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ANTITUMOR AGENTS, 81.[1] JUSTICIDIN-A AND DIPHYLLIN, TWO CYTOTOXIC PRINCIPLES FROM *JUSTICIA PROCUMBENS*

NARIHIKO FUKAMIYA and KUO-HSIUNG LEE*

Natural Products Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27514

The whole plant of *Justicia procumbens* L. (Acanthaceae), known as "Chu Wei Hung" or "Jué Chuáng" in Chinese folklore, is used as an herbal remedy for the treatment of fever, pain due to pharyngo-laryngeal swelling (1-3), and cancer (4). Prior phytochemical studies on this plant have afforded justicidin-C (**4**) and justicidin-D (**3**) (5). As a result of our continuing searches among Chinese medicinal plants for novel, naturally occurring potential antitumor agents (6), the methanolic extract of the whole plant of *J. procumbens* was found to show significant inhibitory activity in vivo against P-388 lymphocytic leukemia growth in BDF_1 male mice (T/C=150% at 50 mg/kg/day, IP) as well as in vitro cytotoxicity in the 9-KB (human nasopharyngeal carcinoma) cell culture assay (7). Bioassay-directed fractionation of the foregoing MeOH extract led to the isolation of five 2,3-naphtalide lignans, which were identified as justicidin-A (**1**), diphyllin (**2**), neojusticin-A (i.e., justicidin-D) (**3**), neojusticin-B (i.e., justicidin-C) (**4**), and justicidin-E (**5**) by spectral analysis and direct comparison with authenic samples of the first four compounds (8,9) as well as data for the last compound reported in the literature (10). Compounds **1-4** were previously isolated from *J. procumbens* var. *leucantha* (8,9). Compound **1** was also reported as a fish-killing component of *Justicia hayatai* var. *decumbens* (11).

A comparison of the cytotoxicity (KB) clearly indicated that the γ-lactone ring carbonyl α to C-3 instead of C-2 is required for potent cytotoxicity. Thus, **1**, and **2**, were active [ED_{50} (KB)<1.0 μg/ml each],[2] while **3-5** were inactive [ED_{50} (KB)=9.0 μg/ml for **3** and >10.5 μg/ml for **4** and **5** each].

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer 257 grating spectrophotometer. ^{1}H-nmr spectra were recorded on a Bruker WM-250 Fourier Transform spectrometer and are given in parts per million (δ) downfield from an internal TMS standard. The abbreviations s and d refer to singlet and doublet, respectively. Mass spectra were determined on an A.E.I. MS-902 instrument at 70 eV using a direct inlet system. Silica gel for column chromatography refers to Merck silica gel 60 (70-230 mesh). Silica gel for preparative tlc refers to Analtech silica gel G (1000 microns). Compounds were visualized by uv light or spraying with 1% $Ce(SO_4)_2$-10% H_2SO_4 solution followed by heating.

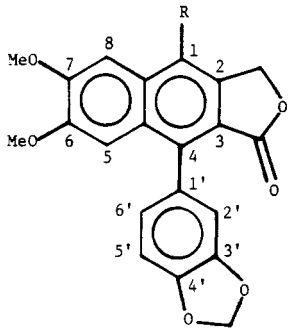
PLANT MATERIAL.—The *J. procumbens* used was from a collection made in the spring of 1979 in Jui-Li, Chia-Yi-Shen, Taiwan, by Professor Huan-Chang Huang. A voucher specimen (HCH-73) is available for inspection at the herbarium of the School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan.

PRELIMINARY EXTRACTION.—The ground, air-dried whole plant (2.27 kg) was exhaustively extracted with hexane and then MeOH. The MeOH extract (132 g) was partitioned between $CHCl_3$ and H_2O. The aqueous layer was extracted with BuOH. The hexane, *n*-BuOH, and the aqueous layer were not examined further as they were not active in the in vitro KB assay.

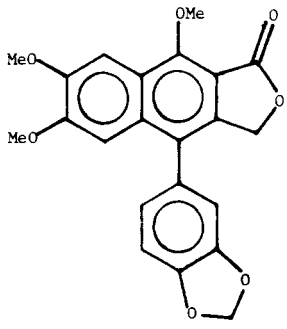
ISOLATION OF JUSTICIDIN-A (**1**), DIPHYLLIN (**2**), NEOJUSTICIN-A (**3**), NEOJUSTICIN-B (**4**),

[1]For Part 80, see G.K. Rice, T. Yokoi, T. Hayashi, H. Suzuki, A.T. McPhail, and K.H. Lee, *J. Chem. Soc. Chem., Commun.*, (submitted).

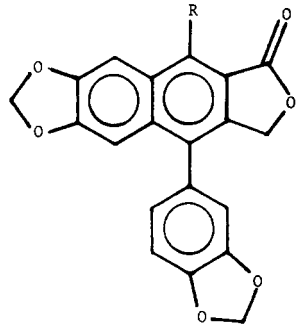
[2]The cytotoxic lignans isolated from *J. procumbens* are **1** and **2**, instead of **3** and **2** as reported by Lee (6), p. 361. The structure of diphyllin reported as **30** in the foregoing References 6 was erroneous and has to be revised to **2**.



1 R=OMe
2 R=OH



4



3 R=OMe
5 R=H

AND JUSTICIDINE-E (**5**).—The KB active $CHCl_3$ extract (36.1 g) was chromatographed on silica gel (2.3 kg) and eluted with $CHCl_3$ (15 liters), Me_2CO (8 liters), and MeOH (8 liters). All fractions were monitored by the foregoing KB cytotoxicity assay. The active $CHCl_3$ fractions (4.2 g) were combined and rechromatographed on silica gel (1.5 kg). Elution of the column with C_6H_6 and C_6H_6 with increasing amount of EtOAc gave six active fractions. Purification of these fractions with preparative tlc [silica gel, $CHCl_3$-Me_2CO (5:1)] yielded five lignans (**1-5**)[3], in which **1** and **2** were cytotoxic while **3-5** were inactive as mentioned above.

Compound **1** (8.1 mg, 0.00036% yield): mp 261-263°; Okigawa *et al.* (8) reported mp 261-263°; ir ($CHCl_3$) 1758 and 1615 cm^{-1}; ^1H nmr ($CDCl_3$) δ 3.80 (3H, s) 4.08 (3H, s), 4.12 (3H, s) (3 x OMe), 5.55 (2H, s, CH_2OCO), 6.04 (1H, d, J=1.0 Hz), 6.06 (1H, d, J=1.0 Hz) (OCH_2O), 6.77 (1H, dd, J=8.0 and 2.0 Hz, H-6′), 6.81 (1H, overlappped d, J=2.0 Hz, H-2′), 6.95 (1H,d, J=8.0 Hz, H-5′), 7.09 (1H, s, H-8) and 7.55 (1H, s, H-5). Compound **1** was identical by tlc, mmp, ir, and nmr spectra with an authenic sample of justicidin-A.

[3]Compounds **1-5** showed Rf values 0.53, 0.25, 0.58, 0.53, and 0.47, respectively, on silica gel in C_6H_6-EtOAc (6:1).

Compound **2** (3.2 mg, 0.00014% yield): mp 284-287°; Okigawa *et al.* (8) reported mp 284-287°; ir (KBr) 3300 and 1730 cm^{-1}; ^1H nmr Me_2CO-d_6) δ 3.71 (3H, s), 3.97 (3H, s) (2 x OMe), 6.05 (2H, s-like, OCH_2O) and 7.67 (1H, s, H-5). This compound was identical with an authenic sample of diphyllin by direct comparision (tlc, mmp, ir, and nmr spectra).

Compound **3** (12.9 mg, 0.00057% yield): mp 273-275°; Okigawa *et al.* (8) reported mp 273-275°; ir ($CHCl_3$) 1755 and 1600 cm^{-1}; ^1H nmr ($CDCl_3$) δ 4.35 (3H, s, OMe), 5.15 (2H, d, J=3.2 Hz, CH_2OCO), 6.09 (4H, m, two OCH_2O, 6.72 (1H, dd, J=8.0 and 2.0 Hz, H-6′), 6.78 (1H, overlapped d, J=2.0 Hz, H-2′), 6.95 (1H, d, J=8.0 Hz, H-5′), 7.00 (1H, s, H-8) and 7.69 (1H, s, H-5). The identity of **3** with an authenic sample of neojusticin-A was established by direct tlc, mmp, ir, and nmr spectral comparison.

Compound **4** (10.2 mg, 0.00045% yield): mp 262-265°; Okigawa *et al.* (8) reported mp 262-265°; ir ($CHCl_3$) 1760 and 1610 cm^{-1}; ^1H nmr ($CDCl_3$) δ 3.84 (3H, s, C_7-OMe), 4.07 (3H, s, C_6-OMe), 4.32 (3H, s, C_1-OMe), 5.15 (2H, s, CH_2OCO), 6.08 (1H, d, J=1.0 Hz), 6.09 (1H, d, J=1.0 Hz) (OCH_2O), 6.80 (1H, dd, J=8.0 and 2.0 Hz, H-6′), 6.83 (1H, overlapped d, J=2.0 Hz, H-2′), 6.90 (1H, d, J=8.0 Hz, H-5′), 6.98 (1H, s, H-8) and 7.69 (1H, s, H-5).

This compound was identical with an authenic sample of neojusticin-B by direct comparison (tlc, mmp, ir, and nmr).

Compound **5** (4.5 mg, 0.0002% yield): mp 271-272°; Wada *et al.* (10) reported mp 265-269°; ir ($CHCl_3$), 1758 (γ-lactone) and 1598 (aromatic ring) cm^{-1}; ^1H nmr ($CDCl_3$)[4] δ 5.19 (2H, d, J=3.2 Hz, CH_2OCO), 6.08 (4H, m, two OCH_2O), 6.77 (1H, dd, J=8.0 and 2.0 Hz, H-6′), 6.81 (1H, overlapped d, J=2.0 Hz, H-2′), 6.98 (1H, d, J=8.0 Hz, H-5′), 7.10 (1H, s, H-8), 7.30 (1H, s, H-5) and 8.24 (1H, s, H-1); ms *m/z* 348.0639 (M^+, 100%; calcd for $C_{20}H_{12}O_6$: *m/z* 348.0632). The comparable melting point of **5** with justicidin-E, coupled with the foregoing spectral data which are consistent with structure **5**, led to the assignment of **5** as justicidin-E (12).

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[4]The different chemical shifts and splitting pattern observed between **5** and neojusticidin-E reported in Reference 10 might be due to the use of different solvents and instruments, in which the former was run in $CDCl_3$ at 250 MHz whereas the latter was measured in DMSO at 100 MHz instrument..