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Aryl naphthalenes norlignans from *Vitex rotundifolia*

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Abstract

Three arylnaphthalene norlignans were isolated from the root of *Vitex rotundifolia*. Their structures were established from spectroscopic evidence as 1-(3,4-dimethoxyphenyl)-7-hydroxy-8- methoxynaphthalene-3-carbaldehyde, 1-(3,4-dimethoxyphenyl)-2,7-dihydroxy-8-methoxynaphthalene-3-carbaldehyde and 2,7-dihydroxy-1,9,10-trimethoxy-7*H*-benzo[*c*]fluorene-6-carbaldehyde, respectively.

Keywords: Vitex rotundifolia; Verbenaceae; Arylnaphthalene; Lignan; Vitrofolal

1. Introduction

Vitex rotundifolia (Verbenaceae) is a common plant growing on East Asian beaches, and its seed is not only used in Japan for a cold remedy, i.e., treatment of headaches, but also used as raw material for Chinese traditional medicine. From this plant, some diterpenoids and sesquiterpenoids have been obtained (Asaka, Kamikawa & Kubota, 1973; Tada & Yasuda, 1984), and interestingly, some insecticidal substances are present in its leaf tissue as well (Watanabe, Takasa, Matsuo & Nishimura, 1995). In this paper, we describe the charactersiation of three norlignans, vitrofolals A, B and C, in roots of *V. rotundifolia*.

2. Results and discussion

A methanol extract obtained from the roots of *V. rotundifolia* was partitioned between EtOAc and water, with the resulting EtOAc soluble fraction subjected to chromatography to obtain each component (see Section 3).

Vitrofolal A (**1**) had a strong UV absorption (269, 224 nm) indicator of a naphthalene chromophore, whereas in the IR spectrum, both hydroxy and carbonyl groups were detected at 3600–2800 and 1687 cm^{-1}, respectively. From its EIMS spectrum, a formula of $C_{20}H_{18}O_5$ was deduced. The ^{1}H-NMR spectrum indicated eight deshielded protons, including a formyl group and three methoxyl groups (Table 1); thus, a naphthalene bearing an aryl ring was proposed for the skeleton of **1**. All C–H correlations were revealed by analysis of a HSQC spectrum. In the ^{1}H–^{1}H COSY, three correlation series were shown, i.e., I: δ 7.83 (H-2) and 8.27 (H-4), II: δ 7.83 (H-5) and 7.39 (H-6), and III: δ 7.03 (H-2′), 6.93 (H-5′) and 7.06 (H-6′). These groups were assigned to three aromatic rings and their position was established by the coupling constants. From the NOESY, the nOe correlations between H-2 and both H-2′ and H-6′, and between H-4 and both H-5 and an aldehyde proton were observed. Thus, an aromatic ring containing series III and the series I and II form a 1-arylnaphthalene skeleton with a formyl group at C-3. The HMBC spectrum showing correlations between the aldehyde proton and C-2 and C-4, H-6 and C-4a, C-7 and C-8, H-2′ and C-1 and C-4′, supported these findings, and assigned three methoxyl groups to C-8, C-3′ and C-4′. Consequently, we were able to assign all proton and carbon signals, and **1** was defined as 1-(3,4-dimethoxyphenyl)-7-hydroxy-8-methoxynaphthalene-3-carbaldehyde.

The ^{1}H-NMR spectral pattern of vitrofolal B (**2**)

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Table 1
^{1}H-NMR spectral data for **1**[a], **2**[a] and **3**[b]

	1	**2**		**3**
2	7.83, 1H, *d*, $J = 1.5$ Hz	–	3	7.40, 1H, *d*, $J = 8.8$ Hz
4	8.27, 1H, *d*, $J = 1.5$ Hz	8.11, 1H, *s*	4	7.83, 1H, *d*, $J = 8.8$ Hz
5	7.83, 1H, *d*, $J = 8.8$ Hz	7.68, 1H, *d*, $J = 8.8$ Hz	5	8.21, 1H, *s*
6	7.39, 1H, *d*, $J = 8.8$ Hz	7.18, 1H, *d*, $J = 8.8$ Hz	7	5.85, 1H, *d*, $J = 3.0$ Hz
2′	7.03, 1H, *d*, $J = 1.9$ Hz	6.99, 1H, *brs*	8	7.34, 1H, *s*
5′	6.93, 1H, *d*, $J = 8.2$ Hz	ca. 7.0, 1H, *m*	11	8.32, 1H, *s*
6′	7.06, 1H, *dd*, $J = 1.9$, 8.2 Hz	ca. 7.0, 1H, *m*	1-OMe	3.69, 3H, *s*
2-OH	–	10.71, 1H, *s*	2-OH	6.52, 1H, *s*
3-CHO	10.12, 1H, *s*	10.05, 1H, *s*	6-CHO	10.14, 1H, *s*
7-OH	6.32, 1H, *s*	6.25, 1H, *s*	7-OH	5.06, 1H, *d*, $J = 3.0$ Hz
8-OMe	3.12, 3H, *s*	3.13, 3H, *s*	9-OMe	4.03, 3H, *s*
3′-OMe	3.90, 3H, *s*	3.89, 3H, *s*	10-OMe	4.03, 3H, *s*
4′-OMe	3.97, 3H, *s*	3.97, 3H, *s*		

[a] Spectra were measured in $CDCl_3$.
[b] Spectra were measured in pyridine-d_5.

was very similar to that of vitrofolal A (**1**), except that it had one D_2O exchangeable singlet signal at low-field (δ 10.71) which resulted in a singlet aromatic methine signal (δ 8.11) appearing instead of two doublet signals coupled with each other.

Furthermore, the formula $C_{20}H_{18}O_6$ suggested that **2** is an oxidized compound of **1**. A proton at δ 10.71 should contribute to forming a hydrogen bond because it appeared at low-field as a sharp singlet signal. This indicated that the hydroxyl group was adjacent to the formyl group. In the nOe experiment, a correlation between H-4 and H-5 (δ 8.11 and 7.68) was observed. From these facts, it was revealed that **2** has a hydroxy group at C-2, and its structure was deduced as 1-(3,4-dimethoxyphenyl)-2,7-dihydroxy-8-methoxynaphthalene-3-carbaldehyde.

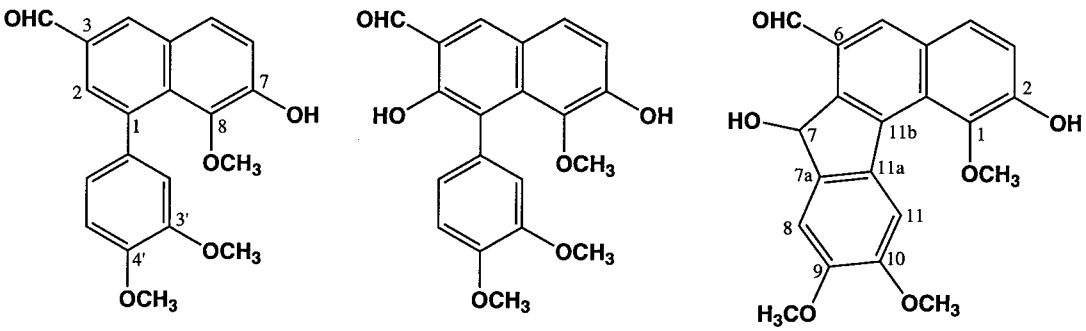
Vitrofolal C (**3**), $C_{21}H_{18}O_6$, displayed analogous spectral data to vitrofolal A (**1**). One doublet methine proton (δ 5.85, $J = 3.0$ Hz) coupled with the hydroxy proton showed long-range correlations with carbon signals at δc 135.8 (C-7a), 132.6 (C-11a), and 139.0 (C-11b) in the HMBC spectrum. Furthermore, in the NOESY spectrum, correlations were observed between H-7 and singlet aromatic proton H-8, and between H-11 and a methoxy group attached to C-1. From these facts, it was revealed that two aromatic rings and the methine carbon make a fluorene ring system. Except for near C-7, the correlation patterns of nOe were close to those of vitrofolal A (**1**). Consequently, vitrofolal C (**3**) was deduced to be 2,7-dihydroxy-1,9,10-trimethoxy-7*H*-benzo[*c*]fluorene-6-carbaldehyde.

Each of the compounds has an arylnaphthalene skeleton that has peviously been only occasionally reported (Rischmann, Mues, Geiger, Laas & Eicher, 1989; Feliciano, Corral, Gordaliza & Castro, 1991). From the viewpoint of their biosynthesis, vitrofolal A (**1**), B (**2**) and C (**3**) should be formed via coniferyl alcohol coupling and subsequent metabolism as described elsewhere (Lewis & Davin, 1999).

3. Experimental

3.1. General

^{1}H- (400 MHz) and ^{13}C-NMR (100 MHz) in $CDCl_3$ and pyridine-d_5 with TMS as internal standard. Column chromatograhpy used silica gel 60 (230-400 mesh, Merck). Silica gel HPLC and GPC (Gel



1 **2** **3**

Table 2
^{13}C-NMR spectral data for **1**[a], **2**[a] and **3**[b]

	1	2		3
1	137.3	120.0	1	140.8
2	127.2	154.6	2	150.9
3	131.5	128.7	3	118.3
4	134.5	138.5	4	129.1
4a	129.9	124.6	4a	130.6
5	128.4	128.7	5	138.2
6	118.3	116.2	6	129.0
7	150.2	151.5	6a	144.5
8	141.0	140.1	7	74.3
8a	128.8	131.6	7a	135.8
1′	134.6	119.7	8	109.8
2′	113.7	114.7	9	149.3
3′	147.7	148.0	10	149.7
4′	148.4	148.3	11	107.9
5′	109.9	110.1	11a	132.6
6′	122.0	123.2	11b	139.0
3-CHO	191.9	196.2	11c	125.7
8-OMe	61.6	61.6	1-OMe	63.0
3′-OMe	56.0	56.0	6-CHO	195.0
4′-OMe	56.1	56.2	9-OMe	56.4
			10-OMe	56.3

[a] Spectra were measured in $CDCl_3$.
[b] Spectra were measured in pyridine-d_5.

Permeation Chromatography) employed SIL-06 (250 × 20 mm, YMC) and H- 2001 (1000 × 20 mm, Shodex), respectively.

3.2. Plant material

Roots of *V. rotundifolia* were collected in 1997, at Tosa-domari beach in Tokushima Prefecture, Japan. The identification of the plant material was comfirmed by Dr. K. Murakami, and a voucher specimen is deposited in the herbarium in Faculty of Pharmaceutical Sciences, University of Tokushima.

3.3. Extraction and fractionation

The MeOH extract from 12.1 kg of air-dried material was partitioned between EtOAc and H_2O to obtain an EtOAc-soluble portion (106 g). This was applied to a silica gel column which was eluted with $CHCl_3$–MeOH (4 : 1) to obtain frs. 1–9. Fr. 2 (19.9 g) was purified by silica gel HPLC (*n*-hexane–EtOAc, 4 : 1) and GPC ($CHCl_3$) to obtain vitrofolal A (**1**, 10.6 mg) and vitrofolal C (**3**, 18.2 mg). Fr. 3 (11.2 g) was purified by GPC to obtain vitrofolal B (**2**, 3.7 mg).

3.4. Vitrofolal A (*1*)

Amorphous yellow powder, HR-EI MS: *m/z* 338.1158 $[M]^+$, calcd. for $C_{20}H_{18}O_5$, 338.1155. IR ν_{max}^{KBr} cm^{-1}: 3600–2800, 1687, 1608, 1511, 1467, 1250, 1157, 1084, 1024. UV λ_{max}^{MeOH} nm (log ε): 329 (4.0), 269 (4.5), 224 (4.5), 208 (4.6). ^{1}H-NMR spectral data (Table 1), ^{13}C-NMR spectral data (Table 2).

3.5. Vitrofolal B (*2*)

Amorphous yellow powder, HR-EI MS: *m/z* 354.1100 $[M]^+$, calcd. for $C_{20}H_{18}O_6$, 354.1103. IR ν_{max}^{KBr} cm^{-1}: 3600–2800, 1656, 1517, 1385, 1252, 1026. UV λ_{max}^{MeOH} nm (log ε): 338 (3.8), 283 (4.2), 206 (4.3). ^{1}H-NMR spectral data (Table 1), ^{13}C-NMR spectral data (Table 2).

3.6. Vitrofolal C (*3*)

Amorphous red powder, $[\alpha]_D$ +3.7° ($CHCl_3$, *c* 0.53), HR-EI MS: *m/z* 366.1096 $[M]^+$, calcd. for $C_{21}H_{18}O_6$, 366.1104. IR ν_{max}^{KBr} cm^{-1}: 3600–2800, 1612, 1499, 1470, 1297. UV λ_{max}^{MeOH} nm (log ε): 365 (4.0), 237 (4.5). ^{1}H-NMR spectral data (Table 1), ^{13}C-NMR spectral data (Table 2).

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