POTENTIAL ANTICANCER AGENTS. XVIII. CONSTITUENTS OF *AMYRIS PINNATA*[1,2]

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Abstract.—Austrobailignan-1 (**1**) was identified as the principal cytotoxic constituent of both the leaves and twigs of *Amyris pinnata* (Rutaceae). Sesalin (**3**), savinin (**4**) and β-sitosterol were also isolated from both plant parts, and imperatorin (**5**), phellopterin (**6**), sventenin (**7**), scopoletin (**2**) and heraclenol (**8**) were isolated from the twigs.

In a continuing search for tumor inhibitors from plants, methanolic extracts of the twigs and the leaves plus inflorescences of *Amyris pinnata* H. B. K. (Rutaceae) were found to possess cytotoxic activity against Eagle's carcinoma of the nasopharynx in cell culture (KB). Marginal activity was observed at 100 and 50 mg/kg against the P–388 lymphocytic leukemia system in mice.[3]

A. pinnata has not been studied previously, but several sesquiterpenes have been isolated from the wood of *Amyris balsamifera* L. (3), and the pyranocoumarin 3-(3,3-dimethylallyl)-xanthyletin has been isolated from the leaves of *A. ignea* Steyerm. (syn. *A. simplicifolia* Karst.) (4). Triterpenes and simple coumarins have been obtained from the stems and leaves of *A. madrensis* S. Wats. (5). More recently, some novel nicotinamide derivatives were obtained from *A. plumieri* DC. (6). None of these constituents however, might be expected to exhibit cytotoxic activity (7, 8).

Bioactivity-directed fractionation of the two plant parts of *A. pinnata* led to the isolation of austrobailignan-1 (**1**) (NSC–294826) and scopoletin (**2**) as the main cytotoxic constituents, exhibiting ED_{50} values of 0.027 and 2.9 μg/ml, respectively, in the 9KB cell culture system. Marginal activity was shown by **1** in the P–388 test system *in vivo* (T/C 125% at 7.5 mg/kg), but no activity was observed against the B16 melanoma test system in the dose range 1.5–12.0 mg/kg.

Austrobailignan-1 (**1**) was previously isolated only from the rare Australian shrub *Austrobaileya scandens* C. T. White (Austrobaileyaceae) (9). During the course of this work, a number of related compounds were isolated and characterized (tables 1 and 2) by comparison with available literature data or with authentic samples.

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[1]For paper XVII in this series, see ref. 1.

[2]Part of this work comprised a portion of the Ph.D. dissertation of A.A.S. accepted by the Graduate College, University of Illinois at the Medical Center, Chicago.

[3]Extracts, fractions and pure compounds were evaluated according to established protocols (2). A compound is considered active *in vivo* if it exhibits a prolongation of life in excess of 25%, and is regarded as cytotoxic if the ED_{50} is less than or equal to 4 μg/ml. For *in vivo* testing, the sample was administered intraperitoneally to six CD_2F_1 male mice over a ten-day period. Evaluation was carried out by comparison of survival time with a control group.

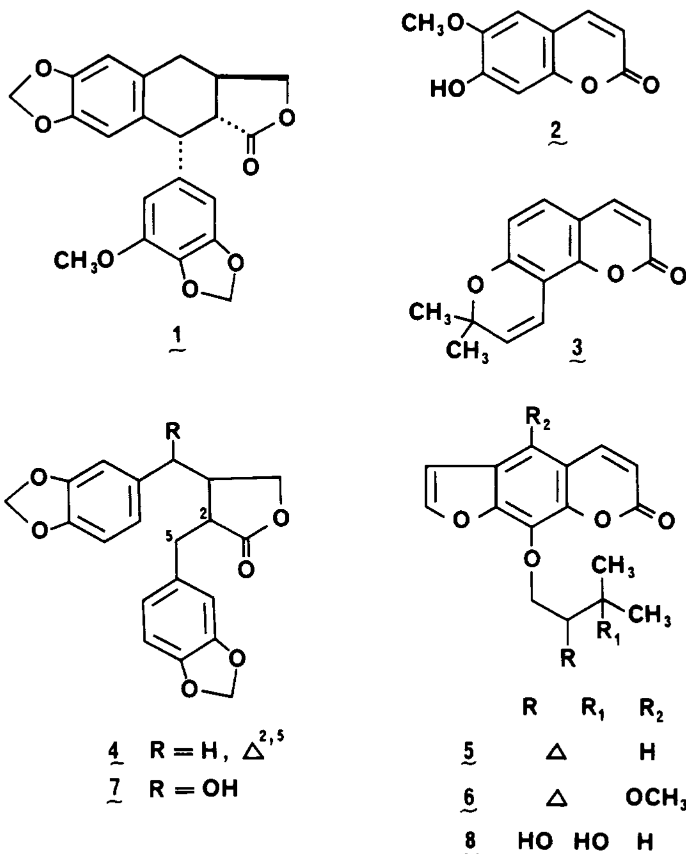
TABLE 1. Isolation of active principles from the twigs of *Amyris pinnata*.

Fraction No.	Eluent	Isolate	Yield		KB activity[a] ED_{50} µg/ml	Characterization	References
			mg	%			
1–12	Benzene	—	—	—	>100*		
13–16	Benzene-$CHCl_3$ (3:1)	Sesalin (**3**)	1300	0.0073	31.0	mp, uv, ir, pmr	10, 11
24	"	Savinin (**4**)	10	0.000057	22.0	mp, uv, ir, pmr, ms	12, 13
26–30	"	Imperatorin (**5**)	20	0.00011	33.0	mp, mmp, pmr, ms	14, 15, 16
		β-Sitosterol	22	0.00012	22.0	mp, mmp	17
36–39	"	Austrobailignan-1 (**1**)	14	0.00008	0.027	mp, mmp, uv, ir, pmr, ms, [α]D	17
		Phellopterin (**6**)	16	0.00009	7.2	mp, uv, ir, pmr, ms	15, 16
40–53	Benzene-$CHCl_3$ (1:1)	—	—	—	16.0*		
54	"	Sventenin (**7**)	10	0.00007	23.0	mp, uv, ir, pmr, ms, [α]D	13
59–61	"	Scopoletin (**2**)	15	0.000085	2.9	mp, uv, ir, pmr, ms	18
62–74	$CHCl_3$	—			22.0*		
75–97	$CHCl_3$-MeOH (99:1)	Heraclenol (**8**)	450	0.0025	>100*	mp, uv, ir, pmr, ms, [α]D	19, 20

[a]Data are for isolated compounds except where indicated by*.

TABLE 2. Isolation of active principles from leaves and inflorescences of *Amyris pinnata*.

Fraction No.	Eluent	KB activity ED_{50} µg/ml	Further Fractionation	Isolate	Yield		Characterization
					mg	%	
5–7	$CHCl_3$-C_6H_6 (2:1)	2.5	Crystallization	Sesalin (**3**)	2000	0.04	mp, uv, ir, pmr, ms
8–13	$CHCl_3$	2.9					
14–17	$CHCl_3$-MeOH (99:1)	2.1		Savinin (**4**)	100	0.002	mp, [α]D, uv, ir, pmr, ms
18–20	$CHCl_3$-MeOH (95:5)	2.0	14–23 combined, chromatographed	Austrobailignan-1 (**1**)	1130	0.025	mp, [α]D, uv, ir, pmr, ms
21–23	$CHCl_3$-MeOH (95:5)	1.6		β-Sitosterol	40	0.0008	mp, uv, ir, pmr, ms



EXPERIMENTAL[4]

PLANT MATERIAL.—Twig and leaf inflorescence samples of *Amyris pinnata* H.B.K. (Rutaceae) were collected in Colombia during March, 1973, and were provided through the auspices of the United States Department of Agriculture under contract from the National Cancer Institute. Voucher specimens representing both samples have been deposited in the Herbarium of the National Arboretum, Agricultural Research Service, U.S.D.A., Washington, D.C. These specimens were verified by Dr. R. E. Perdue, Jr., formely of the Economic Botany Laboratory, Agricultural Research Service, U.S.D.A., Beltsville, MD. Samples were milled to a coarse powder prior to extraction.

FRACTIONATION OF THE TWIG SAMPLE OF *A. pinnata*.—The dried, milled twigs of *Amyris pinnata* (Rutaceae) (17.7 kg) were thoroughly percolated with methanol (30 liters), and the extract was evaporated *in vacuo* at 40°. The total residue (94.9 g) was partitioned between $CHCl_3$ (3 x 3 liters) and water (6 liters), and the $CHCl_3$ phase was dried (Na_2SO_4), filtered, and evaporated *in vacuo* to a brown gum (23.5 g). A sample (23 g) of this fraction was chromatographed on silica gel[5] (750 g), eluted initially with benzene, and subsequently as shown in Table 1. Samples were combined on the basis of tlc comparison with an appropriate solvent system.

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[4]Melting points were determined by means of a Kofler hot plate and are uncorrected. The uv spectra were obtained with a Beckman, model DB-G grating spectrophotometer, and ir spectra with a Beckman, model IR 18-A, spectrophotometer. Proton nmr spectra were recorded in $CDCl_3$ on a Varian T-60A instrument with a Nicolet TT-7 Fourier Transform attachment, operating at 60 MHz. Tetramethylsilane was used as an internal standard. Low resolution mass spectra were obtained at 70 EV on a Perkin Elmer RMU-6D single focussing mass spectrometer or a Varian MAT 112S double focussing mass spectrometer.

FRACTIONATION OF THE LEAF-INFLORESCENCE SAMPLE OF *A. pinnata*.—A sample (5.0 kg) of *A. pinnata* leaves and inflorescences was percolated with petroleum ether (40 liters, bp 40–60°) at room temperature for four days. Removal of solvent *in vacuo* produced 78 g of a petroleum ether extractive (Fraction A, KB, ED_{50} 2.6 μg/ml). Percolation of the marc with methanol (100 liters) for seven days yielded, after evaporation of the solvent *in vacuo*, 386 g of Fraction B (KB, ED_{50} 0.42 μg/ml), which was partitioned between equal volumes of chloroform and water. Evaporation of the chloroform *in vacuo* afforded Fraction C (100 g, KB, ED_{50} 0.78 μg/ml); lyophilization yielded an aqueous soluble Fraction D (270 g, KB, ED_{50} 30.0 μg/ml).

Fraction C (100 g) was dissolved in benzene (200 ml) and chromatographed on Florisil, eluted successively with benzene (2 fractions), benzene-chloroform (1:1) (2), chloroform-benzene (2:1) (3), chloroform (6), chloroform-methanol (99:1) (4), (95:5) (10), (9:1) (2), (4:1) (4), (1:1) (10), and methanol (17). Sixty fractions (1000 ml each) were collected and monitored by tlc on silica gel G plates.[5] Fractions showing similar tlc profiles were pooled and evaluated for cytotoxic activity using the KB *in vitro* system. Subsequent isolations from the active fractions are summarized in table 2.

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