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PLANT ANTICANCER AGENTS, XLII. CYTOTOXIC CONSTITUENTS FROM *WIKSTROEMIA ELLIPTICA*[1]

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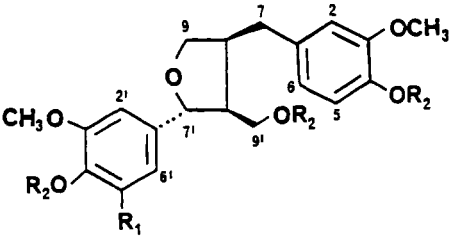
Wikstroemia elliptica Merrill (Thymelaeaceae) represents a genus of some 70 species that is indigenous to parts of Southeast Asia, Australia, and the Pacific (2). This species was selected for study as part of a continuing search for tumor inhibitors from plants when a $CHCl_3$ extract of the combined stem wood and stem bark displayed significant activity against the P-388 lymphocytic leukemia test system in cell culture, when assessed using standard protocols (3). Bioactivity-directed fractionation of this extract has led to the isolation of five cytotoxic substances, namely, the lignans, (±)-5′-methoxylariciresinol (a new compound, **1**), (±)-lariciresinol (**2**), and (±)-syringaresinol, as well as the coumarins, daphnoretin and umbelliferone. No previous phytochemical or biological studies appear to have been performed on extracts of *W. elliptica*.

(±)-5′-Methoxylariciresinol (**1**), $[\alpha]^{25}D$ +0.3° in Me_2CO, was obtained as a resin and exhibited a molecular formula of $C_{21}H_{26}O_7$ as determined by hrms. In the ir spectrum, the absorption bands at 3432, and 1614, and 1517 cm^{-1} were indicative of OH groups and aromatic rings, respectively, and showed the absence of any carbonyl or lactone functionality. The uv, ^1H-nmr, and ^{13}C-nmr spectra indicated that **1** was a lignan of the lariciresinol type (4-6), and, on acetylation with Ac_2O-pyridine under normal conditions, the triacetate **3** was produced. Comparison of the spectral data of **1** and **3**, with those of lariciresinol (**2**) and its triacetate (**4**), indicated that an extra aromatic



	R_1	R_2
1[a]	OCH_3	H
2	H	H
3	OCH_3	$COCH_3$
4	H	$COCH_3$

[a]For simplicity of presentation, only the (−)-enantiomeric forms of compounds **1-4** are represented.

methoxy substituent was present in **1** when compared with **2**. Furthermore, elemental compositions of prominent mass spectral fragments incorporating the phenyl ring directly attached to the furan ring (7-10), observed for **1** at *m/z* 236 ($C_{13}H_{16}O_4$), 183 ($C_9H_{11}O_4$), 182 ($C_9H_{10}O_4$), and 181 ($C_9H_9O_4$) were 30 mass units greater than analogous fragments for **2** at *m/z* 206 ($C_{12}H_{14}O_3$), 153 ($C_8H_9O_3$), 152 ($C_8H_8O_3$), and 151 ($C_8H_7O_3$), respectively. This information suggested that the additional methoxy group of **1** was affixed at some position on the benzene ring attached to C-7′. Observation of a singlet peak in the ^1H-nmr spectrum of **1**, assignable to both the H-2′ and H-6′ protons, because of a symmetrical environment, indicated that the extra methoxy group in

[1]For the previous paper in this series, see Fiebig *et al.* (1).

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1, when compared with **2**, occurred at C-5′.

Final confirmation of the structure of **1** was obtained by comparing its ^{13}C-nmr spectrum with that of **2** and with published ^{13}C-nmr data for other representative lignans (6, 10, 11). The close relationship between such data for **1** and **2** suggested that **1** is based on the same carbon skeleton as **2**, and that the environment of the C-1 through C-9 and the C-7′ through C-9′ carbons is the same in both compounds (Table 1). ^{13}C-Nmr chemical shifts for the C-1′ through C-6′ carbons of **1** were closely comparable with published data for syringaresinol and its analogs (10,11), that also bear a 4-hydroxy-3,5-dimethoxyaryl functionality.

Plants in the genus *Wikstroemia* have afforded a variety of compound classes with antineoplastic activity, comprising lignans (12,13), flavonoids (13), a coumarin (13), and a mixture of daphnane diterpene esters (14). Skin-irritant diterpene esters of the phorbol or daphnane types did not appear to be present in the *W. elliptica* stem wood-stem bark sample investigated here, as a result of the use of mouse ear skin-irritant assay (15) and a droplet counter-current chromatographic procedure designed to concentrate diterpenes of this type (16).

TABLE 1. Comparison of ^{13}C-nmr Data of 5′-Methoxylariciresinol (**1**) and Lariciresinol (**2**)[a]

Carbon	Compound	
	1	**2**
C-1	132.2	132.1
C-2	111.1	111.1
C-3	146.5	146.4[b]
C-4	144.0	143.9
C-5	114.4	114.3[c]
C-6	121.1	121.1
C-7	33.3	33.2
C-8	42.3	42.3
C-9	72.9	72.8
C-1′	133.9[d]	134.6
C-2′	102.4	108.2
C-3′	147.0	146.5[b]
C-4′	134.0[d]	144.9
C-5′	147.0	114.0[c]
C-6′	102.4	118.6
C-7′	83.0	82.7
C-8′	52.6	52.5
C-9′	60.9	60.8
2x-OMe		55.8
3-OMe	55.9	
3′-, 5′-OMe	56.3	

[a]Chemical shifts were determined at 90.8 MHz in $CDCl_3$. The δ values are in ppm downfield of TMS.

[b-d]Signals may be interchanged.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler hot-stage instrument and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. The uv spectra were obtained on a Beckman DB-G grating spectrophotometer, and the ir spectra measured on a Nicolet MX-1 FT-IR interferometer. ^{1}H-Nmr spectra were recorded in $CDCl_3$, using TMS as internal standard, employing either a Nicolet NT-360 instrument (360 MHz) or a Varian T-60A instrument, with a Nicolet TT-7 Fourier Transform attachment (60 MHz). ^{13}C-Nmr spectra were recorded in $CDCl_3$ with a Nicolet NT-360 instrument operating at 90.8 MHz. Low resolution mass spectra were obtained with a Varian MAT 112S instrument, operating at 70 eV.

PLANT MATERIAL.—The combined stem wood and stem bark of *W. elliptica* was collected in Hawaii in 1980. A voucher specimen representing this collection is deposited in the herbarium of the Harold L. Lyon Arboretum, University of Hawaii at Manoa, Honolulu, Hawaii.

ETRACTION AND FRACTIONATION.—The air-dried, milled plant material (11.4 kg) was extracted sequentially with petroleum ether (bp 60-80°) and MeOH. After removal of solvent in vacuo, the MeOH-soluble residue was partitioned between H_2O and $CHCl_3$. The dried $CHCl_3$ extract was found to exhibit activity against the P-388 lymphocytic leukemia system in vitro (3), with an ED_{50} of 3.5 μg/ml. The $CHCl_3$-soluble residue was further partitioned with petroleum ether and MeOH-H_2O (10:1) to produce, on drying, 28.5 g of a cytotoxic aqueous-MeOH extract (P-388, ED_{50}, 2.3 μg/ml).

Column chromatography of this aqueous-MeOH extract over silica gel 60 (Merck, Darmstadt, W. Germany), was undertaken using $CHCl_3$ and $CHCl_3$/MeOH mixtures of increasing polarity. A total of 45 fractions (2 liters each) was collected. Elution by $CHCl_3$-1% MeOH afforded, in turn, daphnoretin and (±)-syringaresinol, while elution with $CHCl_3$-2% MeOH yielded sequentially umbelliferone, (±)-5′-

methoxylariciresinol (**1**), and (±)-lariciresinol (**2**). All five of these isolates were active against the P-388 in vitro test system.

CHARACTERIZATION OF (±)-5'METHOXY LARICIRESINOL (**1**).—This resinous cytotoxic isolate (**1**, 4 mg, 0.000034% w/w) exhibited the following data: $[\alpha]^{25}D$ +0.3° (c 0.33, Me_2CO); uv (MeOH) λmax (log ε) 230 (4.71), 281 nm (4.19); ir (AgCl) νmax 3432, 2928, 1614, 1517, 1464, 1430, 1272, 1237, 1215, 1116, 1036, 758 cm^{-1}; ^1H nmr (360 MHz, $CDCl_3$) δ 2.41 (1H, m, 8'-H), 2.55 (1H, dd, J=13.1, 11.0 Hz, 7-H), 2.74 (1H, m 8-H), 2.91 (1H, dd, J=13.1, 5.3 Hz, 7-H), 3.76 (1H, dd, J=8.7, 5.7 Hz, 9β-H), 3.79 (1H, dd, J=11.0, 6.9 Hz, 9'-H), 3.87 (3H, s, -OCH_3), 3.89 (6H, s, two-OCH_3), 3.93 (1H, dd, J=11.0, 7.0 Hz, 9'-H), 4.06 (1H, dd, J=8.7, 6.6 Hz, 9α-H), 4.80 (1H, d, J=6.2 Hz, 7'-H), 5.51 (1H, br s, phenolic-OH), 6.57 (2H, s, 2'-, 6'-H), 6.68 (2H, m, 2-, 6-H), 6.84 (1H, d, J=8.4 Hz , 5-H); ^{13}C nmr see Table 1; ms *m/z* 390 (M^+, 100%) (found 390.16840), calcd. for $C_{21}H_{26}O_7$, 390.16786), 372 (45), 359 (19), 341 (50), 236 (34) (found 236.10700, calc. for $C_{13}H_{16}O_4$, 236.10486), 183 (57) (found 183.06659, calcd. for $C_9H_{11}O_4$, 183.06574), 182 (54) (found 182.05865, calcd. for $C_9H_{10}O_4$, 182.05791), 181 (61) (found 181.05216, calc. for $C_9H_9O_4$, 181.05009), 167 (54).

ACETYLATION OF (±)-5'-METHOXY LARICIRESINOL (**1**).—(±)-5'-Methoxylariciresinol (**1**, 1 mg) was treated with Ac_2O-pyridine (1:1, 1 ml) at room temperature overnight. Workup in the usual manner afforded a resinous triacetate **3**; $[\alpha]^{25}D$ +0.6° (c 0.17, Me_2CO); uv (MeOH) λmax (log ε) 223 (4.23), 274 (3.61), 282 nm (3.58); ir (AgCl), νmax 2930, 2854, 1765, 1742, 1738, 1732, 1370, 1237, 1220, 1199, 1153, 1131 cm^{-1}; ^1H nmr (360 MHz, $CDCl_3$) δ 2.06 (3H, s, -$OCOCH_3$), 2.31 (3H, s, -$OCOCH_3$), 2.34 (3H, s, -$OCOCH_3$), 2.58 (2H, m, 7-, 8'-H), 2.74 (1H, m, 8-H), 2.87 (1H, dd, J=13.0, 5.0 Hz, 7-H), 3.76 (1H, dd, J=8.6, 7.2 Hz, 9β-H), 3.82 (3H, s, -OCH_3), 3.83 (6H, s, two-OCH_3), 4.10 (1H, dd, J=8.6, 6.8 Hz, 9α-H), 4.23 (1H, dd, J=11.0, 7.6 Hz, 9'-H), 4.38 (1H, dd, J=11.0, 6.8 Hz, 9'-H), 4.83 (1H, d, J=5.0 Hz, 7'-H), 6.57 (2H, s, 2'-, 6'-H), 6.74 (2H, m, 2-, 6-H), 6.96 (1H, d, J=7.6 Hz, 5-H); ms *m/z* 516 (M^+, 29%), 474 (100), 432 (31), 397 (16), 372 (28), 249 (53), 235 (83), 182 (24), 181 (78), 137 (55).

CHARACTERIZATION OF (±)-LARICIRESINOL (**2**).—This resinous cytotoxic isolate (**2**, 27 mg, 0.00023%) exhibited $[\alpha]^{25}D$ −0.2° (c 0.50, Me_2CO) [lit. (−)-lariciresinol, mp 160-162°, $[\alpha]^{25}D$ −17.8° (c 1.4, Me_2CO) (5); (+)-lariciresinol, mp 168-168°, $[\alpha]^{17}D$ 19.7° (c 2.2, Me_2CO) (17)], uv and ir data comparable with published values (4, 5); ^1H nmr (360 MHz, $CDCl_3$) δ 2.42 (1H, m, 8'-H), 2.54 (1H, dd, J=13.5, 10.9 Hz, 7-H), 2.72 (1H, m, 8-H), 2.91 (1H, dd, J=13.5, 5.0 Hz, 7-H), 3.77 (2H, m, 9β-, 9'-H), 3.88 (3H, s, -OCH_3), 3.90 (3H, s, -OCH_3), 3.93 (1H, m, 9'-H), 4.06 (1H, dd, J=8.3, 6.7 Hz, 9α-H), 4.79 (1H, d, J=6.7 Hz, 7'-H), 5.58 (1H, br s, phenolic OH), 6.69-6.86 (6H, m, aromatic H); ^{13}C nmr see Table 1; ms *m/z* 360 (M^+, 100%) (found 360.153372, calcd. for $C_{20}H_{24}O_6$, 360.15731), 342 (75), 329 (2), 311 (70), 206 (43) (found 206.09250, calcd. for $C_{12}H_{14}O_3$, 206.09430), 153 (59) (found 153.05490, calcd. for $C_8H_9O_3$, 153.05517), 152 (56) (found 152.04589, calcd. for $C_8H_8O_3$, 152.04735), 151 (65) (found 151.03037, calcd. for $C_8H_7O_3$, 151.03952), 137 (81). Identification was established by comparison with an authentic sample of (−)-lariciresinol isolated from *Dirca occidentalis* A. Gray (5). The racemate of lariciresinol does not appear to have been found previously as a natural product. (±)-Lariciresinol (**2**, 2 mg) was treated with Ac_2O-pyridine (1:1, 1 ml) at room temperature overnight to generate on workup the resinous (±)-lariciresinol triacetate (**4**), $[\alpha]^{25}D$ −0.3° (c 0.33, Me_2CO), which exhibited comparable spectroscopic data (uv, ir, ^1H nmr, ms) to literature values for (−)-lariciresinol triacetate (4, 6).

(±)-SYRINGARESINOL.—This isolate (25 mg, 0.00022% w/w), mp 173-175°, $[\alpha]^{25}D$ +5.9° (c 0.68, $CHCl_3$) [lit mp 174-176°, $[\alpha]^{24}D$ −2.1° (c 0.8, $CHCl_3$) (4)] exhibited spectral data consistent with literature values (4, 5, 11, 18) and identity was established by direct comparison with (+)-syringaresinol obtained from *D. occidentalis* (5) and *Passerina vulgaris* (18).

DAPHNORETIN.—This isolate (300 mg, 0.0026% w/w), mp 245-247°, exhibited uv, ir, ^1H nmr, and ms values consistent with literature data (19) and identity was established by direct comparison with daphnoretin isolated from *Peddiea fischeri* Engl. (19).

UMBELLIFERONE.—This isolate (34 mg, 0.0003% w/w), mp 228-230°, exhibited uv, ir, ^1H-nmr, and ms values consistent with literature values (19), and its identity was established by direct comparison with umbelliferone isolated from *P. fischeri* (19).

SCREENING FOR THE PRESENCE OF SKIN-IRRITANT DITERPENE ESTERS.—An Me_2CO extract of *W. elliptica* stem bark-stem wood was partitioned between hexane and MeOH/H_2O and between MeOH/H_2O and CH_2Cl_2 as previously described (20). When a portion of the CH_2Cl_2 extract was tested on the inside ears of mice at applied doses of 10 and 20 μg/μl, according to an established protocol (15), no evidence of skin irritation was evident after 24 h. Furthermore, when

the CH_2Cl_2 extract was analyzed by droplet counter-current chromatography using a solvent system known to separate skin-irritant diterpene esters of the tigliane and ingenane types (16, 20), no evidence of such compounds was obtained after tlc analysis. Therefore, it may be concluded that, unlike *Wikstroemia monticola* Skotsberg bark and stems (16), skin-irritant diterpene esters were either not biosynthesized or were in minute concentration in the *W. elliptica* stem sample investigated in this study.

BIOLOGICAL ACTIVITY OF THE ISOLATES.—(±)-5′-Methoxylariciresinol (**1**), (±)-lariciresinol (**2**), (±)-syringaresinol, daphnoretin, and umbelliferone displayed ED_{50} values of 0.33, 0.38, 0.56, 3.4, and 3.1 μg/ml, respectively, in the P-388 lymphocytic leukemia test system in vitro (2, 3). An isolate is considered active in this system if it shows an ED_{50} of ≥4 μg/ml.

None of the isolates obtained in this study were tested for activity in the P-388 in vitro test system (2). However, in previous studies, (+)-syringaresinol, daphnoretin, and umbelliferone have proven to be inactive when tested in this manner (5, 19).

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