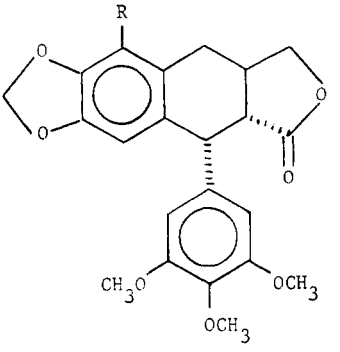
PLANT ANTICANCER AGENTS. X.[1] LIGNANS FROM *JUNIPERUS PHOENICEA*

DAVID A. CAIRNES, OLUSEGUN EKUNDAYO and DAVID G. I. KINGSTON
Department of Chemistry, Virginia Polytechnic Institute and State University, Blackburg, VA 24061, U.S.A.

ABSTRACT.—Desoxypodophyllotoxin and β-peltatin-A methyl ether have been identified as the major cytotoxic components of *Juniperus phoenicea*.

Recent work on *Juniperus phoenicea* has revealed the presence of a variety of diterpenes and diterpene acids in this plant (1, 2), but no antileukemic or cytotoxic compounds have been reported from it, although the presence of desoxypodophyllotoxin in *J. bermudiana* (3) and *J. siliciola* (4) has been reported. In a continuation of our search for anticancer compounds of plant origin, we have examined ethanolic extracts of *J. phoenicea* for cytotoxic and antileukemic constituents.

Extraction of twigs and leaves of *J. phoenicea* with ethanol yielded an extract which showed cytotoxicity in the KB cell culture system. This extract was subjected to repeated chromatography on silica gel to give an active fraction which consisted of a mixture of closely related compounds, inseparable by further normal phase chromatography. Separation was effected readily, however, by reverse phase high performance liquid chromatography (hplc) (5) and yielded two pure cytotoxic compounds. Compound **1** was identified as desoxypodophyllotoxin on



1 R = H

2 R = OCH_3

the basis of its spectroscopic properties; the identification was confirmed by comparison with an authentic sample. Compound **2** was identified as β-peltatin-A methyl ether on the basis of its spectroscopic properties and by comparison with an authentic sample (6).

Various other crude fractions from the initial separations mentioned above also showed cytotoxic activity, but examination of these fractions by hplc indicated that they all contained either desoxypodophyllotoxin or β-peltatin-A methyl

[1]For Part IX, see ref. 9.

ether, or both, and we thus conclude that these two compounds represent the major cytotoxic components of *J. phoenicea*. Although the cytotoxicity of desoxypodophyllotoxin is well known (7), the cytotoxicity of β-peltatin-A methyl ether is reported here for the first time. The *in vivo* antileukemic activity of the crude ethanol extract (T/C 137 in P-388 at 300 mg/kg) is presumably also due to the presence of one or both of these lignans since lignans of this type show activity in the P-388 system (7).

EXPERIMENTAL[2]

PLANT MATERIALS.—Dried twigs and leaves of *Juniperus phoenicea* L. (family Cupressaceae: B 641417, PR-49578) were collected in Israel and authenticated by the Economic Botany Laboratory, USDA, Beltsville, Maryland. An herbarium specimen documenting this collection is deposited in the Herbarium of the National Arboretum, Agricultural Research Service, USDA, Washington, DC. The material was ground in a hammermill.

EXTRACTION AND FRACTIONATION.—A sample of ground twigs and leaves of *J. phoenicea* (2 kg) was extracted exhaustively with ethanol. The combined ethanol extracts were evaporated and partitioned between chloroform and water; partitioning the chloroform fraction between hexane and 10% aqueous methanol yielded 112 g of crude methanol-soluble material with an ED_{50} of 1.2 μg/ml in the KB cell culture system (KB).

Chromatography of the methanol-soluble fraction on silica gel with elution by chloroform and chloroform-methanol yielded an active fraction in the chloroform-methanol (98:2) eluate (17 g, ED_{50} 0.3 μg/ml in KB cell culture). Rechromatography of this fraction on silica gel with elution by chloroform yielded a fraction (5.6 g) with ED_{50} 0.04 μg/ml in KB; rechromatography of a 1 g portion of this fraction on silica gel with elution by chloroform-hexane (3:1) yielded a fraction (0.18 g) with ED_{50} 0.025 μg/ml in KB. Preparative tlc of this fraction on silica gel with development by acetone-hexane (1:3) yielded a single major uv-absorbing band which was shown to consist of two major components by analysis of its proton magnetic resonance (pmr) spectrum. Separation, which was effected by preparative hplc on a 0.7 x 50 cm Partisil-10 ODS column with acetonitrile-water (50:50) as solvent, yielded two homogeneous compounds.

IDENTIFICATION OF β-PELTATIN-A METHYL ETHER (**1**).—The first substance eluted from the column was obtained as a colorless microcrystalline solid, mp 127°. Its mass spectrum showed peaks at *m/z* 428 (M^+, 33), 383(10), 370(24), 261(19), 181(32), 180(44), and 164(34), and it had nmr and ir spectra identical with those published for β-peltatin-A methyl ether $C_{23}H_{24}O_8$ (6, 8). The isolated material also had an identical retention volume on hplc (LiChrosorb RP-8 column, acetonitrile-water, 50:50) as an authentic sample of β-peltatin-A methyl ether.

IDENTIFICATION OF DESOXYPODOPHYLLOTOXIN (**2**).—The second substance was obtained as colorless crystals, mp 166–167°, undepressed in admixture with an authentic sample of desoxypodophyllotoxin, $C_{22}H_{22}O_7$ (9). Its pmr spectrum in $CDCl_3$ showed signals at δ=6.62 (1H, s), 6.48 (1H, s), 6.14 (2H, s) 5.90 (1H, s), 3.76 (3H, s), and 3.71 ppm (6H, s), together with signals assignable to the alicyclic protons of the molecule, and its ir spectrum was identical with that of an authentic sample.

BIOLOGICAL ACTIVITY.—β-peltatin-A methyl ether showed an ED_{50} of 0.026 μg/ml in the KB cell culture system.

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[2]General experimental details are given in Part III of this series: D. G. I. Kingston, B. T. Li, and F. Ionescu, *J. Pharm. Sci.* **66**, 1135 (1977). The Partisil-10 ODS column was obtained from Whatman, Inc.

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