

ISOFRAXIDIN, A CYTOTOXIC COUMARIN FROM *MICRANDRA ELATA* (EUPHORBIACEAE)

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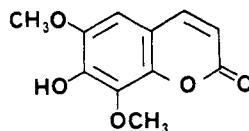
In a continuing search for plants having anticancer activity, the Peruvian plant *Micrandra elata* (Euphorbiaceae) was examined. The chloroform-soluble fraction of the methanol extract of the roots showed activity against the P-388 lymphocytic leukemia in mice,¹ and a previous paper has identified those compounds responsible for most of this activity (2).² 6,8-Dimethoxy-7-hydroxycoumarin (isofraxidin) (I), which displays marginal cytotoxicity, has now been isolated from the twigs of *M. elata*.

EXPERIMENTAL³

PLANT MATERIAL.—The plant material, comprising 31.5 kg of the twigs of *Micrandra*

elata (Didr.) Muell.-Arg. (Euphorbiaceae)⁴ collected in Peru during April of 1976,⁵ was air dried and milled to a coarse powder.

EXTRACTION AND INITIAL FRACTIONATION.—The plant material was exhaustively extracted with petroleum ether (bp 30–60°). After removal of the petroleum ether, the marc was exhaustively extracted with methanol, and the methanol extract was concentrated *in vacuo* at 40°. Three kg of the dark brown, semi-solid residue (3.2 kg total) was partitioned between distilled water (38 liters) and chloroform (95 liters); the chloroform-soluble fraction was dried (Na₂SO₄) and concentrated *in vacuo* at 40° to yield 336 g of a dark-green, solid residue.



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¹The extracts, fractions and compounds were tested under the auspices of the Drug Research and Development Branch of the National Cancer Institute (1). An isolate is considered active if it shows an ED₅₀ ≤ 4 μg/ml in the KB or P-388 cell culture *in vitro*, and a T/C ≥ 130% *in vivo*.

²Since the acceptance of this manuscript the plant material previously identified as *Cumuria spruceana* Baill., has been reidentified as *Micrandra elata* (Didr.) Muell.-Arg.

³Melting points were determined using a Koffler hot plate and are uncorrected. The uv spectra were obtained with a Beckman model DB-G spectrophotometer. The IR spectra were determined with a Beckman model ir-18A spectrophotometer with polystyrene calibration at 1601 cm⁻¹; absorption bands are recorded in wave numbers (cm⁻¹). pmr spectra were recorded in CDCl₃ with a Varian T-60A instrument, operating at 60 MHz with a Nicolet Model TT-7 Fourier Transform attachment. Tetramethylsilane was used as an internal standard and chemical shifts are reported in δ (ppm). Mass spectra were obtained with a Varian MAT-112S double-focusing spectrometer operating at 70 eV and 140°.

SEPARATION OF THE CHLOROFORM FRACTION.—Three hundred and thirty g of the chloroform-soluble fraction was redissolved in chloroform, 350 g of MN-silica gel-P/UV254⁶ was added, the solvent removed *in vacuo*, and the adsorbed extract chromatographed on a column of MN-silica gel-P/UV254 packed in benzene (3.5 kg, 12×90 cm). Elution was initiated with benzene, followed by benzene-chloroform mixtures, chloroform, chloroform-methanol mixtures of increasing polarity, and methanol. A total of 56 fractions (4 liters each) were collected.

⁴The plant material was supplied through the auspices of the Drug Research and Development Program of the National Cancer Institute by the Economic Botany Laboratory, Agricultural Research Service, BARC-East, U.S.D.A., Beltsville, Maryland. A herbarium specimen documenting this collection is deposited in the Herbarium of the National Arboretum, Agricultural Research Service, United States Department of Agriculture, Washington, D.C.

⁵Macherey-Nagel, Duren, West Germany.

ISOLATION OF ISOFRAXIDIN.—Fraction 32 from the column, which was eluted with chloroform:methanol (99:1), was concentrated *in vacuo* at 40° to give 2.5 g of residue. Tlc of the fraction (silica gel,⁶ chloroform:methanol, 97:3) indicated the presence of two major components ($R_f=0.23, 0.18$). Fraction 32 (0.75 g) from the column was rechromatographed on 30 g of MN-silica gel-P/UV254, eluted successively with chloroform-methanol (19:1) (16 fractions, 25 ml each) and methanol (3 fractions). Tlc (silica gel, benzene-acetone, 3:1) indicated the major component to be primarily in fraction 6.

Preparative chromatography of fraction 6 on precoated silica gel 60-F254 plates⁶ (20×20 cm, 2 mm thick), developed twice with benzene-acetone (3:1), followed by processing of an intense blue fluorescent band at $R_f=0.51$, afforded a yellow amorphous solid. Crystallization from benzene afforded amber styloids of isofraxidin (1) mp 146–148° (412 mg, 0.000013%); ir, ν max (KBr) 3340, 1700, 1570 and 1290 cm^{-1} ; uv, λ max (MeOH) 342, 224 and 212 nm; λ max (MeOH+NaOH) 400, 234 and 214 nm; nmr 60 MHz (CDCl_3) δ 3.94 (3H, s, 6-OCH₃), 4.09 (3H, s, 8-OCH₃), 6.26 (1H, d, $J=9.3$ Hz, 3-H), 7.58 (1H, d, $J=9.3$ Hz, 4-H), 6.65 (1H, s, 5-H); ms, m/e 222 (M^+ , 100%), 207 (48), 194 (16), 179 (25), 151 (18) and 123 (34). Mass Measurement: Found 222.0536, Calc. for $\text{C}_{11}\text{H}_{10}\text{O}_5$ 222.0528. These data are in complete agreement with those published for isofraxidin (3).

BIOLOGICAL ACTIVITY.—Isofraxidin (NSC-324637) was inactive in the KB test system ($\text{ED}_{50}>100$ $\mu\text{g/ml}$), but was active in the P-388 lymphocytic leukemia (*in vitro*) test system ($\text{ED}_{50}=1.7$ $\mu\text{g/ml}$).

DISCUSSION

Isofraxidin was first isolated by Späth and Jerzmanowska-Sienkiewiczowa (4) from the bark of the ash tree, *Fraxinus excelsior* L. (Oleaceae), and has subsequently been obtained from other species of *Fraxinus* (5–7), *Artemisia* (8–14) and *Achillea* (15) (Compositae), *Eleutherococcus* (16) (Araliaceae), *Chloranthus* (3) (Chloranthaceae), *Erica* (17) (Ericaceae), and *Parastrephia* (18) (Asteraceae). The compound has been partially and totally synthesized (19, 20).

Preparations containing isofraxidin have been shown to possess choleric

activity in rats. In terms of this activity, isofraxidin is several times more potent than equal amounts of dehydrocholic acid (21).

To our knowledge, this is the first report of a simple coumarin derivative possessing activity in the P-388 lymphocytic leukemia cell culture system.

Neither of the cytotoxic diterpenes obtained from the roots of *M. elata* (2) could be detected by means of thin-layer chromatography of the chloroform extracts of the twigs.

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