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JUGLONE: THE ECHTIOTOXIC PRINCIPLE OF *PTEROCARYA FRAXINIFOLIA*

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Key Word Index—*Pterocarya fraxinifolia*; Juglandaceae; 5-hydroxy-1,4-naphtoquinone; fish poison.

The leaves of *Pterocarya fraxinifolia* (Lam.) Spach. (Juglandaceae), an indigenous plant found on the Caspian shores in the northern part of Iran, have been used by the natives as a fish poison, dyeing agent, antifungal agent, and in the treatment of diarrhoea. The purpose of this investigation was to isolate and identify the echtiotoxic principle.

Fractionation of the leaves of *Pterocarya fraxinifolia*[1] guided by monitoring the echtiotoxic activity indicated that the active component was extracted with water.

Extraction of this aqueous phase with diethyl ether and subsequent column chromatography of the soluble residue on silica gel G led to the isolation of 5-hydroxy-1,4-naphtoquinone (juglone), identified by comparision of its m.p., IR, UV and MS with published data,[2–5] as the active principle.

Juglone has generally reported as a toxic principle in the Juglandaceae and has been exploited for catching fish.[6] It is found in a reduced form as the 4-β-D-glucoside in the green parts of plant and is formed by hydrolysis and oxidation during extraction procedures. The LD_{50} is 0·0013 mg/ml for goldfish (*Carassius auratus*) treated with the juglone solution for 30 min.

EXPERIMENTAL

Mps were determined on a Leitz micro-melting point microscope. IR spectra were recorded in KBr on a Perkin Elmer 257 spectrophotometer, UV spectra on a Beckman DK-2A spectrophotometer, NMR spectra on a Varian HA-100 spectrometer and MS on an A.E.I. MS-902 spectrometer. Evaporation was carried out at less than 45°.

Extraction procedure. Fresh leaves (8·0 kg) were ground and extracted exhaustively with H_2O. The solution was extracted with Et_2O which, after evaporation, yielded a solid (5·0 g). This was chromatographed on a column (60 × 4 cm) containing Keisel gel S (800 g, Riedel-De Haen) using light petrol. (b.p. 40–60°) (1 l.) followed by Et_2O–light petrol. (1:19) (2 l.). The later fractions yielded an orange solid (150 mg) which was recrystallized from light petrol. and sublimed to yield orange needles: m.p. 153–154°. UV: (MeOH) 246, 340, 408, 425 hm ($\epsilon = 12\,900, 2390, 3200$). NMR: ($CDCl_3$): τ −1·84 (*s*, 1H), 2·40 (app. *d*, 2H, *J* 5 Hz), 2·76 (app. *t*, 1H, *J* 5 Hz), 3·06 (s, 2H). MS: *m/e* (rel. int.) 176 (4·5), 175 (12), 174 (100), 173 (22), 146 (12),

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[1] Plant material was collected in Amol (180 km north of Tehran) during May 1970. Voucher specimens were prepared and authenticated as *Pterocarya fraxinifolia* (Lam.) Spach. Specimens representing the collection were deposited in the Herbaria of the Department of Pharmacognosy, University of Tehran, Iran.

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92 (22). The compound gave a positive Borntrager reaction. The echtiotoxic activity of juglone was determined on goldfish and the LD_{50} was calculated according to the method described by Weil.[7]

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TRITERPENES AND FATTY ACIDS FROM *NEPETA ARAGONENSIS*

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Plant. Nepeta aragonensis Lamk. *Source.* Reduced areas in the NE of the Iberian Peninsula. *Previous work.* None.

Present work. From the triterpene fraction, ursolic acid, betulin and uvaol have been isolated; this is the first time that the presence of betulin and uvaol is reported in plants of the *Nepeta* genus. The ursolic acid have been previously isolated from other species of this genus.[1,2]

GLC analysis of the methyl ester of the fatty acids fraction yielded the following results: myristic (17%), palmitic (80%), oleic (0·5%), linoleic (0·47%) and linolenic (1·2%) acids.

EXPERIMENTAL

Extraction. Finely divided aerial parts of *N. aragonensis* were extracted in a Soxhlet with MeOH. The extract was taken to dryness and the residue saponified with 4% ethanolic KOH isolating a neutral and an acid fraction.

Neutral fraction. Following column and preparative thin layer chromatography on SiO_2–$AgNO_3$ impregnated plates, betulin and uvaol were isolated. *Betulin* (most polar compound on SiO_2—$AgNO_3$ plates); m.p. 258–260° (from acetone:*n*-hexane); $[\alpha]_D^{23^\circ}$ + 18° (*c* 1·1, Py). *Diacetyl derivative*: m.p. 220° (from MeOH); $[\alpha]_D^{23^\circ}$ + 21° (*c* 0·8, $CHCl_3$); NMR (τ): 5·34 (2H, *d* with fine splitting, $>C{=}CH_2$), 5·45 (1H, *m*, $>CHOAc$), 5·93 (2H, Q_{AB}, *J* 12 Hz, $=C{=}CH_2OAc$), 7·95 and 7·97 (3H each, *s,s*, two Me–CO–O–), 8·31 (3H, bs, $C{=}C{=}Me$) and methyl singlets at 8·96 (1 –Me), 9·02 (1 –Me) 9·16 (3 –Me). [Found: C, 77·43; H, 10·26. Calc. for $C_{34}H_{54}O_4$: C, 77·52; H, 10·33%]. *Uvaol.* Thick oil; it did not crystallize. *Diacetyl derivative*: m.p. 151–153° (from MeOH); $[\alpha]_D^{23^\circ}$ + 53° (*c* 0·7, $CHCl_3$); NMR (τ): 4·83 (1H, *m*, $W_{\frac{1}{2}}$ 9 Hz, olefinic), 5·47 (1H, *q*, $J_{aa'}$ 8 Hz, $J_{ae'}$ 6 Hz, axial proton $>CHOAc$), 6·15 (2H, Q_{AB}, *J* 12 Hz, $=C{=}CH_2OAc$), 7·97 (6H, *s*, two Me–CO–O–) and methyl singlets at 8·9 (1 –Me), 9·01 (2 –Me) and 9·13 (4 –Me). [Found: C, 77·31; H, 10·29. Calc. for $C_{34}H_{54}O_4$: C, 77·52; H, 10·33%].

Acid fraction. Silica gel column chromatography allows separation of ursolic acid and a mixture of fatty acids. The fatty acids were methylated with diazomethane and analyzed on GLC with standard conditions. The results have been quoted above.

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