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SIMPLEXOLIN, A NEW LIGNAN FROM *JUSTICIA SIMPLEX*

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Key Word Index—*Justicia simplex*; Acanthaceae; ligans; 2,6-di-(3′,4′-methylenedioxyphenoxy)-3,7-dioxabicyclo-[3,3,0]-octane; simplexolin; sesamin; asarinin; sesamolin; structural determination.

INTRODUCTION

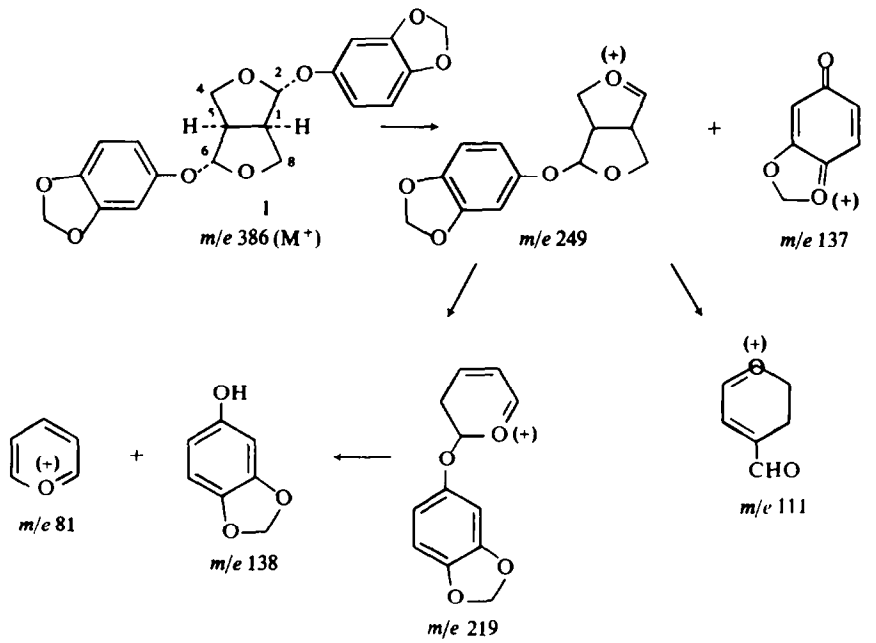
In connection with our interest on biologically active arylnaphthalide lignans [1–4], we investigated the chemical constituents of *Justicia simplex* D. Don. A large number of arylnaphthalide lignans were previously reported [5–8] in two *Justicia* species, viz. *J. hayatai* var. *decumbens* and *J. procumbens* var. *leucantha* of Formosan and Japanese origin. The title species, a native to the Western Himalayas, is used in the Kumaon region, in traditional medicine as an anti-fatigue and stimulating plant drug. To our knowledge, this is the first report of any phytochemical investigation of *Justicia* species of Indian origin. The isolation and characterization of the lignans of *J. simplex* are described in this paper. Additionally, the biological activities of the contained bicyclooctane lignans are mentioned.

RESULTS AND DISCUSSION

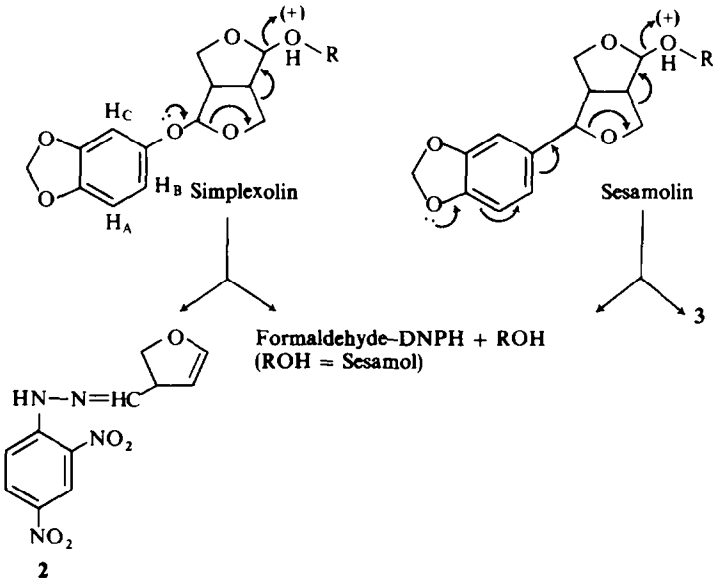
By extensive chromatography of the petrol extract of the whole plant, 3 known lignans, viz. sesamin, asarinin, and sesamolin, and a new one, which we named simplexolin, were isolated. The identity of the known compounds was established by spectral (UV, PMR, MS) evidence, chemical transformation and by direct comparison with authentic compounds where possible. The characterization of only the new lignan is described here.

Simplexolin, $C_{20}H_{18}O_8$ (M^+, 386), mp 160–162°, $[\alpha]_D^{28}$ +254.8° ($CHCl_3$), showed UV maxima characteristic of a 1,3,4-alkoxybenzene. The presence of methylenedioxy groups was indicated from the positive Labat test and from the PMR spectrum of the compound. The MS apart from the M^+ at *m/e* 386, showed significant fragment ions at *m/e* 249, 219, 138, 137, 111, and 81. The pattern of fragmentation is rationalized (Scheme 1) on the basis of a symmetrically substituted 3′,4′-methylenedioxyphenoxy-3,7-dioxabicyclo[3,3,0]-octane, structure, (1) for simplexolin. The genesis of the fragment ions corresponding to *m/e* 249 and 219 is precedented in sesamolin [9] which, having a 3′,4′-methylenedioxyphenyl in place of the aryloxy substituent at C-6, showed the corresponding fragment ions at 16 amu less, i.e. at *m/e* 233 and 203, respectively.

The PMR spectrum of simplexolin (in $CDCl_3$) is also very informative. The methine protons at C-1 and C-5 are equivalent and appeared as a two-proton multiplet centred at δ 3.25 ppm. The chemical shift of these protons is consistent with the presence of aryloxy functions at C-2 and C-6. The two sets of equivalent methylene protons, associated with C-4 and C-8, appeared as a multiplet centred at δ 4.1 ppm. A sharp two-proton singlet appearing at δ 5.53 ppm is ascribable to H-2 and



Scheme 1. MS fragmentation of simplexolin.



Scheme 2. Mechanism of cleavage of simplexolin and sesamolin with 2:4-dinitrophenylhydrazine sulphate.

H-6 .These data suggested that simplexolin belonged to the category of symmetrical bicyclooctane lignans [10, 11] with both aryloxy groups (at C-2 and C-6) equatorially disposed.

The chemical proof of structure 1 for simplexolin was provided by two crucial reactions. Hydrolysis of simplexolin with HCl–HOAc afforded sesamol from the phenolic fraction. The neutral fraction gave a yellow polymeric gummy material. Dimerization of the neutral component, formed from sesamolin under similar conditions, is well documented [12]. In another set of reactions, simplexolin when treated with a methanolic solution of 2:4-dinitrophenylhydrazine sulphate yielded sesamol and formaldehyde dinitrophenylhydrazone. The possible mechanism of the reaction is illustrated in Scheme 2. The dinitrophenylhydrazone 2, formed from the dihydrofuran aldehyde, was unstable and turned into a reddish-orange gummy material on exposure to air. Nevertheless, its identity followed from its MS. Sesamolin under similar conditions of treatment with 2:4-DNP sulphate afforded sesasmol, formaldehyde–DNPH, and another dinitrophenylhydrazone (3), different from 2. This degradation method thus appears to be a general one for detecting aryloxy-bicyclooctane lignans.

The occurrence of a number of bicyclooctane lignans, in appreciable quantities, in *J. simplex* is consistent with its uses as an anti-fatigue and anti-stress agent in medicine. The use of extracts of *Acanthoponax* species, containing related bicyclooctane lignans, as adoptogen, anti-alarm, anti-fatigue and stimulating agents, is well documented [13]. Pharmacological screening of the mixture of lignans from *J. simplex*, in the authors' laboratory, also revealed some significant central nervous system activity in laboratory animals. The details of this finding will be reported elsewhere. Another type of biological activity reported for bicyclooctane lignans was the reversal of sickling and crenation in erythrocytes by plant extracts containing similar bicyclooctane entities [14].

EXPERIMENTAL

All mps were taken on a Köfler block in open capillaries and are uncorr. UV spectra were recorded in MeOH. MS were determined at 70 eV by direct insertion on a probe. PMR spectra were obtained at 60 MHz using TMS as an int. standard. Separation by column chromatography was carried out using Si gel (60–120 mesh) and TLC was carried out on Si gel G. Two solvent systems, C_6H_6–HOAc (50:1, solvent 1) and C_6H_6 (100, solvent 2) were used as developers. I_2, $FeCl_3$ and 2:4-DNPH in MeOH containing traces of H_2SO_4 were used for visualization.

Extraction. Dried and milled whole plant* (1 kg) was continuously extracted in a Soxhlet with petrol (60–80°) for 30 hr. The solvent was evapd to give a yellow gummy material (58 g). It showed *ca* 6 yellow–red spots on analytical TLC when sprayed with 2:4-DNPH reagent. A portion of the gummy material (5 g) was mixed with Si gel (5 g) and chromatographed over a column of Si gel (48 × 3 cm). Elution was carried out with petrol (2 l.), and C_6H_6 (5 l.). Fractions (500 ml) were collected. Fractions 5–7 showed 3 major I_2- and DNPH-positive spots on analytical TLC. These were combined and rechromatographed. Fractions 9–10 contained mainly sesamin and sitosterol. Crystallization from hexane afforded sesamin (27 mg) as colourless needles, mp 110–112° (mp, mmp, co-TLC, $[\alpha]_D$, PMR).

Simplexolin (1). The concentrate from fractions 5–7 was rechromatographed on a Si gel column (34 × 2 cm). Petrol (500 ml), C_6H_6–petrol (1:1, 1 l.), C_6H_6 (2 l.) and C_6H_6–$CHCl_3$ (2:1, 1 l.) were used as eluents. Fractions (100 ml) were collected. Fractions 9–12 were combined and the residue was crystallized from hexane to give simplexolin as colourless crystals (18 mg), mp 160–162°; R_f 0.7 (solvent 1) the spot giving a green colour changing to orange when sprayed with EtOH–H_2SO_4; UV: λ_{max} nm (log ε) 235 (4.12), 292 (4.04); PMR ($CDCl_3$): δ 3.25 (2H, *m*, H-1, H-5), 4.1 (4H, *m*, H-4 and H-8 methylene), 5.53 (2H, *s*, H-2, H-6), 5.9 (4H, O—CH_2—O), 6.5 (2H, *dd*, J = 3.5 and 8.5 Hz, H_B), 6.63 (2H, *d*, J = 3.5 Hz, H_C), 6.69 (2H, *d*, J = 8.5 Hz, H_A); MS: *m/e* 386 (M^+, rel. int., 20%), 249 (65), 219 (82), 151

* The plant material was kindly provided by Mr. V. K. Lal, Central Council of Research in Indian Medicine and Homeopathy, Rainkhet, and was properly identified. A voucher specimen has been preserved at the Pharmaceutical Chemistry Research Laboratory, Department of Pharmaceutics, Banaras Hindu University, Varanasi-5, India.

(48), 138 (98), 137 (62), 111 (55), 81 (100). (Found: C, 62.08; H, 4.34. $C_{20}H_{18}O_8$ requires: C, 62.17; H, 4.66%).

Fractions 15–19 were combined and concd. They showed one major and two minor DNPH-positive (yellow-orange) spots on TLC. The residue crystallized from Me_2CO–hexane to give asarinin, the major component, as colourless needles (45 mg), mp 120–121°. The identity of the compound was established by direct comparison (mp, mmp, co-TLC, PMR) with an authentic sample.

Fractions 30–37 were combined and the residue crystallized from hexane–Me_2CO to give sesamolin as colourless microcrystals (14 mg), mp 90–91°; R_f 0.54 (solvent 1), 0.78 (solvent 2); $[\alpha]_D^{28}$ +188.7° (*c* 0.47, $CHCl_3$); MS: *m/e* 370 (M^+, 18), 233 (65), 203 (47), 138 (100), 137 (88), 135 (78), 69 (52). On acid hydrolysis, according to a previously described procedure [12], it afforded sesamol, mp and mmp (with a synthetic sample) 64°; MS: *m/e* 138 (M^+, 100%).

Acid cleavage of 1. To an ice-cooled soln of 1 (0.11 g), in HOAc (5 ml), conc HCl (0.2 ml) was added. After 10 min, the mixture was diluted with H_2O (20 ml) and extracted with $CHCl_3$ (3 × 25 ml). The phenolic fraction in the $CHCl_3$ soln was extracted with aq. NaOH (1%). The $CHCl_3$ layer was then washed with H_2O, dried and evapd to give a yellow gummy material which did not show an M^+ peak in its MS. The aq. alkaline soln was cooled, acidified and processed in the usual fashion to give sesamol (14 mg), mp and mmp 63–64° (co-TLC, Fe^{3+} reaction).

DNPH cleavage of 1. To a methanolic (10 ml) soln of (98 mg), 2,4-DNPH (0.3 g) soln in MeOH (10 ml) containing H_2SO_4 (01 ml), was added. The mixture was heated at 100° (1 hr) and then kept at room temp. overnight when formaldehyde dinitrophenylhydrazone precipitated as orange-yellow crystals, mp and mmp 163–164° (3 mg); R_f 0.64 (solvent 2). The MeOH mother liquor, after separation of formaldehyde dinitrophenylhydrazone, was concd and passed through a column of acid-washed Al_2O_3. Elution with $CHCl_3$ afforded sesamol (6 mg); R_f 0.55 (solvent 2). The column was washed with MeOH, the MeOH soln concd and then subjected to PLC. The R_f zone 0.4 was eluted with MeOH and the solvent was evapd when dihydrofuran-3-aldehyde dinitrophenylhydrazone was obtained as a reddish-orange gummy material; MS: *m/e* 278 (M^+, 12%), 277 ($M^+ - H$, 28), 250 ($M^+ - C_2H_4$, 14), 249 ($M^+ - CHO$, 7), 248 ($M^+ - CH_2O$, 21), 30 (100).

On TLC plates, sesamin and asarinin showed yellow spots on spraying with DNPH reagent, whilst sesamolin and simplexolin developed pinkish-red spots with the same reagent.

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