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9α-HYDROXYPINORESINOL, 9α-HYDROXYMEDIORESINOL AND RELATED LIGNANS FROM *ALLAMANDA NERIIFOLIA**

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Key Word Index—*Allamanda neriifolia*; Apocynaceae; lignan; 9α-hydroxydioxabicyclo-type lignan.

Abstract—9α-Hydroxypinoresinol and 9α-hydroxymedioresinol were isolated along with seven known lignans, pinoresinol, medioresinol, syringaresinol, and their glucosides, from *Allamanda neriifolia*.

INTRODUCTION

In the course of our studies on the constituents of Apocynaceae plants, we have described the iridoids from *Allamanda neriifolia* Hook [1, 2]. This paper deals with the lignans obtained during the isolation of iridoids.

RESULTS AND DISCUSSION

Three lignans, (+)-pinoresinol (**1**), (+)-medioresinol (**2**), and (+)-syringaresinol (**3**), a monoglucoside of **1** (**4**) and the bis-glucosides of **1**, **2** and **3** (**5**, **6** and **7**, respectively) were isolated from the branches of *A. neriifolia*. Their structures were identified by comparison of their physical and spectral data with those in the literature [3], and by the products released on enzymic hydrolysis.

From the air-dried leaves of the same plant, two lignans (**8** and **9**) were isolated, along with large amounts of **1** and **2**. Lignan **8** had a molecular formula, $C_{20}H_{22}O_7$, based on the molecular ion peak at *m/z* 374.134. A lignan framework of the pinoresinol-type made up of two benzene moieties with two methoxyl groups was suggested by the ^{13}C and ^1H NMR spectra. The presence of three hydroxyl groups was revealed by acetylation to yield a triacetate (**8**-1). One of the hydroxyl groups was assigned to C-9 on the basis of an acetal carbon signal at δ102.6 and an acetal proton signal at δ6.14. Sodium borohydride reduction of **8** afforded a dihydro derivative (**8**-2), in the ^1H NMR spectrum of which a pair of methylene protons at δ4.30 and 4.65, instead of a singlet peak at δ6.14, was assignable to the newly formed primary carbinyl protons. The acetal carbon signal of **8** (δ102.6, *s*) was shifted upfield and was transformed to a primary carbinyl carbon signal (δ60.7, *t*) in the ^{13}C NMR spectrum of **8**-2, suggesting **8**-2 had primary hydroxyl at C-9 arising from cleavage of the furan ring. The orientation of the hydroxyl group at C-9 was assigned as α, since a small coupling constant (J = 1 Hz) was observed between H-9 and H-8 of **8**. Upfield shifts of H-2′ and H-6′ signals in **8**-1 can be explained by the presence of hydrogen bond between the 9α-hydroxyl group and H-2′ or H-6′ in **8**. Lignan **8** was therefore assigned as 9α-hydroxypinoresinol.

The molecular formula of **9** was determined to be

*Part 3 in the series 'Studies on Allamanda'. For Part 2, see ref. [2].

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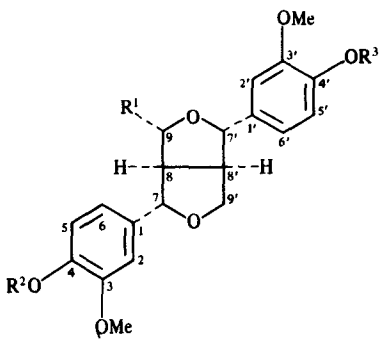
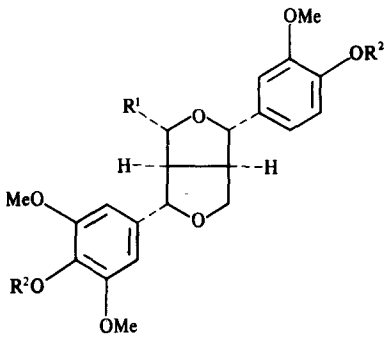
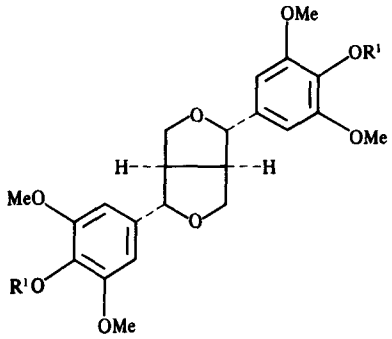


1 R^1 = H, R^2 = R^3 = H
4 R^1 = H, R^2 = glu, R^3 = H
5 R^1 = H, R^2 = R^3 = glu
8 R^1 = OH, R^2 = R^3 = H



2 R^1 = H, R^2 = H
6 R^1 = H, R^2 = glu
9 R^1 = OH, R^2 = H



3 R^1 = H
7 R^1 = glu

$C_{21}H_{24}O_8$, based on the EIMS peak at m/z 404.146 $[M]^+$. Lignan **9** afforded a triacetate (**9**-1) and a dihydro derivative (**9**–2) as in the case of **8**. The carbon peaks due to the two phenolic moieties and the furan ring moiety were almost superimposable to those of **2** and **8**, respectively. NOEs were observed between H-9/H-7, H-7/H-2, H-7/H-6, H-7′/H-2′, and H-7′/H-6′, and the structure of **9** was established as 9α-hydroxymedioresinol.

Homologous 9-hydroxylignans, 9β-hydroxypinoresinol [4] and 9-hydroxysesamine [5] have been isolated. This is the first report on the isolation of 9α-hydroxypinoresinol and 9α-hydroxymedioresinol.

EXPERIMENTAL

General. Mps: uncorr. NMR: 400 and 100 MHz, C_5D_5N, TMS as int. standard; TLC and silica gel CC, the following solvent systems were used. 1: C_6H_6–Me_2CO (10:1–5:1); 2: hexane–AcOEt (2:1–1:1); 3: H_2O–MeOH; 4: $CHCl_3$–MeOH–H_2O (7:2:2, 7:3:1, bottom layer).

Extraction and isolation. Branches of *Allamanda neriifolia*, cultivated at Ibusuki Experimental Station of Kyushu University, were harvested in March, 1982, and the leaves removed. After air-drying, the branches (dry wt 2.6 kg) were powdered, percolated with MeOH and the MeOH percolate concd *in vacuo*. The concd soln (1.5 l) was then diluted with 1.5 l H_2O, and extracted with C_6H_6 (ext. 6.2 g), $CHCl_3$ (15 g), and *n*-BuOH (75.6 g). Lignans **1** (815 mg), **2** (338 mg) and **3** (190 mg) along with tricyclic and tetracyclic iridoids [1, 2] were isolated from the C_6H_6 and $CHCl_3$ extracts by repeated chromatography on silica gel columns with solvents 1 and 2. The BuOH extract was passed through a polystylene column (Mitsubishi Chem. Co., CHP-20P) eluted with solvent 3 containing increasing amounts of MeOH (30–60%). The 40% MeOH effluent was chromatographed on a silica gel column with solvent 4 to afford **4** (220 mg), along with plumieride [1]. From the 50–60% MeOH effluent, protoplumericin A[1] was obtained. The H_2O layer, after extraction with *n*-BuOH, was concd *in vacuo*, and passed through a polystylene column. The fraction eluted with 50% MeOH, was chromatographed on a silica gel column with solvent 4 to give **5** (390 mg), **6** (90 mg), and **7** (80 mg).

Air-dried leaves of the same plant (dry wt 6.4 kg) were powdered and treated in the same manner as described for the stems. From the C_6H_6 extract (17.7 g), **1** (1.1 g) was isolated. From the $CHCl_3$ extract (25.5 g), **2** (1.6 g), **8** (140 mg) and **9** (32 mg) were isolated after chromatography on a polystylene column with solvent 3 and a silica gel column with solvent 1.

Known lignans. (+)-Pinoresinol (**1**): solid, $[\alpha]_D^{24}$ + 71.1° (MeOH; *c* 0.95), EIMS (probe) 70 eV, m/z: 358 $[M]^+$, 205, 163, 151, 137; ^{13}C NMR δ: 133.2 (C-1,1′), 111.0 (C-2,2′), 147.8, 148.8 (C-3, 3′, 4, 4′), 116.4 (C-5, 5′), 119.7 (C-6, 6′), 86.4 (C-7, 7′), 54.8 (C-8, 8′), 71.9 (C-9,9′), 56.0 (–OMe). Acetate of 1 (**1**-1), prisms, mp 160–165°, $[\alpha]_D^{28}$ + 36.7° (MeOH; *c* 0.49), EIMS m/z: 442 $[M]^+$.

(+)-Medioresinol (**2**): prisms from MeOH, mp 170–172°, $[\alpha]_D^{28}$ + 77.7° (MeOH; *c* 0.69), EIMS (probe) 70 eV, m/z: 388 $[M]^+$, 205, 193, 181, 163, 151; ^{13}C NMR δ: 132.3 (C-1), 133.2 (C-1′), 104.9 (C-2, 6), 111.0 (C-2′), 149.3 (C-3, 5), 147.8, 148.8 (C-3′, 4′), 137.4 (C-4), 116.4 (C-5′), 119.7 (C-6′), 86.6, 86.4 (C-7, 7′), 55.0, 54.7 (C-8, 8′), 72.1, 71.9 (C-9, 9′), 56.5 (× 2) (–OMe), 56.0 (–OMe).

(+)-Syringaresinol (**3**): prisms from MeOH, mp 173–174°, $[\alpha]_D^{28}$ + 5.1° (MeOH: *c* 0.5), EIMS (probe) 70 eV, m/z: 418 $[M]^+$, 235, 205, 193, 181, 167; ^{13}C NMR δ: 132.2 (C-1,1′), 104.8 (C-2, 2′, 6, 6′), 149.3 (C-3, 3′, 5, 5′), 137.3 (C-4, 4′), 86.6 (C-7,7′), 72.4 (C-8, 8′), 55.0 (C-9, 9′), 56.6 (–OMe).

4-*O*-Glucoside of **1** (**4**): solid, $[\alpha]_D^{26}$ – 8.7° (MeOH; *c* 1.64). FABMS m/z: 543 $[M + Na]^+$. On hydrolysis with cellulase (10 mg, Sigma) in 20% EtOH at 38°, **4** (5 mg) was hydrolysed to **1** and glucose, which were identified by TLC (solvent 4).

4,4′-*O*-Bisglucoside of **1** (**5**): prisms from MeOH, mp 134–140°, $[\alpha]_D^{28}$ – 29.3° (MeOH; *c* 0.82). FDMS m/z: 682 $[M]^+$, 520, 358. On hydrolysis of **5** (5 mg) with cellulase (10 mg) in the same manner as for **4**, **1** and glucose were detected on TLC (solvent 4).

4,4′-*O*-Bisglucoside of **2** (**6**): prisms from MeOH, mp 232–234°, $[\alpha]_D^{28}$ – 25.8° (MeOH–H_2O; *c* 0.33). FDMS m/z: 712 $[M]^+$, 550, 388. On hydrolysis with cellulase in the same manner as of **4**, **2** and glucose were detected on TLC (solvent 4).

4,4′-*O*-Bisglucoside of **3** (**7**): a crystalline powder from MeOH, mp 235–240°, $[\alpha]_D^{28}$ – 15.9° (MeOH; *c* 0.32). FDMS m/z: 765 $[M + Na]^+$, 580, 418, 163. On hydrolysis with cellulase, **3** and glucose were detected on TLC (solvent 4).

9α-Hydroxypinoresinol (**8**). Solid, $[\alpha]_D^{26}$ + 55.0° (MeOH; *c* 0.95), EIMS (probe) 70 eV, m/z: 374.134, $C_{20}H_{22}O_7$ requires 374.136. ^1H NMR δ: 7.29 (1H, *d*, *J* = 2 Hz, H-2), 7.25 (1H, *d*, *J* = 8 Hz, H-5), 7.16 (1H, *dd*, *J* = 8, 2 Hz, H-6), 7.64 (1H, *d*, *J* = 2 Hz, H-2′), 7.28 (1H, *d*, *J* = 8 Hz, H-5′), 7.30 (1H, *dd*, *J* = 8, 2 Hz, H-6′), 5.29 (1H, *d*, *J* = 6 Hz, H-7), 3.44 (1H, *m*, H-8), 6.14 (1H, *d*, *J* = 1 Hz, H-9), 5.30 (1H, *d*, *J* = 6 Hz, H-7′), 3.45 (1H, *m*, H-8′), 4.25 (1H, *dd*, *J* = 9, 2 Hz, H-9′β), 4.39 (1H, *dd*, *J* = 9, 6 Hz, H-9′α), 3.743, 3.737 (3H each, *s*, –OMe); ^{13}C NMR δ: 134.0, 135.5 (C-1, 1′), 110.8, 111.4 (C-2, 2′), 147.7, 147.8, 148.8, 148.9 (C-3, 3′, 4, 4′), 116.2, 116.5 (C-5, 5′), 119.5, 120.0 (C-6, 6′), 84.2 (C-7), 87.9 (C-7′), 63.2 (C-8), 54.7 (C-8′), 102.6 (C-9), 72.5 (C-9′), 55.9, 55.8 (–OMe). Triacetate of **8** (**8**-1): Formed on acetylation of **8** with C_5H_5N and Ac_2O.

FABMS m/z: 523 ($[M + Na]^+$, $C_{26}H_{28}O_{10}$ + Na), 463, 247, 205, 137; ^1H NMR δ: 2.01, 2.28, 2.29 (–OAc), 6.85 (1H, *s*, 9-H). Lignan **8** was dissolved in MeOH (3 ml) and stirred with $NaBH_4$ (24 mg) at room temp. for 3 hrs. The mixture was then diluted with H_2O and extracted with BuOH. The BuOH extract was purified on a silica gel column with solvent 1 to afford a homogeneous solid (**8**-2), $[\alpha]_D^{26}$ – 68.0° (MeOH; *c* 0.5). FABMS m/z: 399.144, $C_{20}H_{24}O_7Na$ requires 399.142. ^1H NMR δ: 7.36, 7.31 (1H each, *d*, *J* = 2 Hz, H-2, 2′), 7.25, 7.24 (1H each, *d*, *J* = 8 Hz, H-5, 5′), 7.20, 7.16 (1H each, *dd*, *J* = 8, 2 Hz, H-6, 6′), 5.37 (1H, *d*, *J* = 5 Hz, H-7), 3.14 (1H, *m*, H-8), 4.65 (1H, *br t*, *J* = 10 Hz, H-9a), 4.30 (1H, *m*, H-9b), 6.65 (1H, 9-OH), 5.23 (1H, *d*, *J* = 10 Hz, H-7′), 3.35 (1H, *m*, H-8′), 4.04, 3.95 (1H each, *t*, *J* = 9 Hz, H-9′), 3.72, 3.70 (3H each, *s*, –OMe); ^{13}C NMR δ: 135.5, 136.7 (C-1, 1′), 110.0, 110.5 (C-2, 2′), 147.7, 147.4 (C-3, 3′), 148.7 (C-4, 4′), 116.3, 116.2 (C-5, 5′), 119.5, 119.2 (C-6,6′), 84.2 (C-7), 73.0 (C-7′), 50.1 (C-8), 53.2 (C-8′), 60.7 (C-9), 71.0 (C-9′), 55.8 (× 2) (–OMe).

9α-Hydroxymedioresinol (**9**). Prisms from MeOH, mp 210–213°, $[\alpha]_D^{18}$ + 44.0° (MeOH; *c* 0.49), EIMS (probe) 70 eV, m/z: 404.146, $C_{21}H_{24}O_8$ requires 404.147. ^1H NMR δ: 7.04 (2H, *s*, H-2,6), 7.65 (1H, *d*, *J* = 2 Hz, H-2′), 7.27 (1H, *d*, *J* = 8 Hz, H-5′), 7.32 (1H, *dd*, *J* = 8,2 Hz, H-6′), 5.30 (1H, *d*, *J* = 6 Hz, H-7), 3.47 (1H, *t*, *J* = 8 Hz, H-8), 6.18 (1H, *s*, H-9), 5.33 (1H, *d*, *J* = 6 Hz, H-7′), 3.49 (1H, *m*, H-8′), 4.28 (1H, *dd*, *J* = 9,2 Hz, H-9′β), 4.43 (1H, *dd*, *J* = 9, 2 Hz, H-9′α), 3.79 (6H, *s*, –OMe), 3.74 (3H, *s*, –OMe). In the 2D-NOE correlated spectrum of 9 there were cross peaks between the signals; H-9/H-7, H-7/H-2, H-7/H-6, H-7′/H-2′, H-7′/H-9′, H-8/H-9′α, 3,5-OMe/H-2,6. ^{13}C NMR δ: 133.0 (C-1), 135.6 (C-1′), 104.7 (C-2,6), 111.3 (C-2′), 149.3 (C-3,5), 147.7, 148.8 (C-3′, 4′), 137.2 (C-4), 116.2 (C-5′), 120.6 (C-6′), 84.4 (C-7), 87.9 (C-7′), 63.4 (C-8), 54.8 (C-8′), 102.5 (C-9), 72.6 (C-9′), 56.4 (× 2) (–OMe), 55.8 (–OMe). Triacetate of **9** (**9**-1): FABMS m/z: 553 ($[M + Na]^+$, $C_{27}H_{30}O_{11}$ + Na), 488, 471, 381, 359, 294. Reduction of **9** with $NaBH_4$ was carried out in the same manner as for **8**, to afford **9**–2 as a solid, $[\alpha]_D^{25}$ – 24.0° (MeOH; *c* 0.25), FABMS m/z: 429.153, $C_{21}H_{26}O_8Na$ requires 429.153. ^1H NMR δ: 7.07 (2H, *s*, H-2,6), 7.36 (1H, *d*, *J* = 2 Hz, H-2′), 7.23 (1H, *d*, *J* = 8 Hz, H-5′), 7.16 (1H, *dd*, *J* = 8, 2 Hz, H-6′), 5.43 (1H, *d*, *J* = 4 Hz, H-7), 3.19

(1H, *m*, H-8), 4.69, 4.34 (1H each, *dd*, J = 11, 7 Hz, H-9), 5.24 (1H, *d*, J = 10 Hz, H-7′), 3.37 (1H, *m*, H-8′), 4.07, 3.98 (1H each, *t*, J = 9 Hz, H-9′), 3.75 (6H, *s*, –OMe), 3.71 (3H, *s*, –OMe); ^{13}C NMR δ: 134.6, 136.7 (C-1, 1′), 104.3 (C-2, 6), 110.9 (C-2′), 149.2 (C-3, 5), 147.7, 148.7 (C-3′, 4′), 136.7 (C-4), 116.2 (C-5′), 119.8 (C-6′), 84.3 (C-7), 72.8 (C-7′), 50.0 (C-8), 53.2 (C-8′), 60.7 (C-9), 71.0 (C-9′), 56.3 (× 2) (–OMe), 55.8 (–OMe).

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