens. A voucher specimen is on deposit at Eli Lilly and Co., Indianapolis, IN 46206.

[3] Polypenco Nylon 66, Molding Plastics Division, Polymer Corp., Pittsburgh, Pa.



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**Hydrolysis of II**—Compound II (80 mg) was dissolved in 20 ml of 6% aqueous HCl, and the solution was refluxed for 45 min. The solution was cooled and filtered to separate the precipitated I from the soluble sugar. The filtrate was extracted with chloroform (4 × 50 ml), the extract was dried (sodium sulfate) and evaporated to a residue, and the residue was added to the precipitated I to afford a total of 35 mg. The I obtained (mp 300°) was identical in all respects (UV, IR, NMR, and mass spectra, melting point, and mixed melting point) to an authentic sample[4].

The aqueous filtrate was evaporated to dryness to yield a residue (31 mg) of crude L-arabinose; $[\alpha]_D^{29}$ +34.1° (c 1.1, water). TLC of the material (silica gel G, *n*-butanol–acetic acid–ether–water 9:6:3:1) indicated that it was homogeneous. A comparison of the $R_f$ value (0.42) and color reaction with anisaldehyde spray reagent (9) given by the material with known sugars indicated that it was arabinose. This conclusion was confirmed by converting the sugar to its trimethylsilyl derivative (10) and subjecting it, along with the trimethylsilyl derivatives of other known sugars, to GLC analysis. The trimethylsilyl derivative of the isolated sugar showed an identical retention time (7.5 min) to that of the trimethylsilyl derivative of L-arabinose when analyzed individually or as a mixture and was clearly different from the trimethylsilyl derivatives of other sugars.

**5,7,3′,4′-*O*-Methylquercetin (III)**—Compound II (50 mg) was treated for 48 hr with 30 ml of ethereal diazomethane solution prepared from 21.5 g of *N*-methyl-*N*-nitrosó-*p*-toluenesulfonamide[5]. The solution then was evaporated to give a residue (56 mg), the residue was suspended in 10 ml of 7% aqueous $H_2SO_4$, and the suspension was refluxed for 1 hr. The reaction mixture was cooled and extracted with ether (4 × 20 ml), and the ether extract was evaporated to yield 5,7,3′,4′-*O*-methylquercetin (III) (20 mg).

The compound had a melting point of 186–188° [lit. (8) mp 195–196°]; IR (KBr): $\nu_{max}$ 3290, 3000, 2925, 2820, 1635 (sh), 1615, 1570, 1515, 1490, 1090, and 1025 $cm^{-1}$; mass spectrum: *m/e* 358 ($M^+$) (85%), 312 (100), 181 (17), 197 (75), 165 (65), 150 (43), 149 (34), 142 (30), 137 (30), 135 (42), 122 (30), 119 (32), 107 (28), 92 (33), 79 (52), and 77 (48); NMR ($CDCl_3$): δ 3.90 (1H, s), 3.95 (9H, s), 6.26 (1H, d, $J$ = 2 Hz), 6.43 (1H, d, $J$ = 2 Hz), 6.90 (1H, d, $J$ = 9 Hz), 7.70 (1H, d, $J$ = 9 Hz), and 7.73 (1H, bs); UV: $\lambda_{max}$ (methanol) 224 (log ε 4.54), 257 (4.43), 300 (6.65), and 3.61 (4.30) nm; $\lambda_{max}$ (aluminum chloride–methanol) 227 (log ε 4.52), 265 (4.49), and 422 (4.40) nm; and $\lambda_{max}$ (aluminum chloride–hydrochloric acid–methanol) 220 log ε 4.50), 262 (4.38), 360 (4.05), and 420 (4.03) nm. The UV spectrum was indicative of a 3-hydroxy flavonoid (11).

**Avicularin Heptaacetate (IV)**—Compound II (15 mg) was treated at room temperature with acetic anhydride and pyridine (5 ml each) for 16 hr. Water, 2 ml, was added to the reaction mixture, and the mixture was evaporated to a residue. This residue was dissolved in 1 ml of acetone, and 5 ml of hexane was added. The precipitated heptaacetate (IV) (16 mg) was recovered by filtration.

The compound had a melting point of 175° [lit. (4) mp 187°]; $[\alpha]_D^{28}$ −95.1° (c 1.20, chloroform) [lit. (4) $[\alpha]_D$ −136° (c 1.22, chloroform)]; IR (KBr): $\nu_{max}$ 2920, 1725, 1630, 1615, 1500, 1420, 1370, 1215, and 1070 $cm^{-1}$; mass spectrum: *m/e* 728 ($M^+$) (0.2%), 686 (1.0), 644 (0.3), 470 (2.0), 428 (30), 386 (53), 344 (67), 328 (10), 302 (100), 286 (30), 273 (17), 259 (77), 233 (27), 199 (13), 157 (43), 153 (10), 137 (27), 134 (7), and 109 (7). The NMR spectrum ($CDCl_3$) showed singlets at δ 2.10 (12H) and 2.30 (9H), attributable to aromatic and aliphatic acetate groups, respectively.

**Isolation of Gallic Acid (V)**—Elution of the polyamide column with methanol–water (3:1) afforded a fraction (332 mg) containing V. Treatment of this fraction with methanol–chloroform afforded crystals of V (12 mg) with a melting point of 230–235° whose physical properties (IR, UV, NMR, and mass spectra) were identical to those of an authentic sample[5].

[4] K & K Laboratories, Plainview, N.Y.

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

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