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NEOLIGNANS AND A LIGNAN FROM *PIPER CLARKII*

ASHOK K. PRASAD, OM DUTT TYAGI, JESPER WENGEL, PER M. BOLL,* CARL E. OLSEN,† KIRPAL S. BISHT,‡ AMARJIT SINGH,‡ ANTARYAMI SARANGI,‡ RAVINDER KUMAR,‡ SUBHASH C. JAIN‡ and VIRINDER S. PARMAR‡

Department of Chemistry, Odense University, DK-5230 Odense M, Denmark; †Department of Chemistry, Royal Veterinary and Agricultural University, DK-1871 Frederiksberg C, Denmark; ‡Department of Chemistry, University of Delhi, Delhi 110 007, India

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Key Word Index—*Piper clarkii*; Piperaceae; neolignans; lignans; 2,5-diveratryl-3,4-dimethyltetrahydrofuran; benzofuranoid neolignans; bicyclo[3.2.1]octanoid neolignan.

Abstract—Four new compounds, a lignan and three neolignans, together with one known neolignan have been isolated from the leaves and stems of *Piper clarkii*. Their structures were established on the basis of ^1H, ^{13}C and ^1H–^1H COSY NMR, NOE and mass spectral data and CD curves. The novel lignan was identified as (2*S*,5*S*)-diveratryl-(3*R*,4*S*)-dimethyltetrahydrofuran. The structures of the new neolignans were established as (7*R*,8*R*,1′*S*)-$\Delta^{8'}$-1′-methoxy-3,4-methylenedioxy-1′,6′-dihydro-6′-oxo-7.0.4′,8.3′-lignan [2*R*,3*R*,5*S*)-2-(1,3-benzodioxol-5-yl)-3,5-dihydro-5-methoxy-3-methyl-5-(2-propenyl)-6(2H)-benzofuranone], (7*S*,8*S*,3′*R*)-$\Delta^{8'}$-3,3′,4-trimethoxy-3′,6′-dihydro-6′-oxo-7.0.4′,8.3′-lignan [2*S*,3*S*,3a*R*)-2-(3,4-dimethoxyphenyl)-3,3a-dihydro-3a-methoxy-3-methyl-5-(2-propenyl)-6(2H)-benzo-furanone] and rel-(7*S*,8*S*,1′*R*,3′*R*)-$\Delta^{8'}$-5′-methoxy-3,4-methylenedioxy-1′,2′,3′,4′-tetrahydro-2′,4′-dioxo-7.3′,8.1′-lignan [(7*S*,6*S*,5*R*,1*R*)-7-(1,3-benzodioxol-5-yl)-3-methoxy-6-methyl-5-(2-propenyl)bicyclo[3.2.1]oct-3-ene-2,8-dione]. The known neolignan has been identified as denudatin B.

INTRODUCTION

Plants of the genus *Piper* are distributed throughout tropical and subtropical regions of the world. Many *Piper* species have been found to be rich sources of a number of physiologically active compounds [1, 2]. Lignans and neolignans constitute a large fraction of the bioactive compounds [3–5] and their occurrence in Piperaceae has been reviewed recently [6]. As part of our research programme on the isolation and structural elucidation of naturally occurring bioactive compounds from Indian *Piper* species [7, 8], we report the isolation of a new lignan and three new neolignans, together with a known neolignan, from the aerial parts of *P. clarkii*. The biogenetic nomenclature and numbering of neolignans and lignans follow the rules outlined in a review [9] and the systematic names are given in parentheses.

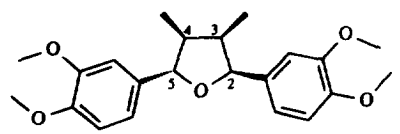
RESULTS AND DISCUSSION

A petrol extract [7] of leaves and stems of *P. clarkii* was subjected to silica gel flash column chromatography with a gradient solvent system of petrol and ethyl acetate. Compounds **1** and **2** were purified by preparative TLC and **3**–**5** by reverse-phase HPLC of the fractions obtained from flash chromatography.

Compound **1** is optically active. Its EI mass spectrum exhibited the $[M]^+$ peak at *m/z* 372 which corresponds to the empirical formula $C_{22}H_{28}O_5$. All the signals in the ^1H NMR spectrum were comparable to those shown by zuionin A [10] except that **1** gives signals for two veratryl units in place of the piperonyl units present in zuionin A. The observed chemical shift values for methyl and methine protons in the ^1H NMR spectrum define the relative stereochemistry as identical to zuionin A having both methyl groups at C-3 and C-4 *cis* and the veratryl groups at C-2 and C-5 *trans* to each other, respectively. In the NOE experiment the 3-Me showed an NOE effect on the 4-Me, the 5-H and the *ortho*-aromatic protons of the vicinal aryl ring, establishing the *cis*-relation of the two methyl groups, as well as of the 4-Me and the 5-H (and, thus, the *trans*- relation between the 4-Me and the vicinal aryl group). It was clear from the NOE effect of the 3-Me on the *ortho*-aromatic protons and the 2-H that the 3-Me is in a *cis*-relation to the vicinal aryl group. In the ^1H NMR spectrum, the 3-Me experienced a shielding effect due to the anisotropic effect of the aromatic rings and gave evidence for the *cis*-relation of the 2-veratryl, 4-Me, 3-Me and the 5-H. The NOE effects due to the methine protons were also in agreement with the configuration given in **1**. The absolute configuration of **1** was established by comparison of its CD curve with that of the *d-epi*-galbacin [11].

The spectral data of **2** were similar to those reported for (7*S*,8*S*,1′R)-$\Delta^{8'}$-1′-methoxy-3,4-methylenedioxy-1′,6′-dihydro-6′-oxo-7.0.4′,8.3′-lignan [12] except that the signals for 2′-H and 5′-H, reported as singlets for one

*Author to whom correspondence should be addressed.



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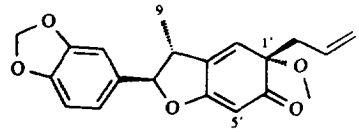

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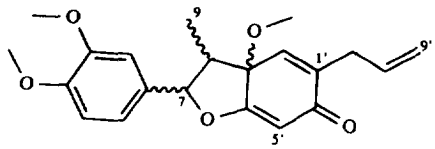
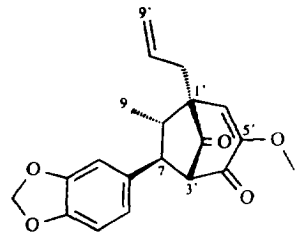


	Ar	Me	OMe
3	α	α	β
4	α	β	β



5

proton each at δ 5.69 and 6.20, respectively, were observed in our spectrum as a doublet (J = 1.9) at δ 6.25 and as a singlet at δ 5.69. This assignment was done on the basis of its NOE and ^{1}H–^{1}H COSY NMR spectrum. In the NOE experiment, the 9-H, 8-H, 7′-H and 1′-OMe showed an NOE effect on the signal at δ 6.25, and vice versa, confirming it as the signal for 2′-H. Furthermore, the signal for 2′-H appears as a doublet due to its coupling with 8-H, as confirmed by observing the expected cross-peak in its ^{1}H–^{1}H COSY NMR. Hence, the singlet at δ 5.69 could be assigned to 5′-H, which is confirmed by observing the NOE effect (1.8%) on irradiation of 1′-OMe. Compound **2** exhibited a specific rotation of the same order of magnitude as that shown by the compound isolated earlier [12], but of opposite sign, suggesting **2** to be the enantiomer. The absolute configuration has been determined from its CD curve comparison with ORD data of its enantiomer [12]. The Cotton effects associated with the benzenoid and enone chromophores in the ORD curve of the reported enantiomer are of opposite sign to the Cotton effects observed for **2**, thus establishing **2** to be the enantiomer of the compound isolated earlier.

Compounds **3** and **4** both analysed for $C_{21}H_{24}O_5$ and their fragmentation patterns indicated that they were isomers. Their ^{1}H NMR spectra were also very similar, the only difference being the chemical shifts of a few signals, thus suggesting **3** and **4** to be diastereisomers. Compound **4** was found to be identical in all respects with denudatin B [13]. On comparison of the ^{1}H NMR spectrum of **4** with that of **3**, the signals for 7-H and 8-H in **3** are comparatively deshielded, whereas the 9-H is shielded and appeared at δ 0.49. It is suggested that **4** and **3** differ in the relative configuration at C-8. The NOE enhancements observed for the 8-H, 2-H and 6-H signals on irradiation of 9-H in **3** establish a *cis*-relation between the veratryl group at C-7 and the methyl at C-8, and a *trans*-relation between the methyl and the 3′-methoxyl group, because no enhancement for the C-3′ OMe signal was observed on irradiation of the protons of the methyl group and vice versa. Furthermore, the *cis*-relation between the 7-veratryl group and the 9-methyl group was confirmed by irradiation of 7-H, which causes an appreciable NOE ($\sim$ 10.3%) in the 8-H signal and vice versa. The observed ^{13}C– and ^{1}H–^{1}H COSY NMR data are in agreement with the proposed structure **3**. The absolute configuration as shown for **3** was established by comparison of its CD curve with the ORD data of 2-*epi*-mirandin A [14]. The CD curve of **3** showed Cotton effects opposite to those of the reported ORD data for 2-*epi*-mirandin A, thus confirming the absolute stereochemistry of **3** to be opposite to that of 2-*epi*-mirandin A. This, to the best of our knowledge, is the first report of its isolation from a natural source.

Compound **5**, obtained as a viscous oil analysed for $C_{20}H_{20}O_5$ from its $[M]^+$ at m/z 340. The ^{1}H NMR, IR, UV and mass spectral data are comparable with those reported for rel-(7*R*,8*R*,1′*S*,3′*S*)-$\Delta^{8'}$-5′-methoxy-3,4-methylenedioxy-1′2′,3′,4′-tetrahydro-2′,4′-dioxo-7.3′,8.1′-lignan [15]. However, the specific rotation was found to be opposite to that reported, suggesting **5** to be its enantiomer. Its relative configuration was established from NOE data. The observed NOE for **5** established the *trans*-relation for the C-7 and C-8 substituents, because irradiation of the methyl proton signal did not cause any enhancement of the aromatic proton signals but, instead, enhancement (14%) for the H-7 and vice versa. However, the enhancement of H-6′ on irradiation of the methyl signal indicates the structure to be as shown in **5**.

EXPERIMENTAL

General. ^{1}H, ^{13}C and 2D NMR and NOE were recorded in $CDCl_3$ using TMS as int. standard on a Brucker AC-250 spectrometer. Silica gel (230–400 mesh, Merck) was used for flash CC and analytical TLC was performed on Merck silica gel 60 F_{254} plates. Spots were visualized under UV light or by spraying with 10% H_2SO_4 in EtOH followed by heating at 120° for a few

min. Prep. TLC was performed on silica gel 60 $F_{254+366}$ or on Merck prep. plates precoated (2 mm) with silica gel 60 F_{254} or with aluminum oxide 60 F_{254}.

Plant material. Leaves and stems of *P. clarkii* C. DC. were collected and identified with the help of the Botanical Survey of India, Eastern Circle Office, Shillong, Meghalaya, India. Details are reported in our previous paper [7].

Extraction and isolation. Minor frs from CC of the petrol extract [7] of leaves and stems with a gradient solvent system of petrol and EtOAC were investigated. Fr. 69, which was eluted with EtOAC–petrol (1:9) as a greenish-yellow oily residue after evapn, was subjected to prep. TLC with EtOAC–benzene (3:7), affording **1** and **2**. Frs 50–55, which were eluted with EtOAc–petrol (2:23), were combined and subjected to prep. TLC to give a fr. containing 3 compounds, which on purification by reverse-phase HPLC (35% aq. EtOH) yielded pure **3**–**5**.

(2S,5S)-*Diveratryl*-(3R,4S)-*dimethyltetrahydrofuran* (**1**). Semi-crystalline solid (7 mg). $[\alpha]_D^{22}$ + 48.6° (*c* 0.14, MeOH). UV λ_{max}^{MeOH} nm: 250, 277. HRMS *m/z*: $[M]^+$ 372.1921 ($C_{22}H_{28}O_5$ requires 372.1937). EIMS *m/z* (rel. int.): 372 ($[M]^+$,28), 342 (4), 287 (4), 206 (100), 195 (14), 191 (36), 178 (22), 175 (33), 165 (16), 151 (8), 138 (8), 129 (8). ^{1}H NMR ($CDCl_3$): δ 0.60 (3H, *d*, *J* = 7, 3-Me), 1.01 (3H, *d*, *J* = 6.3, 4-Me), 2.45 (2H, *m*, 3-H and 4-H), 3.87, 3.88 and 3.90 (12H, 3*s*, 3′-OMe, 3″-OMe, 4′-OMe and 4″-OMe), 4.66 (1H, *d*, *J*, = 9.1, 5-H), 5.46 (1H, *d*, *J* = 5.4, 2-H), 6.82–6.92 (6H, *m*, Ar-H). ^{13}C NMR ($CDCl_3$): δ 9.5 (C-3 Me), 11.9 (C-4 Me), 43.5 (C-3), 47.6 (C-4), 55.9 and 56.0 (C-3′-OMe, C-3″-OMe and C-4′-OMe and C-4″-OMe), 84.8 (C-5), 85.7 (C-2), 109.1, 109.4 (C-2′, C-2″), 110.9, 111.0 (C-5′, C-5″), 118.1 118.5 (C-6′, C-6″), 133.3, 135.7 (C-1′, C-1″), 147.0, 147.5, 148.7, 149.1 (C-3′, C-3″, C-4′, C-4″). CD (5.1 mg in 100 ml, MeOH, 239–400 nm): $[\theta]_{239}$ − 30128, $[\theta]_{243}$ − 20409, $[\theta]_{251}$ 0, $[\theta]_{260}$ + 4373, $[\theta]_{277}$ 0, $[\theta]_{282}$ − 3402, $[\theta]_{292}$ 0, $[\theta]_{300}$ + 5831, $[\theta]_{322}$ + 2916, $[\theta]_{330}$ 0, $[\theta]_{360}$ 0, $[\theta]_{400}$ 0.

(7R,8R,1′S)-$\Delta^{8'}$-1′-*Methoxy*-3,4-*methylenedioxy*-1′,6′-*dihydro*-6′-*oxo*-7.0.4′,8.3′-*lignan*[(2R,3R,5S)-2-(1,3-*benzodioxol*-5-*yl*)-3,5-*dihydro*-5-*methoxy*-3-*methyl*-5-(2-*propenyl*)-6(2H)-*benzofuranone*] (**2**). Oil (12 mg). $[\alpha]_D^{22}$ − 84.3° (MeOH; *c* 1.5). HRMS *m/z*: $[M]^+$ 340.1316 ($C_{20}H_{20}O_5$ requires 340.1311). ^{1}H NMR ($CDCl_3$): δ 1.38 (3H, *d*, *J* = 6.9, 9-H), 2.53 (2H, *m*, 7′-H), 3.02 (1H, *m*, 8-H), 3.14 (3H, *s*, 1′-OMe), 5.03–5.09 (3H, *m*, 9′-H and 7-H), 5.69 (1H, *s*, 5′-H), 5.70 (1H, *m*, 8′-H), 6.00 (2H, *s*, OCH_2O), 6.25 (1H, *d*, *J* = 1.9, 2′-H), 6.82 (3H, *bs*, 2-H, 5-H and 6-H). CD (4.8 mg in 100 ml MeOH, 230–400 nm): $[\theta]_{230}$ + 103380, $[\theta]_{244}$ − 114658, $[\theta]_{260}$ − 58269, $[\theta]_{274}$ 0, $[\theta]_{290}$ + 54510, $[\theta]_{310}$ + 125936, $[\theta]_{328}$ + 54510, $[\theta]_{330}$ 0, $[\theta]_{341}$ − 39473, $[\theta]_{358}$ − 94922, $[\theta]_{383}$ 0, $[\theta]_{400}$ 0.

(7S,8S,3′R)-$\Delta^{8'}$-3,3′,4-*Trimethoxy*-3′,6′-*dihydro*-6′-*oxo*-7.0.4′,8.3′-*lignan*[(2S,3S,3aR)-2-(3,4-*dimethoxyphenyl*)-3,3a-*dihydro*-3a-*methoxy*-3-*methyl*-5-(2-*propenyl*)-6(2H)-*benzofuranone*] (**3**). Oil (5 mg). $[\alpha]_D^{22}$ + 77.8° (MeOH; *c* 0.4). IR γ_{max}^{KBr} cm^{-1}: 1762, 1696, 1672, 1624, 1519. UV λ_{max}^{MeOH} nm: 233, 285. HRMS *m/z*: $[M]^+$ 356.1605 ($C_{21}H_{24}O_5$ requires 356.1625). EIMS *m/z* (rel. int.): 356 ($[M]^+$, 53), 327 (5), 325 (5), 287 (6), 277 (8), 191 (25), 178 (100), 165 (18), 164 (17), 152 (8), 135 (6), 107 (6). ^{1}H NMR ($CDCl_3$): δ 0.49 (3H, *d*, *J* = 7.5, 9-H), 2.67 (1H, *dq*, *J* = 7.5 and 4.6, 8-H), 3.13 (2H, *m* 7′-H), 3.16 (3H, *s*, 3′-OMe), 3.87 and 3.88 (3H each, 2*s*, 3-OMe and 4-OMe), 5.07–5.14 (2H, *m*, 9′-H), 5.88 (1H, *m*, 8′-H), 5.91 (1H, s, 5′-H), 6.10 (1H, *d*, *J* = 4.5,7-H), 6.25 (1H, *bs*, 2′-H), 6.74–6.89 (3H, *m*, 2-H, 5-H and 6-H). ^{13}C NMR ($CDCl_3$): δ 9.7 (C-9), 33.3 (C-7′), 47.3 (C-8), 51.3 (C-3′-OMe), 55.8, 55.9 (C-3-OMe, C-4 OMe), 82.2 (C-3′), 88.0 (C-7), 104.1 (C-5′), 108.8 (C-2), 111.1 (C-5), 117.0 (C-9′), 118.0 (C-6), 129.0 (C-1), 132.0 (C-8′), 135.1 (C-2′), 143.3 (C-1′), 148.79, 149.0 (C-3, C-4), 173.0 (C-4′), 187.3 (C-6′). CD (4.9 mg in 100 ml MeOH, 244–400 nm): $[\theta]_{244}$ 0, $[\theta]_{255}$ + 12775, $[\theta]_{275}$ 0, $[\theta]_{280}$ − 15249, $[\theta]_{284}$ 0, $[\theta]_{308}$ + 162968, $[\theta]_{388}$ 0, $[\theta]_{355}$ − 51464, $[\theta]_{384}$ − 9530, $[\theta]_{400}$ 0.

(7S,8R,3′R)-$\Delta^{8'}$-3,3′4-*Trimethoxy*-3′,6′-*dihydro*-6′-*oxo*-7.0.4′,8.3′-*lignan* (*denudatin B*) (**4**). Oil (15 mg). $[\alpha]_D^{22}$ + 18.7° (MeOH; *c* 0.17) ; lit. [13] $[\alpha]_D$ + 82.7°, *c* 2.67, MeOH). Spectral data (^{1}H, ^{13}C NMR, UV, IR and EIMS) as previously reported [13].

Rel-(7S,8S,1′R,3′R)-$\Delta^{8'}$-5′-*Methoxy*-3,4-*methylenedioxy*-1′,2′,3′,4′-*tetrahydro*-2′,4′-*dioxo*-7.3′,8.1′-*lignan* [(7S,6S,5R,1R)-7-(1,3-*benzodioxol*-5-*yl*)-3-*methoxy*-6-*methyl*-5-(2-*propenyl*)*bicyclo*[3.2.1]*oct*-3-*ene*-2,8-*dione*] (**5**). Oil (4 mg). $[\alpha]_D^{22}$ + 97.4° ($CHCl_3$; *c* 0.57). HRMS *m/z*: $[M]^+$ 340.1328 ($C_{20}H_{20}O_5$ requires 340.1311). ^{13}C NMR ($CDCl_3$): δ 13.9 (C-9), 35.4 (C-7′), 46.8 (C-8), 50.0 (C-7), 55.8 (C-5′-OMe), 57.3 (C-1′), 70.1 (C-3′), 101.1 (OCH_2O), 107.2 (C-2), 108.4 (C-5), 117.9 (C-9′), 119.0 (C-6′), 120.5 (C-6), 133.0 (C-8′), 135.1 (C-1), 146.8, 148.1 (C-3, C-4), 152.4 (C-5′), 190.1 (C-4′), 202.5 (C-2′).

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