LIGNANS FROM *JUSTICIA FLAVA*

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ABSTRACT.—Three lignans have been isolated from the leaves of *Justicia flava* Vahl. Two of these have been identified as helioxanthin (**1**) and (+)-isolariciresinol (**5**). The structure of the third lignan (**2**), for which the name justicinol is proposed, has been established primarily by spectral correlation to helioxanthin (**1**) and by chemical interconversions to common derivatives. In addition, docosanoic acid and β-sitosterol-β-D-glucoside have been isolated and identified.

A number of arylnaphthalide lignans have previously been reported (1–5) in three *Justicia* species, viz: *J. hayati* var. *decumbens*, *J. procumbens* var. *leucantha*, and *J. simplex*. In a previous report (6) we described the isolation and identification of four sterols and salicylic acid from the petroleum ether extracts of the leaves, stems, and roots of *J. flava* Vahl (Acanthaceae). As part of our studies on Nigerian plants of medicinal interest, and in continuation of our investigation on *J. flava*, we have examined the methanolic extract of the leaves of this plant. The present paper reports on the isolation and the structural elucidation of a new lignan named justicinol (**2**), together with the previously known lignans (**1**) and (**5**) from this plant.

RESULTS AND DISCUSSION

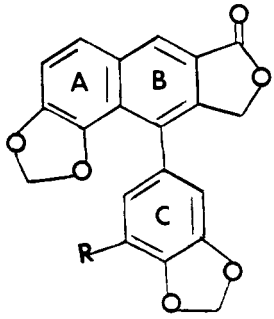
The acidic fraction of the methanolic extract of the leaves of *J. flava* was purified by column chromatography to yield a number of fractions. Fraction A gave a dark green semi-solid (0.3 g), which was shown on tlc to be a mixture of several compounds; this fraction has not been further investigated.

Fraction B yielded a solid, which was homogeneous on tlc and was designated compound **1**. It crystallized from methanol-chloroform, and high resolution mass spectral analysis gave the molecular formula $C_{20}H_{12}O_6$ for this compound. Its solution gave a strong blue fluorescence and a positive Labat's test for the methylenedioxy group (7). Ir absorption peaks at 1750 and 930 cm^{-1} suggested the presence of γ-lactone and methylenedioxy groups, respectively. Comparison of the spectral data (uv, ir, ms) of this compound with those published for helioxanthin (8) suggested their identity. A study of the pmr of the compound lent support to this view. Signals in the pmr spectrum of compound **1** were observed at δ 8.42 (1H, *s*, H–4), 7.70 (1H, *d*, $J=8$Hz, H–5), 7.30 (1H, *d*, $J=8$Hz, H–6), 6.86–6.73 (3H, *m*, H–2', 5', 6'), 6.08 (2H, *d*, $J=1$Hz, ring C methylenedioxy), 5.96 (2H, *s*, ring A methylenedioxy) and 5.21 (2H, *s*, lactone methylene). Direct comparison (mmp, co-tlc, ir, ms) of compound **1** with an authentic sample[1] established its identity as helioxanthin (**1**). Helioxanthin (**1**) had previously been isolated from two plant sources (8, 9). This, however, is the first reported isolation of helioxanthin from a genus of the family Acanthaceae.

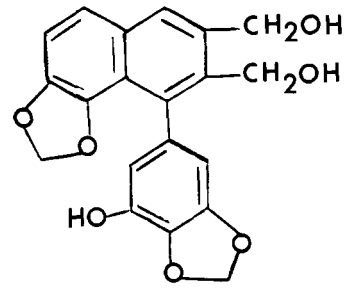
[1]We are extremely grateful to Prof. L. Crombie of the Dept. of Chemistry, University of Wales, Cardiff, for a generous sample of helioxanthin.

Column fraction C on recrystallization from methanol-chloroform furnished a pale yellow compound designated compound **2**, for which the name justicinol is proposed. Its solution gave a blue fluorescence, a deep blue color for the Labat's test, indicative of the presence of a methylenedioxy unit; further support for this assignment came from the pmr spectrum of the compound and a purple color with sulfuric acid, characteristic of arylnaphthalide lignans (9). Its uv spectrum was similar to that of helioxanthin (**1**); the ir spectrum showed characteristic absorption for hydroxyl (3450 cm^{-1}) (absent in (**1**)), γ-lactone (1760 cm^{-1}) and methylenedioxy (935 cm^{-1}). The pmr spectra confirmed the presence of two methylenedioxy groups, which exhibited signals at δ6.00 (2H) and 5.93 (2H). Accurate mass measurement established the molecular formula as $C_{20}H_{12}O_7$ (M^+, 364). The parent ion was 16 mass units higher than that of (**1**), requiring the placement of an additional oxygen atom in the molecule. Acetylation of compound **2** yielded a monoacetate, $C_{22}H_{14}O_8$ (M^+, 406), while methylation with excess diazomethane afforded a monomethyl ether, $C_{21}H_{14}O_7$ (M^+, 378), thus confirming the existence of a phenolic hydroxyl group in (**2**).

A careful analysis and comparison of the pmr signals contributed by the arylnaphthalide γ-lactone moiety of helioxanthin (**1**) and justicinol (**2**) clearly indicated



1; R = H
2; R = OH
3; R = OAc



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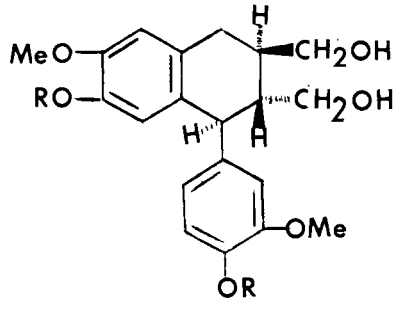

5; R = H
6; R = Me

FIG. 1. Structures of lignans isolated from *Justicia flava* and other compounds cited in the text.

that the A, B, as well as the methylenedioxy and lactone rings of both compounds were identical. However, the pmr spectrum of (**1**) showed a multiplet centered at δ 6.80 integrating for three protons, H-2', 5' and 6', in contrast to (**2**) which exhibited a two-proton singlet appearing at δ 6.86; this is representative of the equivalent H-2' and H-6' aromatic protons (10). This leaves the C-5' position for the hydroxyl substitution. Aside from the C-5' position's being the most sterically desirable and biogenetically most favorable of the three possible positions, C-2', 5' and 6', further support for a C-5' OH was obtained from the pmr spectrum of justicinol acetate (**3**) in which the signals for the H-2' and H-6' protons were observed as a pair of unresolved doublets at δ 6.78 and δ 7.10, respectively. This indicates that on acetylation of the phenolic hydroxyl at C-5', the H-2' and H-6' protons, which are *meta* coupled, are no longer equivalent.

The mass spectrum of justicinol (**2**), aside from a strong molecular ion at *m/z* 364, showed loss of an oxygen atom (*m/z* 348, M^+–16) and a characteristic fragmentation pattern resembling that of helioxanthin (**1**), in which each oxygen carrying a carbon associated with the methylenedioxy is lost in turn (8, 9). Chemical evidence for the presence of the lactone ring was obtained from $LiAlH_4$ reduction of justicinol (**2**), which gave the corresponding triol (**4**), $C_{21}H_{20}O_6$ (M^+, 368). The evidence presented here leads us to propose that justicinol has structure (**2**). As far as we are aware, justicinol has not previously been isolated from a natural source.

The residue from column fraction D yielded, upon recrystallization from methanol-chloroform, a known lignan (**5**), mp 158–160°, which analyzed for $C_{20}H_{24}O_6$ (M^+, 360). This compound was identified as (+)-isolariciresinol (**5**) by its ir, uv, pmr and mass spectral properties and by direct comparison (mmp, co-tlc, ir, ms) with an authentic sample.[2] The dimethyl ether (**6**) and the tetraacetate of (**5**) gave analytical results consistent with those reported in the literature (11–13). This is the first reported occurrence of (+)-isolariciresinol in the Acanthaceae.

The co-occurrence of the lignans of types (**1**), (**2**) and (**5**) in the same plant species has not previously been reported. Simultaneous production of these lignan types can be viewed as suggestive evidence for a direct biogenetic relationship between them.

In addition to the lignans described above, the acidic fraction yielded docosanoic (behenic) acid, which was identified by direct comparison (mmp, co-tlc, ir) with a recrystallized authentic sample[3], and comparison of its methyl ester with a sample obtained by methylation of an authentic sample. The neutral fraction afforded β-sitosterol-β-D-glucoside as the only identifiable component (mmp, co-tlc, ir, ms with an authentic sample).

The lignans (**1**), (**2**) and (**5**) were screened for pharmacological activity by the method of Sandberg (16). Apart from their noticeable mild effect on the central nervous system, demonstrated by decrease in motor activity and ataxia, none of the lignans showed any significantly observable pharmacological activity in mice.

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[2]We thank Prof. T. Popoff of the Dept. of Chemistry, Agricultural College of Sweden, Uppsala, for an authentic sample of (+)-isolariciresinol.

[3]Commercial sample from British Drug Houses Ltd. (B.D.H.).

EXPERIMENTAL[4]

PLANT MATERIAL.—The plant material used for this study was collected at the University of Ife in July 1977 and authenticated by Dr. O. A. Olantunji of the Botany Department, University of Ife, Ile-Ife, Nigeria. A voucher specimen is kept in the University of Ife Herbarium. The leaves were dried at 40° and then powdered.

EXTRACTION AND CHROMATOGRAPHY.—The dried, pulverized leaf material (2 kg) was extracted with methanol in a Soxhlet for 24 h. After removal of the solvent, the dark green residue was resolved into basic, acidic, and neutral fractions. These fractions yielded, upon evaporation of solvent, 0.8, 22.0 and 8.0 g of residue, respectively.

The acidic residue (20 g) was dissolved in chloroform and adsorbed on silica gel (20 g). The solvent was removed *in vacuo*, and the slurry in petroleum ether (bp 40–60°) was poured onto a column of silica gel (300 g) in petroleum ether. Elution was commenced with petroleum ether and continued stepwise through petroleum ether-chloroform mixtures, chloroform and chloroform-methanol mixtures. The eluate was collected in 20 ml fractions and the fractions were combined into five main groups, A-E, having different tlc patterns on silica gel (methanol-chloroform, 1:9), to yield the following compounds:

HELIOXANTHIN (**1**).—Fraction B, upon recrystallization from methanol-chloroform, gave pale yellow crystals as prisms (260 mg), mp 241–242° (lit. (8) 240–241°); ir: ν max 2880, 2770, 1750, 1630, 1580, 1530, 1490, 1440, 1370, 1260, 1230, 1070, 1030, 930, 800 cm^{-1}; uv: λ max 268 (log ϵ 4.68), 295 (3.82), 360.5 (3.92) nm; ms (*m/z*, %): 348 (100, M^-), 319 (19), 291 (7), 261 (5), 233 (6), 205 (5), 176 (7), 174 (9), 116 (5), 88 (10); high resolution ms: found *m/z* 348.0639 (M^-). Calc. for $C_{20}H_{12}O_6$: 348.0634. Comparison of (**1**) with authentic helioxanthin[5] (mmp, co-tlc, ir, ms) established their identity.

JUSTICINOL (**2**).—Fraction C was recrystallized twice from methanol-chloroform to furnish compound **2** as pale yellow prisms (140 mg), mp 250–252°. Tlc of the isolate on silica gel gave R_f 0.56, using methanol-chloroform (1:9), and R_f 0.22, using ethyl acetate-toluene (1:4) as solvent systems. It gave a purple color with sulfuric acid, a deep blue color in the Labat's test and a negative ferric chloride test in addition to: ir: ν max 3450, 1755, 1620, 1580, 1525, 1495, 1440, 1310, 1215, 1060, 1030, 960, 935, 800 cm^{-1}; uv: λ max 269.5 (log ϵ 4.70), 297 (3.80), 361 (3.93) nm; ms (*m/z*, %): 364 (100, M^-), 348 (27), 335 (4), 334 (4), 320 (35), 319 (36), 318 (28), 317 (12), 307 (2), 291 (19), 290 (28), 277 (5), 262 (17), 261 (16), 249 (4), 233 (21), 205 (17), 189 (4), 176 (27), 175 (14), 174 (21), 163 (8), 116 (15), 88 (30); high resolution ms: found *m/z* 364.0581 (M^-), $C_{20}H_{12}O_7$ requires 364.0583; pmr: δ ($CDCl_3$+MeOD) 8.35 (1H, *s*, H-4), 7.71 (1H, *d*, J=8.5 Hz, H-5), 7.35 (1H, *d*, J=8.5 Hz, H-6), 6.86 (2H, *s*, H-2', H-6'), 6.00 (2H, *s*, ring C methylenedioxy), 5.93 (2H, *s*, ring A methylenedioxy), and 5.15 (2H, *s*, lactone methylene). Acetylation of (**2**) (30 mg) with pyridine (1 ml) and acetic anhydride (3 ml) at room temperature for 12 hr gave the monoacetate, $C_{22}H_{14}O_8$, which crystallized from methanol as pale yellow needles (20 mg), mp 243–244°: ir: ν max 1765 (γ-lactone), 1750 (acetate), 1620, 1585, 1530, 1495 (arom.), 935 (methylenedioxy) cm^{-1}; ms (*m/z*, %): 406 (100, M^-), 364 (7), 363 (10), 348 (17), 347 (71), 318 (13), 317 (20), 291 (5), 290 (15), 289 (11), 261 (17), 233 (11), 205 (10), 176 (15), 175 (15), 174 (14), 116 (7), 88 (10); pmr: δ 8.36 (1H, *s*, H-4), 7.71 (1H, *d*, J=8.5 Hz, H-5), 7.32 (1H, *d*, J=8.5 Hz, H-6), 7.10 (1H, unresolv. *d*., H-6'), 6.78 (1H, unresolv. *d*., H-2'), 6.03 (2H, *s*, ring C methylenedioxy), 5.94 (2H, *s*, ring A methylenedioxy), 5.13 (2H, *s*, lactone-methylene), 1.92 (3H, *s*, acetate). Methylation of (**2**) with excess diazomethane yielded the monomethyl ether, $C_{21}H_{14}O_7$ (M^-, 378).

$LiAlH_4$ REDUCTION OF COMPOUND **2**.—A solution of the lactone (**2**) (39 mg) in dry THF (5 ml) was added dropwise to a suspension of $LiAlH_4$ (75 mg) in THF (10 ml). The mixture was stirred at room temperature for 3 hr and diluted with ethyl acetate. Excess reagent was destroyed by addition of dilute HCl. The organic layer was separated, washed with water, and dried (Na_2SO_4); removal of the solvent gave a residue which was purified by preparative tlc over silica gel using methanol-chloroform (15:85) as the mobile phase and elution with chloroform to yield the triol (**4**), (8 mg), $C_{21}H_{20}O_6$, mp 166–168°; ir: ν max 3300 (broad, OH),

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[4]Melting points were determined with a Leitz mikroskopheitztisch 350. Ir spectra (KBr discs) were obtained on a Jasco-IRA-1-spectrophotometer, uv spectra were recorded in chloroform, unless stated on a Shimadzu MPS-5000 UV-VIS spectrophotometer. Pmr spectra were recorded at 100 MHz in $CDCl_3$, unless otherwise stated, on a Jeol 100 MHz instrument and chemical shifts (δ) are in ppm relative to internal tetramethylsilane. Mass spectra were determined on an LKB 9000 or AEI MS 50 spectrometer at 70 eV with direct inlet. Optical rotations were measured on a Zeiss polarimeter. Thin-layer chromatography was carried out on either precoated silica gel 60 plates (0.25 mm silica gel F_{254}, E Merck) or on a 0.50 mm layer of silica gel GF_{254} (type 60, E. Merck) spread on 20 x 20 cm glass plates. Silica gel 60 (70–230 mesh, E. Merck) was used for column chromatography.

1630, 1595, 1570, 1500, 1490 (arom.), 930 (methylenedioxy) cm^{-1}; uv: λ max 250 (log ϵ 4.26), 271 (4.12), 278 (4.08), 299 (3.94), 314 (3.82), 355 (3.36) nm; ms (*m/z*, %): 368 (100, M^+), 352 (25), 334 (16), 306 (16), 306 (16), 305 (11), 291 (5), 277 (5), 276 (8), 275 (13), 248 (5), 247 (10), 233 (5), 219 (7), 190 (6), 189 (17), 176 (8), 138 (6), 94 (13), 85 (40), 83 (73). Acetylation of the triol with acetic anhydride and pyridine furnished the triacetate, $C_{27}H_{26}O_9$ (M^+, 494).

(+)-ISOLARICIRESINOL (**5**).—The product obtained from column fraction D was recrystallized from methanol-chloroform to give colorless needles (300 mg), mp 158–160° (lit. (11) 157–158°) or from aqueous methanol, mp 114–115° (lit. (14) 112°); $[\alpha]^{20}D$+64.6° (*c* 1.5 in Me_2CO); ir: ν max 3400, 3300 (OH), 1600, 1580 (Arom.) cm^{-1}; uv: λ max 222 (log ϵ 4.56), 285 (4.23), 290 (4.16); pmr: δ 6.84 (1H, *d*, *J*=8 Hz), 6.69 (1H, *d*, *J*=2 Hz), 6.67 (1H, *s*), 6.58 (1H, *dd*, *J*=2 Hz, 8 Hz), 6.28 (1H, *s*), 3.85 (3H, *s*), 3.82 (3H, *s*), 3.44–3.79 (5H, *m*), 2.91 (2H, *m*), 1.62–2.10 (2H, *m*); ms (*m/z*, %): 360 (100, M^+), 342 (10), 325 (12), 311 (91), 297 (9), 284 (27), 279 (10), 271 (16), 255 (17), 241 (32), 211 (7), 197 (7), 187 (20), 175 (38), 137 (38), 131 (7), 115 (9).

The compound was identical (mmp, ir, ms, co-tlc) with an authentic sample[4] of (+)-isolariciresinol (**5**). It formed a tetraacetate, mp 167–169° (lit. (11) 164–165°), $C_{28}H_{32}O_{10}$ (M^+, 528), $[\alpha]^{20}D$−3.68 (*c* 1.05 in $CHCl_3$, lit. (11) −3.36°), and a dimethyl ether, $C_{22}H_{28}O_6$ (M^-, 388), mp 176–178° (lit. (11) −176–178°), $[\alpha]^{20}D$+15.4° (*c* 1.0 in $CHCl_3$) lit. (11) +14.8°).

Fraction E yielded, on recrystallization from methanol, docosanoic acid (behenic acid) (35 mg), mp 78–80° (lit. (15) 79–80°, $C_{22}H_{44}O_2$ (M^-, 340); ir: ν max 3250–2500 (broad, -COOH), 1700 (acid carbonyl), 1460, 1430 (CH_2- str.), 730, 720 (*n*-alkane chain) cm^{-1}; ms (*m/z*, %): 340 (32, M^-), 326 (5), 312 (21), 297 (7), 284 (10), 185 (11), 129 (33), 115 (12), 111 (11), 97 (22), 43 (100). The compound was identical (mmp, ir, ms, co-tlc) with a recrystallized commercial sample of docosanoic acid[3]. Methylation of the acid with diazomethane furnished the methyl ester, $C_{23}H_{46}O_2$, mp 53°, identical (mmp, ir) with a methylated authentic sample of docosanoic acid.

The methanol-chloroform (1:4) eluate of the neutral fraction (8 g), when subjected to column chromatography over silica gel, afforded a product (2.2 g) shown to be identical to authentic β-sitosterol-β-D-glucoside (**6**) by mp 290–292°, mmp, co-tlc, and ir. It formed a tetraacetate, mp 162–163°, and on hydrolysis (dil. HCl) afforded glucose (paper chromatography) and β-sitosterol, mp 135–137° (from MeOH), identical with an authentic sample (tlc, ir, mmp).

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