FICUSINS A AND B, TWO NEW CYCLIC-MONOTERPENE-SUBSTITUTED ISOFLAVONES FROM *FICUS SEPTICA* BARM. F.[1]

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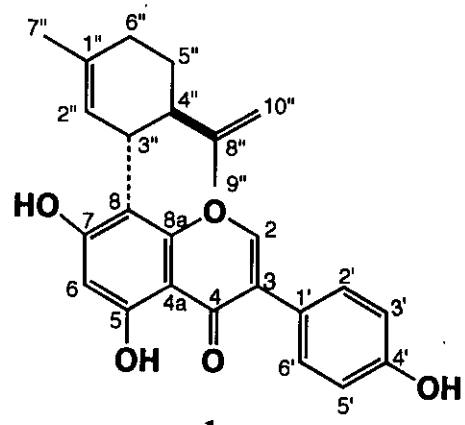
Abstract - Two new cyclic-monoterpene-substituted isoflavones, ficusins A (**1**) and B (**2**) were isolated from the Indonesian moraceous plant, *Ficus septica* Barm. F. The structures of ficusins A and B were shown to be **1** and **2**, respectively, on the basis of spectroscopic data.

Previously we reported the structure determination of isoprenoid-substituted phenolic compounds isolated from Indonesian moraceous plant, such as *Artocarpus heterophyllus*,[2-7] *A.communis*,[8] *A.rigida*,[9,10] *Antiaris toxicaria*,[11-13] and *Paratocarpus* (=*Artocarpus*) *venenosa*.[1,14] In the course of our studies on the constituents of the moraceous plants, we examined the constituents of *Ficus septica* Barm. F. collected in Bogor, Indonesia. This paper deals with the characterization of the two new cyclic-monoterpene-substituted isoflavones, ficusins A (**1**) and B (**2**) as well as the isolation of a known compound, genistein (**3**).

Ficusin A (**1**), pale yellow amorphous powder, $[\alpha]_D^{23}$+34°, $C_{25}H_{24}O_5$, gave a dark green coloration with methanolic ferric chloride. The ir spectrum of **1** disclosed absorption bands due to hydroxyl, conjugated carbonyl, and benzene ring moieties. The uv spectrum of **1** exhibited maxima at 204, 266, and 333 nm, and was similar to those of isoflavones.[15] From this result, compound (**1**) seems to be an isoflavone derivative. The ^{1}H nmr spectrum (400 MHz) of **1** was analyzed with the aid of the 2D ^{1}H-^{1}H COSY spectrum and showed the signals of the following protons (δ in acetone-*d*6) : protons in an isopropenyl group, δ 1.66 (3H, s), 4.66 (2H, br s), methyl protons, δ 1.69 (3H, br s), protons in two sets of methylene protons, δ 1.73-1.83 (2H, m), 2.05 (1H, m), 2.22-2.23 (1H, br), two methine protons, δ 2.95 (1H, td, J = 10 and 4 Hz), 4.12 (1H, br d, J =



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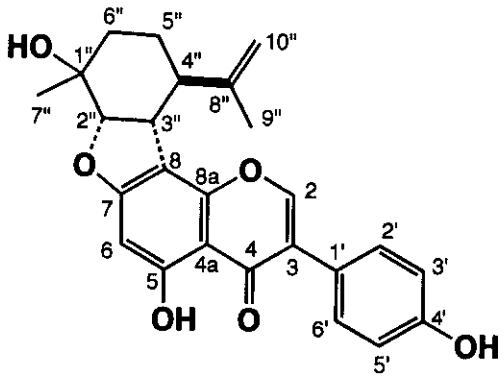

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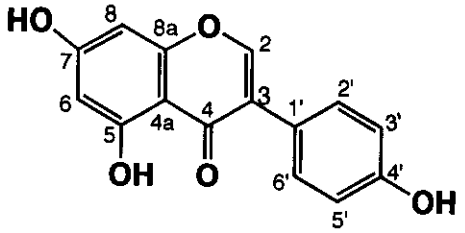

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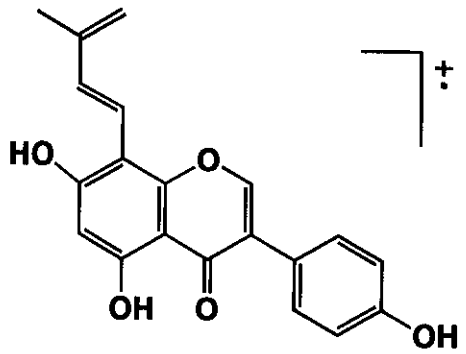

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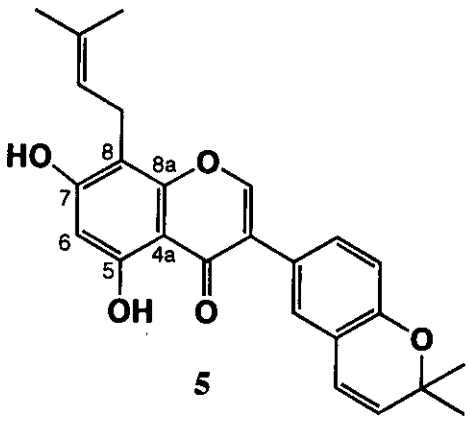

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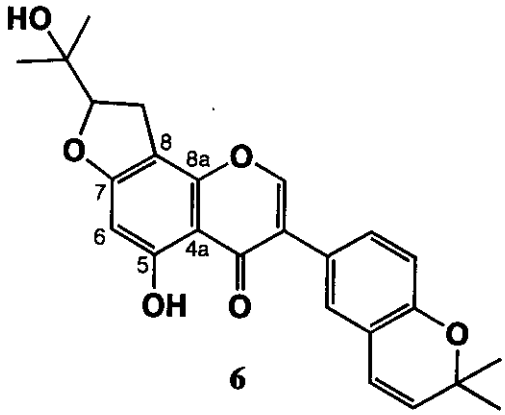

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Figure 1

Table 1 ^{13}C Nmr Chemical Shifts of **1**, **2** and **3** (δ in acetone-*d6*)

Carbon	**1**	correlated proton	**2**	correlated proton	**3**
2	154.11	8.22 (s)	153.76	8.14 (s)	154.76
3	123.15		123.02		123.08
4	182.05		182.06		181.65
4a	106.28		106.50		106.00
5	161.61	13.08# (s, OH)	164.42	13.36# (s, OH)	163.93
6	99.68	6.31 (s)	95.67	6.33 (s)	99.86
7	162.90		166.37		165.01
8	109.99		111.74		94.49
8a	154.11		153.76		159.06
1'	123.52		124.03		124.06
2'	131.20	6.90 (d, J = 8 Hz)	131.20	6.90 (d, J = 8 Hz)	131.18
3'	115.97	7.47 (d, J = 8 Hz)	116.00	7.45 (d, J = 8 Hz)	115.99
4'	158.39	8.50# (br s, OH)	158.50	8.61# (br s, OH)	158.45
5'	115.97	7.47 (d, J = 8 Hz)	116.00	7.45 (d, J = 8 Hz)	115.99
6'	131.20	6.90 (d, J = 8 Hz)	131.20	6.90 (d, J = 8 Hz)	131.18
1"	133.82		68.47	4.06# (s, OH)	
2"	125.50	5.29 (br s)	92.52	4.33 (dd, J = 5.5, 1.5 Hz)	
3"	36.69	4.12 (br d, J = 10 Hz)	40.95	3.56 (dd, J = 11, 5.5 Hz)	
4"	46.22	2.95 (td, J = 10, 4 Hz)*	51.45	1.86 (dd, J = 11, 3 Hz)	
5"	30.29	1.73-1.83 (2H, m)	25.60	1.26-1.33 (m)	
				2.01 (td, J = 13, 3 Hz)	
6"	31.22	2.05 (m)	35.69	1.68 (td, J = 13, 3 Hz)	
		2.22-2.33 (br)		1.79 (dtd, J =13, 3, 1.5 Hz)	
7"	23.60	1.69 (3H, br s)	28.18	1.45 (3H, s)	
8"	149.37		148.40		
9"	19.95	1.66 (3H, s)	18.24	1.87 (3H, s)	
10"	111.01	4.66 (2H, br s)	112.27	4.42, 4.60 (each 1H, br s)	

* measured at 60 °C

These hydroxyl groups were assigned by HMBC spectrum

10 Hz), an olefinic proton, δ 5.29 (1H, br s), an aromatic proton, δ 6.31 (1H, s), A_2B_2 type aromatic protons, δ 6.90, 7.47 (each 2H, d, J = 8 Hz), an olefinic proton, δ 8.22 (1H, s), proton in a hydrogen-bonded hydroxyl group, δ 13.08 (1H, s). The ^{13}C nmr spectrum of **1** showed the signals of the 25 carbon atoms, and was analyzed by comparing with that of genistein (**3**), along with the aid of the 2D ^{1}H-^{13}C correlation COSY spectrum (Table 1). In the ^{13}C nmr spectrum of **1**, the chemical shifts of all the carbon atoms in the isoflavone moiety except those of C-5, C-7, C-8, C-8a were similar to those of the relevant carbon atoms of **3**. This finding supported the presence of 8-substituted genistein moiety in the structure of **1**. The location of the substituent on genistein moiety was confirmed by the ^{1}H-detected heteronuclear multiple bond connectivity

(HMBC) spectrum (Figure 2). In the spectrum, the hydrogen-bonded hydroxyl group at δ 13.08 (C-5-OH) shows long-range correlation with the carbon at δ 99.68 (C-6) and the quaternary carbon at δ 106.28 (C-4a), while the proton at δ 6.31 (s, C-6-H), assignment of which was supported by 2D ^{1}H-^{13}C COSY spectrum, shows long-range correlation with the quaternary carbon at δ 109.99 (C-8) and the carbon at δ 106.28 (C-4a). The remaining part of the C-8 substituent, consisting of the $C_{10}H_{15}$ portion in the structure of **1**, was indicated by the ^{1}H nmr spectrum to contain an isopropenyl group, an olefinic proton, a methyl group on a double bond, two sets of methylene protons, and two methine protons. Comparison of the ^{13}C nmr spectrum of the substituent with those of cyclic-monoterpene type derivatives reported in the literatures[16] revealed that the chemical shifts of the carbon atoms of the substituent were similar to those of the relevant carbon atoms of 1,8-*p*-menthadiene (=limonene) skeleton. Furtheremore, the structure of the C-8 substituent of the $C_{10}H_{15}$ was supported by the HMBC spectrum as shown in Figure 2. The EI-ms of **1** exhibited the characteristic retro Diels-Alder type fragment ions at m/z 336 (M^{+}-C_5H_8, 4).[17] Considering the HMBC spectrum and the mass fragmentation pattern, the isoflavone moiety in **1** was linked at the C-3" carbon atom of 1,8-*p*-menthadiene structure. The stereochemistry of the 1,8-*p*-menthadiene structure was supported by the ^{1}H nmr spectrum of **1**. The olefinic proton ascribed to C-2" position was observed as a broad singlet at δ 5.29 and the coupling constant between the C-3"-H (δ 4.21) and C-4"-H (δ 2.95) was 10 Hz, demonstrating that the hydrogens are *trans* oliented. From above results, we propose the formula **1** for the structure of ficusin A.

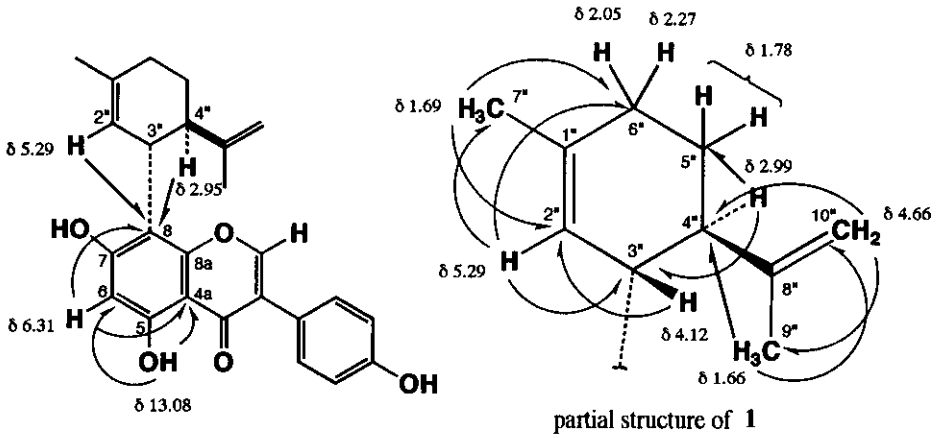
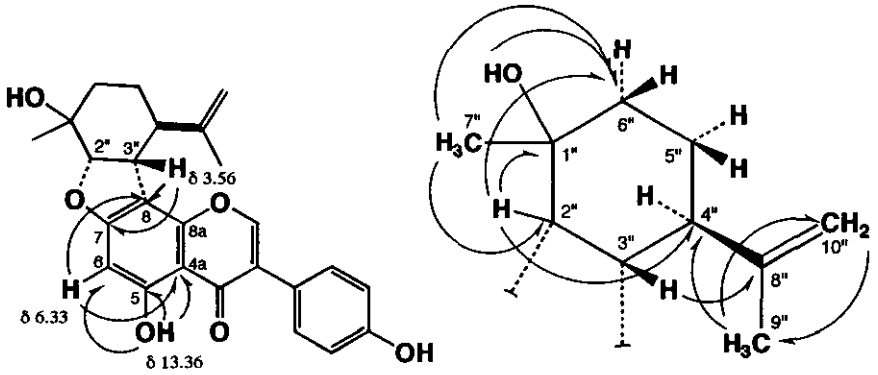


Figure 2 HMBC spectrum of **1** (δ in acetone-d_6)

Ficusin B (**2**), pale yellow needles, mp 124-126 °C, $[\alpha]_D^{23}$ -173°, $C_{25}H_{24}O_6$, gave a dark green coloration with methanolic ferric chloride. The ir spectrum of **2** disclosed absorption bands due to hydroxyl, conjugated

carbonyl, and benzene ring moieties. The uv spectrum of **2** exhibited maxima at 203, 220(sh), 265, and 335 nm, and was similar to that of **1**. The ^{1}H nmr spectrum of **2** was analyzed with the aid of the 2D ^{1}H-^{1}H COSY spectrum and showed the signals of the following protons (δ in acetone-d_6) : protons in an isopropenyl group, δ 1.66 (3H, s), 4.42, 4.62 (each 1H, br s), methyl protons, δ 1.45 (3H, s), protons in two sets of methylene protons, δ 1.26-1.33 (1H, m), 2.01 (1H, td, J = 13 and 3 Hz), 1.68 (1H, td, J = 13 and 3 Hz), 1.79 (1H, dtd, J = 13, 3 and 1.5 Hz), three methine protons, δ 1.86 (1H, dd, J = 11 and 3 Hz), 3.56 (1H, dd, J = 11 and 5.5 Hz), 4.33 (1H, dd, J = 5.5 and 1.5 Hz), protons in two hydroxyl groups, δ 4.06, 8.61 (each 1H, s, exchangeable with D_2O), A_2B_2 type aromatic protons, δ 6.90, 7.45 (each 2H, d, J = 8 Hz), an aromatic proton, δ 6.33 (1H, s), an olefinic proton, δ 8.14 (1H, s), a proton in hydrogen-bonded hydroxyl group, δ 13.36 (1H, s). The ^{13}C nmr spectrum of **2** was analyzed by comparing with that of **1**, along with the aid of the 2D ^{1}H-^{13}C COSY spectrum (Table 1). In the ^{13}C nmr spectrum of **2**, the chemical shifts of all the carbon atoms in the isoflavone moiety except those of C-6, C-7, C-8, C-8a were similar to those of the relevant carbons of **3**. This result suggested that **2** is a C-8-substituted genistein derivative. The location of the substituent at the C-8 position was confirmed by the HMBC spectrum as follows (Fig.3). The signal at δ 6.33 (C-6-H) showed long-range correlation with the quaternary carbons at δ 106.50 (C-4a), 164.42 (C-5), 166.37 (C-7), and 111.74 (C-8). Therefore the signal at δ 6.33 could be assigned to the proton at C-6 position. The methine proton at δ 3.56 (C-3"-H) in the $C_{10}H_{16}O$ moiety shows long-range correlation with the quaternary carbons at δ 111.74 (C-8), 166.37 (C-7), and 148.40 (C-8"). The C-8 substituent, consisting of the $C_{10}H_{16}O$ moiety, was indicated by the



partial structure of **2**

Figure 3 HMBC spectrum of **2** (δ in acetone-d_6)

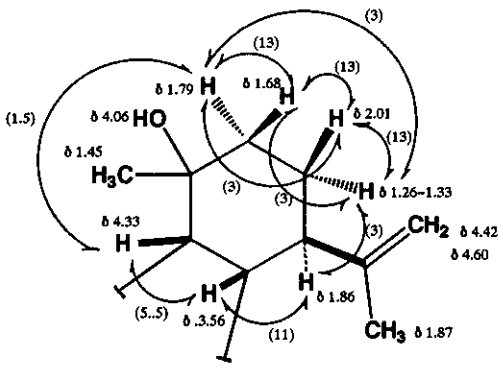


Figure 4 ^{1}H -^{1}H COSY spectrum of **2** (monoterpene moiety) and coupling constants (Hz) (δ in acetone-d_6)

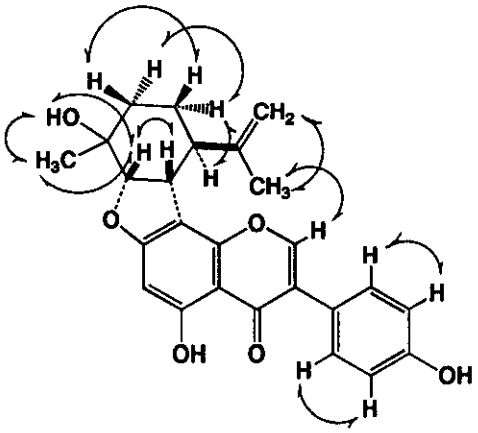


Figure 5 NOESY spectrum of **2** (measured in acetone-d_6)

^{1}H nmr spectrum to contain an isopropenyl group, a methyl group, two sets of methylene protons, three adjacent methine protons, and a hydroxyl group. The proton signals of the moiety were assigned with the aids of the 2D ^{1}H-^{1}H COSY spectrum as well as the 2D ^{1}H-^{13}C COSY spectrum as shown in Figure 4. Furthermore, comparison of the ^{13}C nmr spectrum of **2** with those of cyclic-monoterpene type derivatives[16] revealed that the structure of the $C_{10}H_{16}O$ moiety seems to be 2,3-disubstituted 8-*p*-menthen-1-ol. Comparing the ^{13}C nmr spectrum of **2** with that of **1**, the chemical shift of the C-6 signal of **2** was observed in higher field than the relevant carbon of **1** (+4.01 ppm, Table 1). On the other hand, in the ^{1}H nmr spectrum of **2**, the proton signal of the hydrogen-bonded hydroxyl group was observed in lower field than the relevant proton signal of **1** (-0.28 ppm, Table 1). The similar results have been reported in the case of 8-prenylisoflavone (**5**) and its derivative (**6**) as follows[18] : compound **5**, δ 99.7 (C-6), δ 12.79 (C-5-OH); compound **6**, δ 94.3 (C-6), δ 13.03 (C-5-OH). The stereochemistry of the $C_{10}H_{16}O$ moiety was supported by the NOESY spectrum of **2** (Figure 5), along with the consideration of the coupling constants of relevant protons (Figure 4). From above results, we propose the formula **2** for the structure of ficusin B.

While cyclic-monoterpene-substituted flavonoids have been isolated from the Lauraceae plants,[19,20,21] ficusins A (**1**) and B (**2**) are unique isoflavone derivatives with a cyclic-monoterpene-substituent.

EXPERIMENTAL

Abbereviations: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad, sh = shoulder, infl = inflection. The general procedures followed and instruments used are described in our previous papers.[7]

Plant material : Bark and root bark of *Ficus septica* was collected in the Botanical Garden of Bogor, Indonesia, in October 1991, and was identified by the members of Botanical Garden of Bogor.

Isolation of Ficusins A (1), B (2), and genistein (3) from the root bark

The dried root bark of *F. septica* (1 kg) was finely cut and extracted for three days at room temperature with *n*-hexane (3 l x 3), benzene (3 l x 3), and acetone (3 l x 3), successively. Evaporation of *n*-hexane, benzene, and acetone solutions to dryness yielded 11 g, 13 g, and 14 g of the residue, respectively. The acetone extract (14 g) was chromatographed over silica gel (250 g) using benzene, benzene - acetone (19 : 1, 9 : 1, 4 : 1, 3 : 1, 2 : 1), and then acetone. The fraction eluted with benzene - acetone (19 : 1) was evaporated to give the residue (1.8 g), which was fractionated by preparative tlc [*n*-hexane - ethyl acetate (3 : 2), *n*-hexane - acetone (2 : 1)] to give ficusin A (1, 4 mg) and ficusin B (2, 0.5 mg). The fraction eluted with benzene - acetone (9 : 1) was evaporated to give the residue (1.3 g) which was fractionated by preparative tlc [chloroform - acetone (4 : 1), *n*-hexane - acetone (3 : 2)] to give genistein (3, 5 mg).

Isolation of Ficusin B (2) from the bark

The dried bark of *F. septica* (1 kg) was finely cut and extracted for three days at room temperature with *n*-hexane (3 l x 3), benzene (3 l x 3), and acetone (3 l x 3), successively. Evaporation of *n*-hexane, benzene, and acetone solutions to dryness yielded 9 g, 17 g, and 11 g of the residue, respectively. The acetone extract (11 g) was chromatographed over silica gel (140 g) using benzene, benzene - acetone (19 : 1, 9 : 1, 4 : 1, 3 : 1, 2 : 1), and acetone to prepare frs.1 - 75. Each fraction (300 ml) was monitored by tlc. The fraction eluted with benzene - acetone (19 : 1, frs. 9 - 10, 64 mg) was fractionated by preparative tlc [*n*-hexane - ether (2 : 1), chloroform - methanol (20 : 1)] to give ficusin B (2, 2 mg).

Ficusin A (1)

Compound (1) was obtained as pale yellow amorphous powder. $FeCl_3$ test : positive (dark green). $[\alpha]_D^{23}$ + 34° (c = 1.52, MeOH). EI-ms : m/z (rel. int.) 404 (M^+, 13%), 336 (73), 321 (100), 283 (25), 270 (15), 203 (9.5), 174 (15). HR-ms : m/z 404.1574 (M^+, $C_{25}H_{24}O_5$ requires 404.1624), m/z 336.0923 ($C_{20}H_{16}O_5$ requires 336.0997), m/z 321.0772 ($C_{19}H_{13}O_5$, requires 321.0763), 283.0574 ($C_{16}H_{11}O_5$, requires 283.0607). Ir ν_{max}^{KBr} cm^{-1} : 3600 - 3000 (br), 1680 (sh), 1650 (sh), 1640, 1610, 1500, 1420. Uv λ_{max}^{MeOH} nm (log ε) : 335 (3.33), 266 (4.38), 204 (4.35).

Ficusin B (2)

Compound (2) was obtained as a pale yellow needles from benzene, mp 124 - 126 °C. $FeCl_3$ test : positive (dark green). $[\alpha]_D^{23}$ - 173° (c = 0.52, MeOH). EI-ms : m/z (rel. int.) 420 (M^+, 40%), 337 (55), 295 (100), 176 (16). HR-ms : m/z 420.1602 (M^+,$C_{25}H_{24}O_6$, requires 420.1573), m/z 337.0659 ($C_{19}H_{13}O_6$, requires 337.0712), m/z 295.0576 ($C_{17}H_{11}O_5$, requires 295.0607). Ir ν_{max}^{KBr} cm^{-1} : 3600 - 3000 (br), 1680 (sh), 1650, 1640, 1610, 1510, 1420. Uv λ_{max}^{MeOH} nm (log ε) : 335 (3.44), 265 (4.48), 220 (sh,4.42), 203 (4.40).

Genistein (3)

Compound (3) was obtained as a pale yellow needles from methanol, mp 285 °C. $FeCl_3$ test : positive (dark green). EI-ms : m/z 270 (M^+). Uv λ^{MeOH} nm (log ε) : 261 (4.58), 210 (4.46). ^1H nmr δ (acetone-d_6) : 6.29 (1H, d, J = 2 Hz), 6.42 (1H, d, J = 2 Hz), 6.90 (2H, d, J = 8 Hz), 7.45 (2H, d, J = 8 Hz), 8.17 (1H, s), 8.59 (1H, br s), 13.03 (1H, s)

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