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7,9′-EPOXYLIGNAN AND OTHER CONSTITUENTS OF *ZANTHOXYLUM CULANTRILLO*

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Key Word Index—*Zanthoxylum culantrillo*; Rutaceae; 3,4-dimethoxy-3′,4′-methylenedioxy-7,9′-epoxylignan-9-ol; lignans; flavonol rhamnosides.

Abstract—Methylpiperitol, sitosterol, skimmianine, afzelin and quercitrin were isolated from the leaves of *Zanthoxylum culantrillo*, while the stem bark yielded (+)-methylpiperitol, sitosterol, lupeol, (+)-sesamin and the new tetrahydrofuran lignan 3,4-dimethoxy-3′,4′-methylenedioxy-7,9′-epoxylignan-9-ol. Their structures were elucidated by NMR, including 2D techniques and comparison with authentic samples.

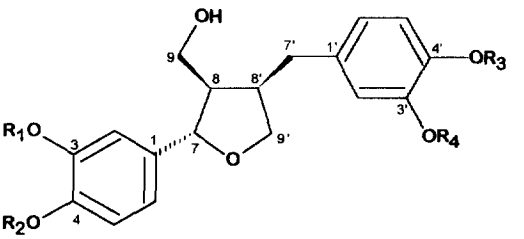
INTRODUCTION

Zanthoxylum culantrillo (H.B.K.) Krug and Urban is a medium to large tree distributed throughout Central and South America and it has many uses [1]. In Colombia, for example, leaves and bark are used by the native population of the Baudo region for the preparation of a textile dye. The only one previous chemical investigation [1] has shown the presence of eight alkaloids and two lignans. In this paper, we report on the isolation and identification of five known compounds from the leaves, and four known compounds and one new lignan (**1a**) from the stem bark.

RESULTS AND DISCUSSION

Chromatographic separation of the ethanolic extract of the leaves of *Z. culantrillo*, resulted in the isolation of five known compounds: (+)-methylpiperitol [2, 3], sitosterol [4], skimmianine [5] (also isolated in the previous work [1]), afzelin (kaempferol 3-rhamnoside) [6] and quercitrin (quercetin 3-rhamnoside) [7]. Similar treatment of the stem bark afforded four known compounds: (+)-methylpiperitol, sitosterol, lupeol [8, 9], (+)-sesamin [3, 10], as well as the new 7,9′-epoxylignan **1a**. The known compounds were characterized by spectroscopic methods and comparison with authentic samples, and/or literature data.

The new epoxylignan **1a** in its HREI mass spectrum exhibited a $[M]^+$ ion at *m/z* 372.1442 corresponding to the formula $C_{21}H_{24}O_6$ (calcd. 372.1566). Its UV spectrum did not exhibit bathochromic shift on addition of MeONa and its IR spectrum revealed the presence of a hydroxyl (3400 cm^{-1}) assignable to an alcoholic function. The ^1H NMR spectrum showed signals in the aliphatic region similar to those observed for the 7,9′-epoxylignans **1b** [11], **1c** [12], **1d** [13] and **1e** [13]: a multiplet at δ 2.43 (H-8); two nonequivalent methylene protons (H-7′a and H-7′b) at δ 2.58 (*dd*, 13.5 and 10.7 Hz) and 2.92 (*dd*, 13.5 and 5.3 Hz); a multiplet at δ 2.74 (H-8′); one oxymethylene proton δ 4.09 (*dd*, 8.3 and 7.0 Hz, H-9′a or H-9′b); a multiplet at δ 3.72–3.98 (3H, 2H-9a/b, H-9′a or H-9′b); and a doublet signal for an oxygenated benzylic proton at δ



	R_1	R_2	R_3	R_4
1a	$-CH_3$	$-CH_3$	$-CH_2-$	
1b	$-CH_2-$		$-CH_3$	$-CH_3$
1c	$-CH_2-$		$-CH_2-$	
1d	$-CH_3$	$-CH_3$	$-CH_3$	$-CH_3$
1e	$-CH_3$	$-CH_3$	$-OH$	$-CH_3$

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Table 1. ^{13}C NMR spectral data for **1(a–e)***

C	**1a**	**1b** [11]	**1c** [11, 12]	**1d** [11, 14]	**1e** [3, 15]
1	135.3	137.0	137.1	135.9	135.2
2	108.8[a]	106.3	106.2	108.9	108.8
3	149.0	147.8[a]	147.8	149.0	148.8
4	148.3	147.4[a]	147.8	148.9	148.2
5	110.9	108.0	108.2[a]	110.9[a]	111.0[a]
6	117.9	119.1	119.0	117.9	117.8
7	82.8	82.8	82.8	82.7	82.6
8	52.4	52.7	52.6	52.5	52.4
9	60.9	60.9	60.8	60.8	60.8
1′	134.1	132.9	134.2	132.9	132.0
2′	108.3[a]	111.3[b]	108.0[a]	111.3[a]	110.9[a]
3′	147.7	148.9	145.9	148.4	146.4
4′	145.9	146.9	145.3	147.4	143.8
5′	108.9[a]	111.9[b]	108.9	111.9[a]	114.2
6′	121.4	120.5	121.3	120.5	120.9
7′	33.3	33.1	33.3	33.2	33.2
8′	42.3	42.3	42.3	42.3	42.3
9′	72.8	72.9	72.9	72.9	72.7
—OCH_3	55.8	55.9	—	55.8	55.8
	55.9			55.9	
—OCH_2O—	100.9	100.9	100.9	—	—
			100.8		

[a,b] Assignments may be reversed.

* 25.2 MHz for **1c** and **1e**, 50.3 MHz for **1b** and **1d**, and 75.0 MHz for **1a**, in $CDCl_3$, TMS.

4.84 (6.3 Hz, H-7). Additionally, its ^1H NMR spectrum showed the presence of a methylenedioxy group (δ 5.96, *s*, 2H), two methoxyl groups (δ 3.89, 3.92, 2*s*, 3H each) and six aromatic protons (δ 6.64–6.92), indicating two trisubstituted aromatic rings. The EI-mass spectral fragment at *m/z* 135 (100%) assignable to a 3,4-methylenedioxytropylium ion and the fragment at *m/z* 151 (23%) of the 3,4-dimethoxybenzoyl ion; suggested the location of the piperonyl group at C-7′ and the veratryl group at C-7, contrary to that reported for compound **1b** [11]. The ^{13}C NMR spectral data (Table 1) obtained for the aliphatic carbons of **1a** are in good agreement with those of the 7,9′-epoxylignans **1b–1e**. The chemical shifts for aromatic carbons of **1a** were assigned by comparison with those of dihydrosesamin (**1c**) (C-1′ to C-6′) [11, 12], lariciresinol dimethyl ether (**1d**) (C-1 to C-6) [11, 14] and lariciresinol 4-monomethyl ether (**1e**) (C-1 to C-6) [3, 15], as well as by the selective INEPT experiments. Selective irradiation of the methylenedioxy signal (δ 5.96) enhanced the resonances at δ 147.7 and 145.9 while irradiation of both methoxyl signals (δ 3.89 and 3.92) enhanced the resonances at δ 149.0 and 148.3. The close agreement of the ^1H NMR chemical shifts and coupling constants in conjunction with the positive optical rotation suggests for **1a** the 7*S*,8*R*,8′*R* configuration, similar to **1b**.

The genus *Zanthoxylum s. l.* is well known for the production of benzo[*c*]phenantridine alkaloids such as dihydrochelerythrine and chelerythrine [16], and it is somewhat strange that this species, *Z. culantrillo*, does not appear to produce this type of alkaloid. These alkaloids are well known cytotoxins/antibiotics and the lack of antimicrobial activity in the crude extracts of the leaf and stem bark of *Z. culantrillo* bears this out.

EXPERIMENTAL

General. Mps: uncorr.; ^1H NMR: 300 and 600 MHz in $CDCl_3$ with TMS as int. standard; ^{13}C NMR: 75 MHz; EI-MS: direct inlet system at 70 eV; UV: in MeOH; CC: Silica gel 60: silica gel (Kieselgel 60, Merck). TLC: silica gel G 60 HF_{254}.

Plant material. A sample of leaves and stem bark of *Zanthoxylum culantrillo* (H.B.K.) Krug and Urban. was collected at Guandal (Urabá-Antioqueño) Colombia, in August 1992 by Dr Rodrigo Caballero. A voucher specimen, No. 034583, is deposited at the Herbarium MEDEL, Universidad Nacional seccional Medellín, Colombia.

Extraction and isolation. Dried and powdered leaves (1.7 kg) were exhaustively extracted with EtOH at room temp. The thick viscous residue (10.5 g) was suspended in H_2O and extracted with $CHCl_3$, EtOAc and *n*-BuOH, successively. Extended CC (silica gel; C_6H_6–EtOAc, 9:1) of the $CHCl_3$- soluble portion (3.14 g) gave sitosterol (380 mg), (+)-methylpiperitol (205 mg) and skimmianine (52 mg). Purification (silica gel; $CHCl_3$–MeOH, 9:1, and Sephadex LH20; MeOH) of

the EtOAc (0.70 g) and *n*-BuOH (3.30 g) extracts afforded afzelin (44 mg) and quercitrin (51 mg).

The EtOH extract (10 g) from the stem bark (2.5 kg) was fractionated by CC with a gradient of EtOAc in C_6H_6 to give sitosterol (400 mg), lupeol (1.16 g), fatty material, and a mixt. of methylpiperitol, sesamin and **1a**. Further purification of the mixt. by centrifugal PTLC (C_6H_6–EtOAc, 19:1), followed by CC (silica gel; C_6H_6–EtOAc, 4:1) yielded (+)-methylpiperitol (232 mg), (+)-sesamin (91 mg) and **1a** (48 mg).

Biological activity. In a preliminary screening for biological activity, the EtOH extracts of leaves and stem bark were tested against four bacteria (*Enterococcus faecalis* 29212, *Staphylococcus aureus* ATCC 65380, *Salmonella tiphymurium* 14028s and *S. tiphymurium* MS 7953), but were found to be inactive.

Identification of known compounds. Sitosterol, lupeol, (+)-sesamin, (+)-methylpiperitol, skimmianine, afzelin and quercitrin were identified by comparison (TLC, NMR) with authentic specimens available in our laboratory.

(7S,8R,8′R)-3,4-*dimethoxy*-3′,4′-*methylenedioxy*-7,9′-*epoxylignan*-9-*ol* (**1a**). Viscous oil; $[\alpha]_D^{25}$ +14 ($CHCl_3$, *c* 1.70); IR ν_{max}^{film} cm^{-1}: 3400, 2850, 1610, 1512, 1465, 1144, 992 and 826; UV λ_{max}^{MeOH} nm (log ε): 229 (3.06), 281 (2.98), $\lambda_{max}^{MeOH+MeONa}$ no change; High Resolution MS: Found $[M]^+$ *m/z* 372.1442 $C_{21}H_{24}O_6$, requires 372.1566; EIMS (70 eV) (rel. int.) *m/z*: 372 $[M]^+$ (15), 151 (23), 135 (100). ^1H NMR (600 MHz $CDCl_3$, TMS): δ 2.43 (1H, *m*, H-8), 2.58 (1H, *dd*, J = 13.5, 10.7 Hz, H-7′a or H-7′b), 2.74 (1H, *m*, H-8′), 2.92 (1H, *dd*, J = 13.5, 5.3 Hz, H-7′a or H-7′b), 3.72–3.98 (3H, *m*, 2H-9, H-9′a or H-9′b), 3.89 (3H, *s*, OMe), 3.92 (3H, *s*, OMe), 4,09 (1H, *dd*, J = 8.3, 7.0 Hz, H-9′a or H-9′b), 4.84 (1H, *d*, J = 6.3 Hz, H-7), 5.96 (2H, *s*, —OCH_2O—), 6.66 (1H, *dd*, J = 7.9, 1.0 Hz, H-6′), 6.71 (1H, *d*, J = 1.0 Hz, H-2′), 6.76 (1H, *d*, J = 7.9 Hz, H-5′), 6.82–6.92 (3H, *m*, H-2,5,6); ^{13}C NMR (75 MHz, $CDCl_3$): Table 1.

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