Helichrysum vestitum (*Herbar Nr.* 73/49). 1 kg Wurzeln ergab 1 mg **6**, 1 mg **7** und 15 mg **3**.

5-*Hydroxyobliquin* (**4**). Farblose Kristalle aus Ether, Schmp. 211°. IR cm^{-1}: 3560 (OH); 1740 (Cumarin). MS: M^+ *m/e* 260.068 (100%) ($C_{14}H_{12}O_5$); −Me 245 (5); 2 mg **4** versetzte man mit etherischer Diazomethan-Lösung. Nach 5 min Stehen bei 25° wurde eingedampft. Nach DC (Ether–Petrol, 1:1) erhielt man neben Ausgangsmaterial *ca* 1 mg **5**, farblose Kristalle aus Ether–Petrol, Schmp. 110–15°, IR- und ^{1}H-NMR-Spektren identisch mit denen des Naturstoffs.

5-*Methoxyobliquin* (**5**). Farbloses, nicht völlig frei von **3** erhaltenes Öl, IR: 1740 (Cumarin); MS: M^+ *m/e* 274.084 (100%) ($C_{15}H_{14}O_5$); $-{}^{\cdot}Me$ 259 (5); $-{}^{\cdot}C_4H_2$ 219 (22).

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NEW FUROFURANO LIGNANS FROM *JUSTICIA SIMPLEX**

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Key Word Index—*Justicia simplex*; Acanthaceae; 3,7-dioxabicyclo[3,3,0]octane lignan; justisolin; lignan *O*-glucoside; simplexoside; isolation; structure elucidation; biological function.

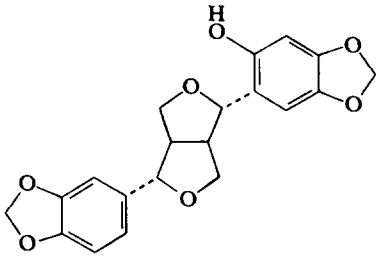
Abstract—A new 3,7-dioxabicyclo[3,3,0]octane lignan, named justisolin, and a new lignan *O*-glucoside, named simplexoside, were isolated from the whole plant of *Justicia simplex* D. Don. (Acanthaceae), collected at fruiting. The structure of the free lignan was established as 2*e*-(3,4-methylenedioxy-6-hydroxy)-phenyl-6*e*-piperonyl-3,7-dioxabicyclo[3,3,0]octane (**1**) and that of the glucoside as 2*e*-(3-methoxy-4-*O*-β-D-glucopyranosyl)-phenyl-6*e*-piperonyl-3,7-dioxabicyclo[3,3,0]octane (**2**) on the basis of chemical transformation and spectral evidence. The biological functions of these and related lignans are appraised.

Justicia simplex D. Don. (Acanthaceae), a native to the Western Himalayas, is used in popular medicine in the Kumaon region of Uttar Pradesh, as an anti-stress and anti-fatigue plant drug. Recently, we reported the isolation and structural elucidation of a number of new and known 3,7-dioxabicyclo[3,3,0]octane lignans from a petrol extract of this species collected at flowering [1]. An EtOH extract of the defatted plant gave two glucosyloxy-arylnaphthalide lignans as minor entities [2]. Since plants at different stages of growth are known to elaborate different chemical constituents having different biological functions [3,4], it was thought worthwhile to examine the chemical characters of the species collected at fruiting.

A petrol extract of the dried and milled plant on column chromatography and fractional crystallization afforded a new lignan (**1**) in addition to sesamin [**1**], asarinin [1], and sitosterol [1]. An EtOH extract of the defatted marc, on the other hand, gave a new lignan glucoside (**2**). The two new compounds were characterized as follows.

Compound **1**. The compound, mp 146–148°, $C_{20}H_{18}O_7$ (M^+, 370), showed UV maxima characteristic of furofurano lignans [2]. UV maxima showed a bathochromic shift in the presence of NaOMe. In the MS, it exhibited significant fragment ions, resulting from the dual breakdown [5,6] at *m/e* 149, 135, 121 corresponding to the piperonyl moiety, and *m/e* 166, 165, 151, and 137 ascribable to a hydroxymethyl-enedioxyphenyl moiety. The latter fragment ion peaks were shifted by 14 amu in the corresponding Me ether. The 90 MHz ^{1}H NMR spectrum of the compound, in $CDCl_3$, confirmed and amplified the conclusions drawn above. The protons associated with the

*Part 2 in the series 'Chemical Constituents of *Justica*'. For Part 1 see ref. [1].



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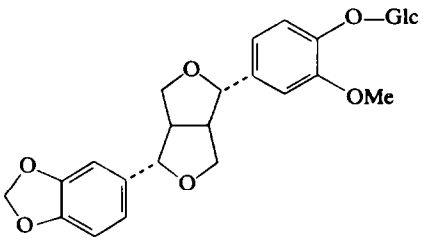

2

piperonyl ring showed a typical ABC pattern [1], whilst the two protons in the hydroxymethylenedioxyphenyl ring did not split and were therefore in *para* disposition. The resonances of the two sets of methylene protons appeared between δ 4.5 and 3.75 indicating [6] diequatorial configuration of the aryl rings. Corroboration for this was also available from its optical rotation [7]. On methylation with ethereal CH_2N_2, it afforded a mono Me ether whose physical and spectral properties were very similar to those reported for sesangolin [8]. The Me ether on oxidation with $KMnO_4$, in Me_2CO, afforded piperonylic acid and 6-methoxypiperonylic acid. These data suggest 2*e*-(3,4-methylenedioxy-6-hydroxy)-phenyl-6*e*-piperonyl-3,7-dioxabicyclo[3,3,0]octane as the structure (**1**) for this compound which we named justisolin.

Compound **2**. This compound, which we named simplexoside, mp 205–210°, $C_{26}H_{30}O_{11}\cdot H_2O$, showed UV maxima characteristic of furofurano lignans. The maxima positions remained unaltered in presence of the usual shift reagents. Its glycosidic nature was indicated by its hydrolysis with emulsin when a lignan aglucone, mp 160–162°, $C_{20}H_{20}O_6$ (M^+, 356) and glucose were obtained. The glucoside formed a tetraacetate. As expected for an *O*-glucoside, it did not exhibit an M^+ peak in its MS but produced an abundant fragment ion peak at *m/e* 356 due to the lignan moiety. The tetraacetate, however, gave a small but identifiable M^+ peak in its MS. In the ^1H NMR spectrum of the tetraacetate, the H-5′ (aromatic) and H-2 (benzylic) signals showed a considerable downfield shift suggesting the assignment of the glucosyloxy function at $C_{4'}$-position in simplexoside. In the ^1H NMR spectrum of the aglucone, the two sets of methylene proton signals appeared in the range δ 4.25–3.88 ppm thereby ruling out the possibility of an axial–equatorial stereochemistry of the aglucone as also of the glucoside. The mono Me ether of the aglucone showed physical and spectral properties closely similar to those reported for methylpiperitol [6, 9]. These data suggest 2*e*-(3-methoxy-4-*O*-β-D-glucopyranosyl)-phenyl-6*e*-piperonyl-3,7-dioxabicyclo-[3,3,0]octane (**2**) as the structure for simplexoside. Recent investigations have provided ample evidence for glycosidation–deglycosidation phenomena in polyphenolics during ontogeny of plants [10–12]. This information is particularly germane to the use of plant extracts for therapeutic purposes since phenolic glycosides and aglucones produce different biological actions in laboratory animals [12–16]. Some years ago, a study on the antihypertensive–sedative principle of Tu-chung, located the activity in (±)-pinoresinol-di-β-D-glucoside [17]. Interestingly, also simplexoside produced a weak central nervous depressant action in albino mice and rats, whilst the free lignans of the title species produced CNS stimulant action. Different proportions of mixtures of simplexoside and the free lignans showed a complex psychopharmacological action as was observed before with mixtures of swertiamarin and mangiferin [18]. The opposite pharmacological actions produced by glycosides and aglucones would thus seem to be a general phenomenon not restricted to a particular class of polyphenolic compounds, e.g. polyoxygenated xanthones [3]. A preliminary investigation with the lignan glucoside and aglucones in seed germination and growth promotion/retardation of seedlings of lettuce and radish indicated that these compounds also have different growth regulator properties in plants.

EXPERIMENTAL

The general methods are the same as those reported recently [19].

Extraction. Dried and milled whole plant* (*ca* 500 g) was continuously Soxhlet extracted with petrol (60–80°) and then with EtOH (30 hr each). The two extracts were seperately processed.

Petrol extract. Solvent was evaporated to give a yellow gummy material (27 g). It showed *ca* 4 yellow–red spots on analytical TLC when sprayed with 2,4-DNPH regent [1], and *ca* 12 blue–violet spots after spraying with H_2SO_4 followed by heating (100–110° for 10 min). A portion of the gummy material (5 g) was mixed with Si gel (5 g) and chromatographed on a column of Si gel (50×3 cm). Elution was carried out with petrol (2 l.), C_6H_6 (4 l.), and C_6H_6–$CHCl_3$ (9:1, 2 l.). Fractions (100 ml) were collected and monitored by analytical TLC. Fractions 25–29 showed 3 major I_2-positive spots, 2 of which, on a separate TLC plate, turned yellow when sprayed with DNPH reagent. Fractions were combined and concentrated. The concentrate on rechromatography and crystallization from C_6H_6–MeOH afforded sesamin (14 mg), asarinin (11 mg) and sitosterol (23 mg).

Justisolin **1**. Combined fractions 48–55 were rechromatographed on a Si gel column (28×2 cm). C_6H_6 (1 l.) and C_6H_6–$CHCl_3$ (1:1, 1 l.) were used as eluents. Fractions (100 ml) were collected. Fractions 14–16 were combined and

*The plants, at fruiting, were collected from Ranikhet, District Almorah, Uttar Pradesh, and were properly identified. A voucher specimen has been preserved at the Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Banaras Hindu University, Varanasi-5, India.

the solvent was evaporated. The residue crystallized from C_6H_6 to give justisolin as colourless crystals (9 mg), mp 146–148°; R_f 0.34 (C_6H_6–HOAc, 19:1), the spot when sprayed with conc H_2SO_4 followed by heating gave was violet changing to dull grey; $[\alpha]_D^{28}$ +42.7° (*c* 0.21, $CHCl_3$); UV: λ_{max}^{MeOH} nm (log ε): 228 (4.32), 282 (3.78); $\lambda_{max}^{MeOH-NaOMe}$ nm: 245 sh, 295–300; ^{1}H NMR ($CDCl_3$): δ 6.84 (1H, *d*, *J* = 8.5 Hz, H-5″), 6.8 (1H, *s*, H-2′), 6.72 (1H, *d*, *J* = 3.5 Hz, H-2″), 6.65 (1 H, *dd*, *J* = 3.5 and 8.5 Hz, H-6″), 6.42 (1 H, *s*, H-5′), 5.82 (2H, O—CH_2—O), 5.8 (2H, O—CH_2—O), 4.75 (1H, *d*, *J* = 4.5 Hz, H-6), 4.57 (1H, *d*, *J* = 5 Hz, H-2), 4.52 (2H, *m*) and 3.75 (2H, *m*) (C-4 and C-8 methylene protons), 3.01 (2H, *m*, H-1 and H-5). (Found: C, 64.42; H, 4.70. $C_{20}H_{18}O_7$ requires: C, 64.8; H, 4.8%). On methylation with CH_2N_2–Et_2O, justisolin gave a mono Me ether, mp 88–90°; *m/e* 384 (M^+); ^{1}H NMR data in $CDCl_3$ were indistinguishable from those reported for sesangolin in the literature [8].

$KMnO_4$ *oxidation of justisolin*-O-Me *ether.* The ether (35 mg) when oxidized according to ref. [6] yielded 3,4-methylenedioxy-6-methoxybenzoic acid (2 mg), mp 148° [20] and piperonylic acid (1.5 mg), mp 235–237°.

EtOH *extract.* The extract was evaporated to a small vol. under red. pres., diluted with H_2O (200 ml) and filtered. The residue (fraction A) was kept for further processing at a later time. The clarified aq. soln was extracted successively (5 × 250 ml-protons each) with petrol (fraction B), $CHCl_3$ (fraction C), and EtOAc (fraction D). Fractions B and C showed similar TLC compositions and were therefore combined. Column chromatography of the combined concentrate on Si gel afforded further quantities of (+)-sesamin (32 mg), (+)-asarinin (27 mg), and justisolin (3 mg) together with a partially characterized aryl-naphthalide lignan as evidenced from its bright blue fluorescence under a short-wave UV lamp, colour reactions [2] and UV maxima [2]. Fraction D: The EtOAc concentrate was subjected to column chromatography on a column of Si gel (34 × 3 cm) using $CHCl_3$–MeOH–H_2O (95:4:1) as eluent. Fractions (50 ml) were collected and monitored by analytical TCL for glucosides using benzidine–meta periodate as the staining reagent.

Simplexoside (**2**). Fractions 7–14 were combined and rechromatographed twice, as described above, to give a brown solid which crystallized from EtOH as straw-coloured crystals (58 mg), mp 205–210°; $[\alpha]_D^{28}$ −12.5 (*c* 0.34, MeOH); UV: λ_{max} nm (log ε): 228 (4.26), 278–280 (3.65); ^{1}H NMR (CD_3OD): δ 6.95–6.6 (6H, Ar—H), 5.98 (2H, O—CH_2—O), 5 (1H, glucosyl H-1), 4.3 (2H, *m*, H-2 and H-6), 3.88 (3H, *s*, OMe), 3.82 (2H, *m*). (Found: C, 58.0; H, 5.38. $C_{26}H_{30}O_{11}\cdot H_2O$ requires: C, 58.22; H, 5.97%). Hydrolysis of **2** with emulsin, according to ref. [21], afforded glucose (PPC) and a lignan aglucone, mp 160–162°; $[\alpha]_D^{28}$ +62.8° (*c* 0.27, $CHCl_3$); MS *m/e* 356 (M^+, 88%), 355 (26), 325 (12), 257 (3), 232 (6), 205 (19), 204 (18), 203 (20), 152 (47), 151 (82), 150 (73), 149 (100). Treatment of the aglucone with Et_2O–CH_2N_2 gave a mono Me ether, mp 75–76°; MS *m/e* 370 (M^+, 100%), 220 (5), 219 (18), 207 (22), 204 (7), 203 (37), 177 (48), 166 (52), 165 (88), 151 (70), 150 (87), 149 (98), 137 (14), 136 (64), 122 (12), 121 (6). The physical and spectral properties of this compound are indistingushable from those reported for methylpiperitol [6, 9]. Acetylation of **2** with Ac_2O–C_5H_5N, at room temp. for 7 days and work-up in the usual fashion afforded a tetraacetate which crystallized from aq. EtOH as colourless crystals, mp 117–120°; MS *m/e* 686 (M^+, 1.8%), 356 (1), 355 (8), 331 (82), 271 (100), 243 (14), 211 (22), 169 (74), 115 (11); ^{1}H NMR ($CDCl_3$): δ 7.14 (1H, *d*, *J* = 8.5 Hz, H-5′), 6.8–6.5 (5H, *m*, Ar—H), 5.9 (2H, *s*, O—CH_2—O), 5.1 (1H, *d*, *J* = 4 Hz, H-2), 4.76 (1H, *d*, *J* = 4 Hz, H-6), 4.34 (2H, *q*), 3.88 (3H, *s*, OMe), 3.8 (2H, *q*), 2–1.74 (12H, OAc).

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