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THE ISOLATION AND STRUCTURE OF THREE NEW LIGNANS FROM *JUSTICIA PROCUMBENS* LINN. VAR. *LEUCANTHA* HONDA

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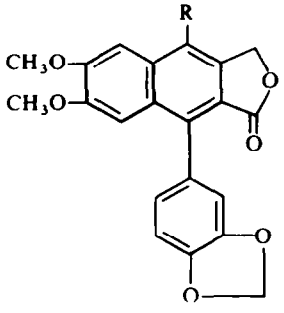
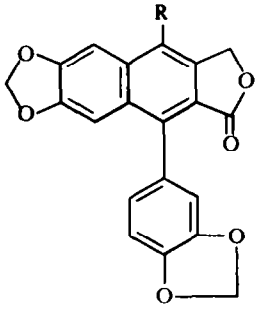
Abstract—Six lignans were isolated from *Justicia procumbens* var. *leucantha*. Three of them, neojusticin A (V) and B (VI) and taiwanin E methyl ether (IV), are new compounds: the structure VI was verified by comparison with a synthetic sample. The others were justicidin A (I) and B (II) and diphyllin (III). Neojusticin A and B are the first examples of naturally occurring 1-oxygenated-2,3-naphthalide lignans.

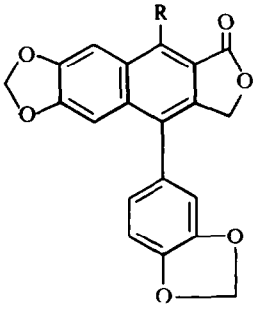
MUNAKATA *et al.* reported[1] fish-killing lignan components, justicidin A (I) and B (II) from the Formosan plant, *Justicia Hayatai* var. *decumbens* (Acanthaceae). There has been little study[2] of the ingredients of the Japanese plant, *J. procumbens* Linn. var. *leucantha* Honda (Japanese name, kitsunenomago). We have however isolated six



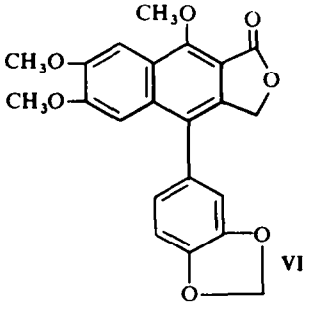
I R = OCH_3
II: R = H
III: R = OH



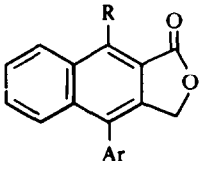
IV: R = OCH_3
VII: R = OH



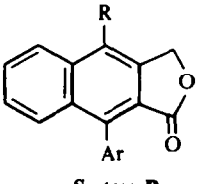
V: R = OCH_3
VIII: R = OH



VI



System A



System B

lignans from this plant.* They are justicidin A[3] and B,[1b] diphyllin (III),[3, 5] taiwanin E methyl ether (IV) and the newly named neojusticin A† (V) and B (VI), the last three being new compounds.

These lignans were isolated from the ether extract of the dried plant by column chromatography and preparative thin layer chromatography (TLC) as described in the experimental part. Diphyllin (III) was identified by comparison with the authentic sample (IR and NMR) and was methylated with diazomethane to give justicidin A (I). The UV, IR and NMR spectra of justicidin A and B were in good accord with the reported data.[1b, 3]

The formula $C_{21}H_{14}O_7$ of neojusticin A (V), m.p. 273–275° followed from mass spectral (M^+ 378·074) and elemental analysis. The IR spectrum contained bands associated with an aromatic-γ-lactone (1760 cm^{-1}) and a methylenedioxy ether (933 cm^{-1}). The UV spectrum contained the same absorption bands as that of justicidin A. The NMR spectrum contained the signals of five aromatic protons (7·70 (s), 6·99 (s), 6·95 (d), 6·78 (s) and 6·72 ppm (d)). These observations implied a similar substitution pattern for neojusticin A to that of justicidin A. The NMR signal of the γ-lactone methylene group (5·10 ppm) was shifted to higher field than in justicidin A (5·47 ppm), which suggested anisotropic effects of an aryl nucleus whose orientation is perpendicular to the naphthalene ring. Horii *et al.*[4] reported that the NMR signal of a lactone methylene proton in system **A** appears between 5·08 and 5·23, whereas it is 5·32–5·52 ppm in system **B**. The data listed in Table 1 show that neojusticin A and B should belong to system **A** but the others to system **B**. Further evidence to support the assignment of system **A** to neojusticin A and B is given by the methoxy signals (4·31 and 4·37 ppm) of these compounds, found in much lower field than the others because of the deshielding effect of lactone carbonyl.

TABLE 1. NMR SIGNALS OF LACTONE METHYLENE, METHOXY AND METHYLENEDIOXY GROUPS

Compounds	$-CH_2-O-CO-$	$-OCH_3$	$-O-CH_2-O-$
Neojusticin A (V)	5·10	4·31	6·06 (4H)
Neojusticin B (VI)	5·11	4·37, 4·05, 3·83	6·07 (2H)
Justicidin A (I)	5·47	4·08, 4·02, 3·77	5·99 (2H)
Justicidin B (II)	5·36	4·04, 3·81	6·05 (2H)
Diphyllin (III)	5·34	3·97, 3·71	6·05 (2H)
Taiwanin E methyl ether (IV)	5·49	4·07	6·07 (4H)

Diphyllin was measured in CD_3COCD_3 and the others in $CDCl_3$.

Neojusticin B (VI), $C_{22}H_{18}O_7$ (M^+ 394·104), m.p. 262–265° belongs to system **A** since it exhibits spectral data very similar to those of neojusticin A except that two methoxy groups (3·83 and 4·05 ppm) appear instead of the one methylenedioxy (6·06 ppm) of neojusticin A (Table 1). Of the two possible structures (VI and an isomer in which two methoxy and a methylenedioxy are interchanged) formula VI is preferable

* Recently, Munakata *et al.* have isolated seven lignans from the same plant (private communication), but the results were obtained by entirely independent work.

† This compound was reported as neojusticin in a preliminary communication (Chem. Pharm. Bull. Tokyo **18**, 862 (1970)).

since a mass peak (*m/e* 121·027) is found caused by the methylenedioxyphenyl group of formula VI in the mass spectrum (methylenedioxyphenyl cation, $C_7H_5O_2^+$ has mass 121·029). This formula (VI) is that of the structure of justicidin A formerly proposed by Govindachari[6] and synthesized by Horii.[3] The identity of both the natural and synthetic compounds was fully established by direct comparison.

Taiwanin E has been reported as a fifth minor constituent of heartwood extract obtained from the tree *Taiwania cryptomeriodes* Hayata (Taxodiaceae).[7] Neojusticin A (V) has the structure of the methyl ether of VIII formerly proposed as the structure of taiwanin E. However, the structure of taiwanin E was recently revised[4] from VIII to VII. The spectral data from a compound that we obtained, $C_{21}H_{14}O_7$ (M^+ 378·071), m.p. 227–230°, revealed that it should be taiwanin E methyl ether (IV), having one methoxy (4·07 ppm), two methylenedioxy (6·07 ppm) and an γ-lactone methylene (5·49 ppm suggesting system **B**) group and the same substitution pattern (NMR spectrum of aromatic protons and UV spectrum).

Neojusticin A (V) and B (VI) are the first examples of naturally occurring 1-oxygenated-4-aryl-2,3-naphthalides; the others are 1-aryl-4-oxygenated-2,3-naphthalides except justicidin B. The fish-killing activity of these compounds will be reported elsewhere.

EXPERIMENTAL

All m.ps were measured on a Yanagimoto micro-melting point determination apparatus and are uncorrected. NMR spectra were recorded on a Hitachi H-60 instrument. Chemical shifts are expressed as parts per million (ppm) with TMS as internal standard. Mass spectra (MS) were determined on a JEOL JMS-OISG double focus high resolution spectrometer with a direct inlet system and operating at an ionization energy of 75 eV. IR spectra were taken with a Nihon-Bunko DS-301 spectrometer, UV spectra on a Hitachi ESP-2 recording spectrophotometer. TLC analysis was performed on silica gel G according to Stahl (Merck) and using ether as a developing agent. For column chromatography, standardized aluminium oxide (Merck) and Mallinckrodt silicic acid (100 mesh) were used.

Isolation

The dried and cut plants (3 kg) of *Justicia procumbens* Linn. var. *leucantha* Honda. collected in August at Nagasaki, were extracted with boiling ether three times (10 l/each). The combined ether extract on concentration to about 500 ml gave pale brown ppts (mixture A, 9 g) and a dark green filtrate (F1). The mixture A (3·5 g) was treated with boiling 0·5 N NaOH in EtOH (80 ml) for 40 min. Water was added and insoluble materials filtered off. The filtrate was washed with ether, acidified with HCl and allowed to stand for two days, giving pale brown ppts (mixture B, 1·3 g). A CH_2Cl_2 soln of mixture B (820 mg), on the addition of ether gave ppts (mixture C, 580 mg), in which five components were detected by TLC. R_f: 0·68 (neojusticin A), 0·61 (neojusticin B), 0·55 (taiwanin E methyl ether), 0·43 (justicidin A) and 0·37 (justicidin B). The CH_2Cl_2–Et_2O mother liquor, separated from mixture C on evaporation under reduced press, gave a pale yellow residue (210 mg), chromatography of which on 100 g of silica gel, eluting with ether, gave neojusticin A (18 mg), justicidin A (10 mg) and justicidin B (6 mg). Two other components were detected (R_f: 0·61 and 0·55), but in too small amounts to isolate.

Neojusticin A (V)

Colourless needles from $CHCl_3$–Et_2O, m.p. 273–275°; MS, 378·074. (Found: C, 66·26; H, 3·70. Calc for $C_{21}H_{14}O_7$: C, 66·67; H, 3·73%; MW, 378·074). NMR ($CDCl_3$): 4·31 (3H, s, methoxy), 5·10 (2H, s, lactone methylene), 6·06 (4H, s, two methylenedioxy groups), 6·72 (1H, d, J = 7 cs), 6·78 (1H, s), 6·95 (1H, d, J = 7 cs), 6·99 (1H, s) and 7·70 (1H, s, five aromatic hydrogens). UV ($CHCl_3$) mμ (log ε): 263·5 (4·69), 298 (4·01), 319 (4·03), 355 (3·47). IR (KBr) cm^{-1}: 1760 (γ-lactone), 1605 (aromatic), 933 (methylenedioxy), 875, 807.

Justicidin A (I)

Colourless needles from $CHCl_3$–Et_2O, m.p. 261–263°; MS, 394·100. (Found: C, 66·68; H, 4·57. Calc for $C_{22}H_{18}O_7$: C, 67·00; H, 4·60%; MW, 394·105). NMR ($CDCl_3$): 3·77, 4·02, 4·08 (3H each, s), 5·47 (2H, s), 5·99 (2H, q, J = 1 cs) 6·69 (1H, d, J = 8 cs), 6·75 (1H, s), 6·98 (1H, d, J = 8 cs), 6·99 (1H, s), 7·47 (1H, s). UV ($CHCl_3$) mμ (log ε): 265 (4·75), 296 (4·06), 314 (4·07), 355 (3·86). IR (KBr) cm^{-1}: 1748, 1595, 925, 871, 809. This compound is identical with the methyl ether of diphyllin in all respects

Justicidin B (II)

Colourless plates from ethyl acetate–benzene, m.p. 235–238°; MS, 364·092. (Found: C, 68·98; H, 4·35. Calc for $C_{21}H_{16}O_6$: C, 69·22; H, 4·43%; MW, 364·095). NMR ($CDCl_3$): 3·81, 4·04 (3H each, s), 5·36 (2H, s), 6·05 (2H, q, J = 1 cs), 6·79 (1H, d, J = 8 cs), 6·85 (1H, s), 6·96 (1H, d, J = 8 cs), 7·10 (1H, s), 7·18 (1H, s), 7·69 (1H, s). UV ($CHCl_3$) mμ (log ε): 260 (4·78), 295 (4·13), 310 (4·13), 350 (3·41). IR (KBr) cm^{-1}: 1760, 1620, 931, 873, 811.

Taiwanin E methyl ether (IV)

Mixture C (150 mg) was chromatographed on silica gel (100 g) eluting with ether. Early fractions gave neojusticin A (8 mg). The following fractions afforded a mixture (15 mg) of taiwanin E methyl ether, neojusticin A and B, which was purified by preparative TLC to give taiwanin E methyl ether (5 mg), m.p. 227–230°. TLC R_f: 0·55. MS, found: 378·071; Calc for $C_{21}H_{14}O_7$: 378·074. NMR ($CDCl_3$): 4·07 (3H, s, methoxy), 5·49 (2H, s, lactone methylene), 6·07 (4H, s, two methylenedioxy groups), 6·75 (1H, d, J = 8 cs), 6·78 (1H, s), 6·94 (1H, d, J = 8 cs), 7·06 (1H, s), 7·54 (1H, s, five aromatic protons). UV ($CHCl_3$) mμ (log ε): 265 (4·53), 297·5 (3·97), 321 (3·92), 355 (3·61). IR (KBr) cm^{-1}: 1755 (γ-lactone), 1605 (aromatic), 930 (methylenedioxy), 845, 795.

Diphyllin (III)

F1 on evaporation *in vacuo* gave a dark green residue (mixture D, 29 g), 24 g of which was chromatographed on silica gel (350 g) eluting with CH_2Cl_2 (2 l.), 2:1 CH_2Cl_2–Et_2O (500 ml), and Et_2O successively. The CH_2Cl_2 portion gave a semi-solid mass which was not further studied. Diphyllin was isolated from the later ether fractions. TLC R_f: 0·28. Pale yellow needles (120 mg) from EtOH, m.p. 284–287°; MS, 380·082. (Found: C, 65·89; H, 4·17. Calc for $C_{21}H_{16}O_7$: C, 66·31; H, 4·24%; MW, 380·090. NMR (CD_3COCD_3): 3·71, 3·97 (3H each, s), 5·34 (2H, s), 6·05 (2H, s), 6·76 (1H, d, J = 8 cs), 6·82 (1H, s), 7·13 (1H, d, J = 8 cs), 7·32 (1H, s), 7·67 (1H, s). UV ($CHCl_3$) mμ (log ε): 262 (4·54), 269 (4·56), 292 (3·98), 320 (3·94), 355 (3·72). IR (KBr) cm^{-1}: 3300 (broad), 1723, 1620, 927, 855, 810. IR and NMR spectra of this comp were identical with those of the authentic sample of diphyllin. On methylation with CH_2N_2 in ether soln followed by purification by passing through alumina (5 g), 15 mg of III gave justicicin A (10 mg).

Neojusticin B (VI)

Mixture D (40 g) was treated with boiling 0·5N NaOH in EtOH (800 ml) for 50 min and concentrated *in vacuo* to approx 100 ml. Water was added and the soln was extracted with CH_2Cl_2. The aqueous layer was acidified with HCl and again extracted with CH_2Cl_2. The latter CH_2Cl_2 extract, on evaporation, gave a dark green viscous residue (27 g), 10 g of which was subjected to chromatographic separation on silica gel (300 g) using ether as an eluting solvent. Neojusticin A (42 mg) was obtained from earlier fractions and from later fractions neojusticin B, which on purification by passing a $CHCl_3$ soln over alumina (4 g) followed by recrystallization from $CHCl_3$–Et_2O gave colourless needles (45 mg), m.p. 262–265°; MS, 394·105. TLC R_f: 0·61. (Found: C, 66·51; H, 4·43. Calc for $C_{22}H_{18}O_7$: C, 67·00; H, 4·60%; MW, 394·105). NMR ($CDCl_3$): 3·83, 4·05, 4·37 (3H each, s, three methoxy groups), 5·11 (2H, s, lactone methylene), 6·07 (2H, s, methylenedioxy), 6·77 (1H, d, J = 8 cs), 6·82 (1H, s), 6·98 (1H, s), 6·98 (1H, d, J = 8 cs), 7·69 (1H, s, five aromatic protons). UV ($CHCl_3$) mμ (log ε): 264·5 (4·75), 320 (4·05), 355 (3·75). IR (KBr) cm^{-1}: 1745 (γ-lactone), 1605 (aromatic), 930 (methylenedioxy), 865, 807. This compound was identical with 1,6,7-trimethoxy-4-(3',4'-methylenedioxy)-phenyl-2,3-naphthalide (VI), m.p. 264–267°, synthesized by Horii *et al.*[3] (mixed m.p., IR and NMR).

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