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TWO DIARYLHEPTANOIDS AND A LIGNAN FROM *CASUARINA JUNGHUHNIANA*

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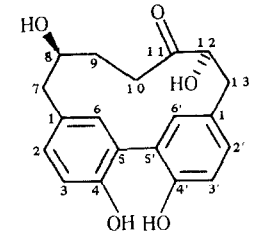
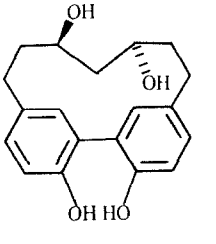
Abstract—A new diarylheptanoid, casuarinondiol, and two known compounds, alnusdiol (a further diarylheptanoid) and (±)-lyoniresinol 2α-*O*-rhamnoside (a lignan), have been isolated from the roots of *Casuarina junghuhniana* and characterized spectroscopically. These are the first representatives of these compound classes to have been obtained from a plant in the Casuarinaceae

INTRODUCTION

Casuarina junghuhniana Miquel roots were collected in Thailand as part of a continuing project to discover novel antineoplastic agents of plant origin This species does not appear to have been investigated phytochemically before, but the various plant parts of another member of the genus, *C. equisetifolia* L., have afforded several common benzenoids and flavonoids [1–3]. Although no biologically active compounds were obtained in the present study, we wish to report the isolation and structure elucidation of a new diarylheptanoid, casuarinondiol (**1**). This substance was accompanied by two known compounds, alnusdiol (**2**) and (±)-lyoniresinol 2α-*O*-rhamnoside (**3**) Compound **2** has been isolated previously from *Alnus japonica* Steud. [4, 5] and the (+)-enantiomer of **3** has been found to occur in the heartwood of *Ulmus thomasii* Sarg. [6] Certain additional NMR data have been obtained for **2** and **3**, as well as for the hydrolysis product of **3**, (±)-lyoniresinol (**4**).



1



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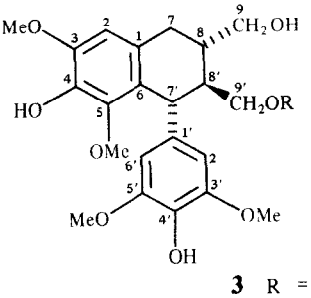

3 R = α-L-Rha
4 R = H

RESULTS AND DISCUSSION

The molecular formula of **1** was determined as $C_{19}H_{20}O_5$ (*m/z*: 328.1308) in its high-resolution mass spectrum. The presence of a phenolic ring was suggested by the absorption maxima at 247 and 300 nm in the UV spectrum and at 3200 (OH), 1510 cm^{-1} (benzene ring) in the IR spectrum. In the ^{13}C NMR spectrum, **1** exhibited 19 carbons, indicating that its skeleton might be that of a phenylheptanoid [4, 5, 7]. Furthermore, the EI mass spectrum base peak at *m/z* 213 suggested the possibility that **1** was a biphenolic heptanoid Two typical phenolic ABX patterns (6H) were observed at δ6.56 (*d*, *J* = 2.1 Hz) and 6.73 (*d*, *J* = 2.1 Hz), 6.82 (*d*, *J* = 8.1 Hz) and 6.83 (*d*, *J* = 8.3 Hz), and 7.03 (*dd*, *J* = 8.2, 2.1 Hz) and 7.05 (*dd*, *J* = 8.1, 2.1 Hz) in the ^1H NMR spectrum In addition, two downfield phenolic carbon atom signals at δ152.3 and 152.7 appeared in the ^{13}C NMR spectrum. The chemical shifts of the seven aliphatic ring carbons of **1** at δ31.8 (*t*), 39.5 (*t*), 40.0 (*t*), 41.7 (*t*), 72.8 (d), 77.7 (*d*) and 220.1 (*s*) in the ^{13}C NMR spectrum implied that this system contained two secondary hydroxy groups and one keto group. The position of these functionalities was confirmed by the selective INEPT experiment which identifies vicinal ^{13}C–^1H coupling [8] Thus, in this experiment, irradiation of the hydroxy-group bearing protons centred at δ4.41 (H-12) and δ4.05 (H-8) ($^3J_{CH}$ = 6 Hz) gave selective enhancements at δ129.4 (C-1′) and δ130.6 (C-1), respect-

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ively. Therefore, the positions of these two hydroxy groups were confirmed at C-8 and C-12. The position of the keto group was proposed as occurring at C-11, because the C-12 methine proton (δ4.41) appeared as a doublet of doublets. To confirm the position of this carbonyl functionality, assignments of all of the alicyclic protons in the molecule of **1** were determined by homo-spin-decoupling, ^{1}H-^{1}H COSY and ^{1}H–^{13}C HETCOR NMR experiments. As a result, the methylene proton signal resonating at δ1.95 was assigned to H-9 from evidence that the multiplet centred at δ4.05 (H-8) was converted to a doublet of doublets (J = 9.4, 3.6 Hz) after spin decoupling. The presence of vicinal ^{13}C–^{1}H coupling between the H-9 protons (δ1.84–2.05) and the keto carbon (δ220.1) was confirmed by a selective INEPT NMR experiment ($^3J_{CH}$ = 4 Hz). The configurations of the two secondary hydroxy groups of **1** were established by a NOESY NMR experiment, in which NOEs were observed between H-6 aromatic (δ6.73) and H-8 (δ4.05), H-10α (δ3.53) and H-9 (δ1.95); H-6′ aromatic (δ6.56) and H-13β (δ3.50) and H-9 (δ1.95); H-12 (δ4.41) and H-13β (δ3.50), H-13α (δ2.88) and H-10β (δ2.92); H-8 (δ4.05) and H-10α (δ3.53); H-7α (δ3.04) and H-9 (δ1.95); H-2′ aromatic (δ7.03) and H-13α (δ2.88), and H-2 aromatic (δ7.05) and H-7β (δ2.81). Therefore, the two hydroxy groups at C-8 and C-12 were confirmed as possessing β- and α-configuration, respectively. It was also noted that the H-13β proton resonated at a lower field (δ3.50) when compared with the H-7α proton (δ3.04). This observation may be explained by the fact that the two phenyl functions of **1** twist somewhat (30–40 degrees) at the biphenyl bond, which forces the alicyclic chain to form a rigid shape [7]. This new diarylheptanoid, to which we have accorded the trivial name, casuarinondiol, therefore possesses the structure represented by **1**.

Compound **2** exhibited a UV spectrum (λ_{max} 248, 302 nm) identical to that of **1**, suggesting that it was also a diarylheptanoid. Its ^{13}C NMR spectrum (10 overlapping resonances), as well as its EI mass spectrum [*m/z* 314 [M]$^+$, 211 (base peak)], indicated that **2** was symmetrical and consisted of the same biphenolic structure as **1**, but possessed no keto function. In the ^{1}H–^{1}H COSY NMR spectrum, the signal (2H, H-9, H-11) at δ3.95–4.05 (*m*) showed a correlation with the peak (2H) at δ1.90–2.00 (*m*), which was confirmed as H-10 by a ^{1}H–^{13}C HETCOR experiment. Therefore, **2** was identified as alnusdiol [4, 5]. More detailed ^{1}H and ^{13}C NMR data for **2** than published previously have been obtained in the present investigation.

Compound **3** showed maxima at 280 nm in the UV spectrum and at 1500–1510 cm^{-1} in the IR spectrum, thereby suggesting the presence of an aromatic moiety. The ^{1}H and ^{13}C NMR spectra (C_{12}–C_6 pattern) indicated this isolate to be a mixture of two lignan rhamnoside enantiomers, occurring in a ratio of *ca* 3 to 2. Hydrolysis of **3** with HCl afforded only one genin, which was identified as (±)-lyoniresinol (**4**) by comparison (mp, $[\alpha]_D$, UV, ^{1}H NMR) with literature values [6, 9, 10], and after appropriate ^{1}H–^{1}H COSY, ^{1}H–^{13}C HETCOR, selective INEPT, and NOESY NMR experiments. Therefore, **3** was considered as a *rac*-lyoniresinol rhamnoside [6], with the rhamnose unit affixed to either the C-9 or C-9′ primary hydroxy group. The rhamnose position was determined as C-9′ by comparison with ^{13}C NMR data of related substances [11], and observation of the glycosylation shift of the α-carbon of the aglycone [12, 13]. The configuration of rhamnose was identified as α, by analysis of coupling constants at the rhamnosyl C-1 carbon [$^1J_{CH}$ = 167.4 Hz (δ102.2) and 166.9 Hz (δ101.9) in the proton-coupled ^{13}C NMR spectrum] [13]. (±)-Lyoniresinol 2α-*O*-rhamnoside does not appear to have been characterized as a plant constituent before, although its (+)-enantiomer has been isolated from *Ulmus thomasii* Sarg. [6].

EXPERIMENTAL

Mps: uncorr. UV. EtOH. IR: KBr disc. ^{1}H and ^{13}C NMR data were recorded on 300 or 360 MHz instruments with TMS as int. std. EIMS (70 eV) data were measured with a direct probe.

Plant material The roots of *C. junghuhniana* Miquel (Casuarinaceae) were collected in June 1988, in Saraburi Province, Thailand A voucher specimen representing this collection has been deposited at the Herbarium of the Royal Forestry Department, Bangkok, Thailand.

Extraction and isolation procedure. The dried roots of *C. junghuhniana* (1.4 kg) were subjected to exhaustive Soxhlet extraction with 95% EtOH (6 l) to afford 42.0 g of residue on removal of solvent under vacuum This extract was taken up in MeOH (20 ml), and the resultant suspension in H_2O (500 ml) was treated successively with $CHCl_3$ (500 ml × 3) and EtOAc (500 ml × 3). The EtOAc layer was concd to dryness and the residue (10 g) was chromatographed batchwise on silica gel with $CHCl_3$–MeOH (5:1), and $CHCl_3$–EtOAc–MeOH (15 5:1) to afford **1** (380 mg, 0.027% w/w) and alnusdiol (**2**, 40 mg, 0.003% w/w) The H_2O layer was evapd to dryness after a ppt. was removed by filtration and the filtrate was dried (14.5 g) and subjected to CC on silica gel by elution with $CHCl_3$–MeOH–H_2O (40:10:1) and EtOAc–MeOH–H_2O (200 20 1), to afford (±)-lyoniresinol 2α-*O*-rhamnoside (**3**, 460 mg, 0.033% w/w).

Casuarinondiol (**1**). Prisms (MeOH), mp 243–244°, $[\alpha]_D^{20}$ +10.0° (EtOH; *c* 0 2). UV λ_{max} nm (log ε): 247 (sh, 4.11), 300 (3.94); IR ν_{max} cm^{-1}: 3500–3000, 1710, 1510, 1080, 800; ^{1}H NMR [CD_3OD–$CDCl_3$ (5·1)]: δ1.84–2.05 (2H, *m*, H-9), 2.81 (1H, *dd*, J = 15.8, 9 7 Hz, H-7β), 2.88 (1H, *dd*, J = 15 1, 6.5 Hz, H-13α), 2.92 (1H, *ddd*, J = 19.8, 11.9, 8.3 Hz, H-10β), 3.04 (1H, *dd*, J = 15 8, 3.2 Hz, H-7α), 3.50 (1H, *d*, J = 13 9 Hz, H-13β), 3.53 (1H, *ddd*, J = 19.3, 10.9, 2 7 Hz, H-10α), 4.01–4.08 (1H, *m*, H-8α), 4.41 (1H, *dd*, J = 6.4, 1.7 Hz, H-12β), 6.56 (1H, *d*, J = 2.1 Hz, H-6′), 6.73 (1H, *d*, J = 2.1 Hz, H-6), 6.82 (1H, *d*, J = 8 1 Hz, H-3′), 6.83 (1H, *d*, J = 8.3 Hz, H-3), 7.03 (1H, *dd*, J = 8.2, 2.1 Hz, H-2′), 7.05 (1H, *dd*, J = 8 1, 2.1 Hz, H-2); ^{13}C NMR [CD_3OD–$CDCl_3$ (5 1)]: δ31.8 (*t*, C-9), 39 5 (*t*, C-10), 40.0 (*t*, C-13), 41.7 (*t*, C-7), 72.8 (*d*, C-8), 77.7 (*d*, C-12), 116.6 (*d*, C-3), 116.7 (*d*, C-3′), 126.6 (*s*, C-5′), 127 8 (*s*, C-5), 129 4 (*s*, C-1′), 130.2 (*d*, C-2), 130.6 (*s*, C-1), 131 4 (*d*, C-2′), 134.4 (*d*, C-6′), 135.3 (*d*, C-6), 152 3 (*s*, C-4), 152.7 (*s*, C-4′), 220.1 (*s*, C-11), EIMS *m/z* (rel int.) 328 ([M]$^+$, 5), 310 ([M − H_2O]$^+$, 5), 213 (100); HRMS: mass measurement, found, 328.1308, calcd for $C_{19}H_{20}O_5$, 328.1311

Alnusdiol (**2**) Prisms (MeOH), mp 293–298° (dec), $[\alpha]_D^{20}$ −45.3° (EtOH; *c* 0.15) {lit mp >300°, $[\alpha]_D$ −46 7° (EtOH; *c* 0.52) [4]}. UV λ_{max} nm (log ε). 248 (sh, 4.02), 302 (3.98), IR ν_{max} cm^{-1}: 3500–2900, 1510, 1250–1230, 940, 800, ^{1}H NMR [CD_3OD–$CDCl_3$ (5:1)]: δ1 70–1.85 (2H, *m*, H-8α, 12α), 1.90–2 00 (2H, *m*, H-10), 2 30–2.45 (2H, *m*, H-8β, 12β), 2.80–3 04 (4H, *m*, H-7, 13), 3 95–4.05 (2H, *m*, H-9, 11), 6.83 (2H, *d*, J = 8 9 Hz, H-3, 3′), 7 03 (2H, *br s*, H-6, 6′), 7.04 (2H, *d*, J = 8 3 Hz, H-2, 2′); ^{13}C NMR [CD_3OD–$CDCl_3$ (5:1)]: δ27 2 (*t*, C-7, 13), 35.5 (*t*, C-8, 12) 51.3 (*t*, C-10), 67.0 (*d*, C-9, 11), 116 7 (*d*, C-3, 3′), 126.8 (*s*, C-5, 5′), 130.2 (*d*, C-2, 2′), 131.7 (*s*, C-1, 1′), 134.6 (*d*, C-6,

6'), 151 8 (*s*, C-4, 4'), EIMS *m/z* (rel int.): 314 ([M]$^+$, 100), 296 ([M $-H_2O$]$^+$, 3), 211 (75).

(±)-*Lyoniresinol 2α-O-rhamnoside* (**3**). Amorphous powder, $[\alpha]_D^{20} -34.7°$ (EtOH, *c* 0.08) UV λ_{max} nm (log ε). 280 (3 51), IR ν_{max} cm^{-1} 3600–3000, 1510, 1500, 1100–1000, 800, ^{1}H NMR (CD_3OD): δ1 21* (*d*, *J* = 6.1 Hz) and 1.31 (*d*, *J* = 6 1 Hz) (3H, Rha-Me), 1.60–1 73 (1H, *m*, H-8), 2 05–2 14 (1H, *m*, H-8'), 2 60–2.78 (2H, *m*, H-7), 3 35 (3H, *s*, 5-OMe), 3 74 (6H, *s*, 3', 5'-OMe), 3 84 (3H, *s*, 3-OMe), 4 31 (1H, *d*, *J* = 5 8 Hz, H-7'), 4 68 (*br s*) and 4 74* (*br s*) (1H, Rha C_1-H), 6.36* (*s*) and 6 38 (*s*) (2H, H-2', 6'), 6.58 (1H, *s*, H-2), ^{13}C NMR (CD_3OD): δ17.9* and 18 1 (*q*, Rha C-6), 33.5 (*t*, C-7), 40.6 and 40.9* (*d*, C-8), 42 9 (*d*, C-7'), 46.4* and 46.5 (*d*, C-8'), 56.6 (*q*, 3-OMe), 56 9 (*q*, 3', 5'-OMe), 60 1 (*q*, 5-OMe), 66.2* and 66.3 (*t*, C-9), 69 7* and 70 0 (*t*, C-9'), 70 2 (*d*, Rha C-5), 72.2 (*d*, Rha C-1), 72.5 (*d*, Rha C-3), 73.9 (*d*, Rha C-4), 101.9* and 102 2 (*d*, Rha C-1), 106.6* and 106 8 (*d*, C-2', 6'), 107 7 (*d*, C-2), 125.9* and 126 1 (*s*, C-6), 130.1 (*s*, C-1), 134 5 (*s*, C-4'), 138 8 (*s*, C-4), 139 1* and 139 2 (*s*, C-1'), 147 4* and 147 5 (*s*, C-5), 148.6 (*s*, C-3), 148.9 (*s*, C-3', 5') (Peaks marked with an asterisk were of lesser intensity than their companion peaks of similar resonance.), EIMS *m/z* (rel. int.) 566 ([M]$^+$, 21), 419 ([M – Rha]$^+$, 49), 401 ([M – Rha – H_2O]$^+$, 56), 167 (100)

Hydrolysis of (±)-*lyoniresinol 2α-O-rhamnoside* (**3**) Compound **3** (50 mg) was hydrolysed with 2M HCl/EtOH–H_2O (1 1) at 80° for 2 hr to give (±)-lyoniresinol (**4**, 20 mg), prisms (MeOH), mp 189–191°, $[\alpha]_D^{20}$ 0° (EtOH, *c* 0 15) {lit mp 193–194°, $[\alpha]_D$ 0° [6]} UV λ_{max} nm (log ε) 281 (3.55); IR ν_{max} cm^{-1}, 3500–3000, 1520, 1510, 1200, 1100; ^{1}H NMR [CD_3OD–$CDCl_3$ (5:1)] δ1 61–1.75 (1H, *m*, H-8), 1.91–2 01 (1H, *m*, H-8'), 2.58 (1H, *dd*, *J* = 15.1, 11.4 Hz, H-7), 2.69 (1H, *dd*, *J* = 15 1, 5 0 Hz, H-7'), 3 36 (3H, *s*, 5-OMe), 3 48–3 62 (4H, *m*, H-9, 9'), 3 77 (6H, *s*, 3', 5'-OMe), 3 88 (3H, *s*, 3-OMe), 4 23 (1H, *d*, *J* = 5.8 Hz, H-7'), 6.38 (2H, *s*, H-2', 6'), 6.54 (1H, *s*, H-2), ^{13}C NMR [CD_3OD–$CDCl_3$ (5 1)] δ33 3 (*t*, C-7), 40.4 (*d*, C-8), 42.1 (*d*, C-7'), 48 9 (*d*, C-8'), 56 2 (*q*, 3-OMe), 56 5 (*q*, 3', 5'-OMe), 59 9 (*q*, 5-OMe), 63 8 (*t*, C-9'), 66.4 (*t*, C-9), 106 0 (*d*, C-2', 6'), 107 1 (*d*, C-2), 125 3 (*s*, C-6), 129 3 (*s*, C-1), 133 5 (*s*, C-4'), 138 0 (*s*, C-4), 138 5 (*s*, C-1'), 146 7 (*s*, C-5), 147 6 (*s*, C-3), 147 9 (*s*, C-3', 5'), CIMS *m/z* 420 [M]$^+$

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