

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

Miwa Aida, Yoshio Hano, and Taro Nomura*

Faculty of Pharmaceutical Sciences, Toho University, 2-2-1, Miyama,
Funabashi, Chiba 274, Japan

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**).

Ficuin A (**1**), pale yellow amorphous powder, $[\alpha]_D^{23} +34^\circ$, C₂₅H₂₄O₅, 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 ¹H nmr spectrum (400 MHz) of **1** was analyzed with the aid of the 2D ¹H-¹H 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 =

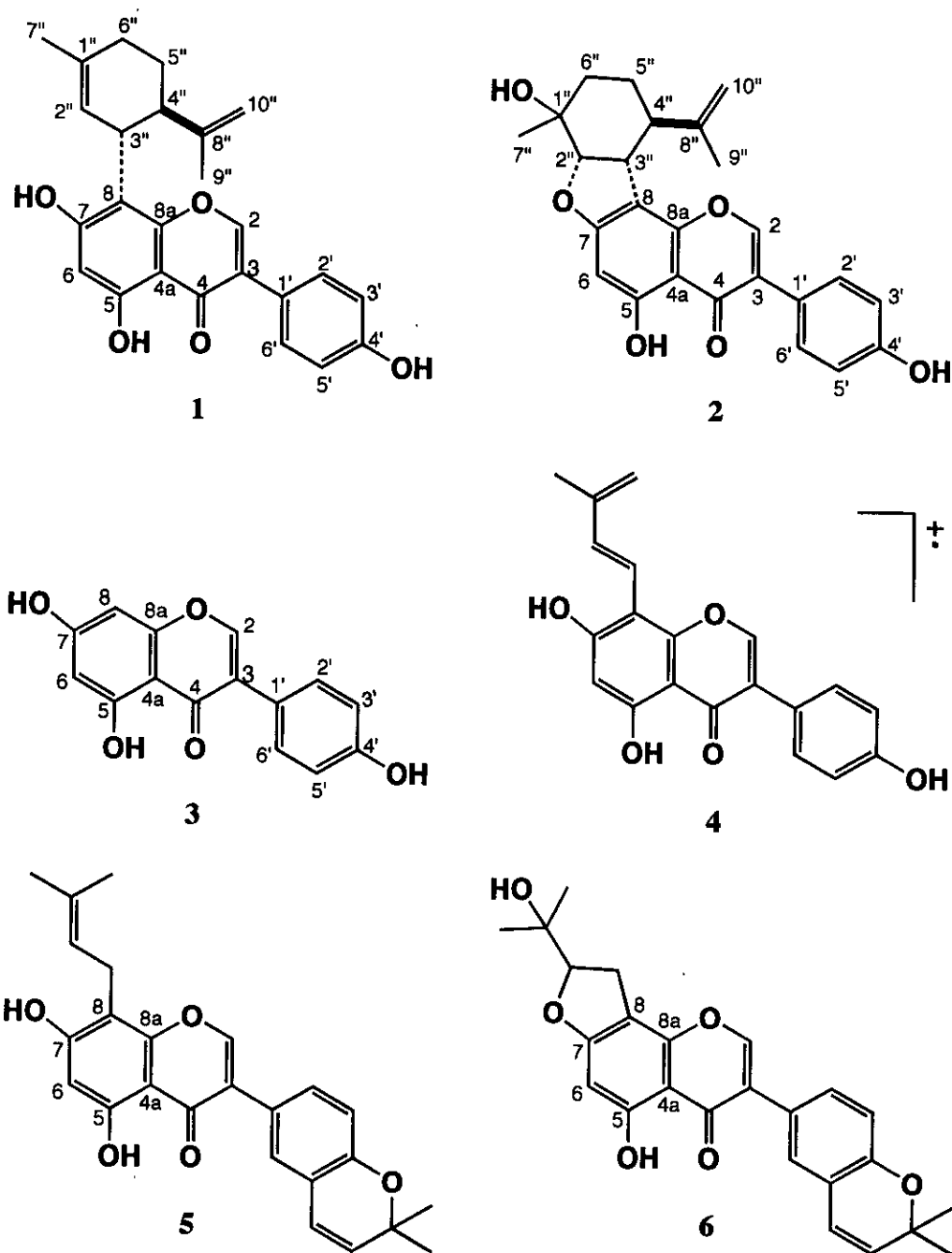


Figure 1

Table 1 ^{13}C Nmr Chemical Shifts of 1, 2 and 3 (δ in acetone- d_6)

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)	
6''	31.22	2.05 (m)	35.69	2.01 (td, J = 13, 3 Hz)	
7''	23.60	2.22-2.33 (br)		1.68 (td, J = 13, 3 Hz)	
8''	149.37	1.69 (3H, br s)	28.18	1.79 (dtd, J = 13, 3, 1.5 Hz)	
9''	19.95	1.66 (3H, s)	18.24	1.45 (3H, s)	
10''	111.01	4.66 (2H, br s)	112.27	1.87 (3H, s)	
				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), A₂B₂ 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 ^1H - ^{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 ^1H -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 ^1H - ^{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 $\text{C}_{10}\text{H}_{15}$ portion in the structure of **1**, was indicated by the ^1H 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 literatures¹⁶ 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. Furthermore, the structure of the C-8 substituent of the $\text{C}_{10}\text{H}_{15}$ 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 ($\text{M}^+ - \text{C}_5\text{H}_8$, **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 ^1H 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* oriented. From above results, we propose the formula **1** for the structure of ficusin A.

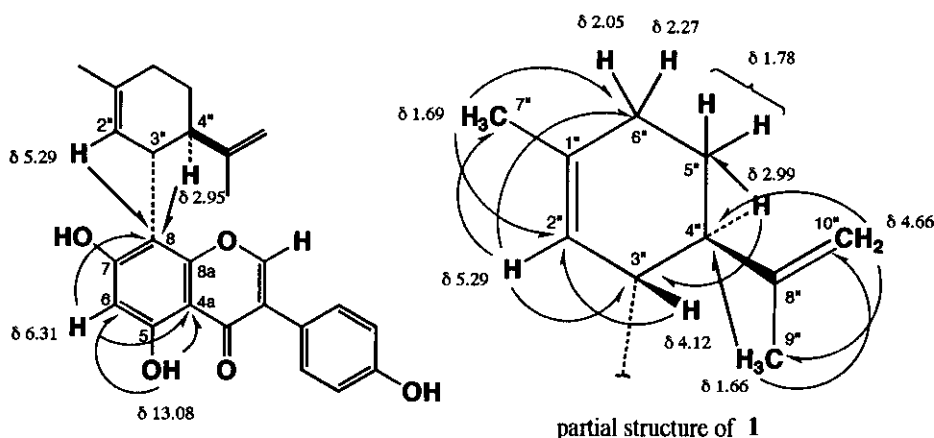


Figure 2 HMBC spectrum of **1** (δ in acetone- d_6)

Ficusin B (**2**), pale yellow needles, mp 124-126 °C, $[\alpha]_{\text{D}}^{23} -173^\circ$, $\text{C}_{25}\text{H}_{24}\text{O}_6$, 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 ^1H nmr spectrum of **2** was analyzed with the aid of the 2D ^1H - ^1H 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 $_2$ B $_2$ 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 ^1H - ^{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 $\text{C}_{10}\text{H}_{16}\text{O}$ 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 $\text{C}_{10}\text{H}_{16}\text{O}$ moiety, was indicated by the

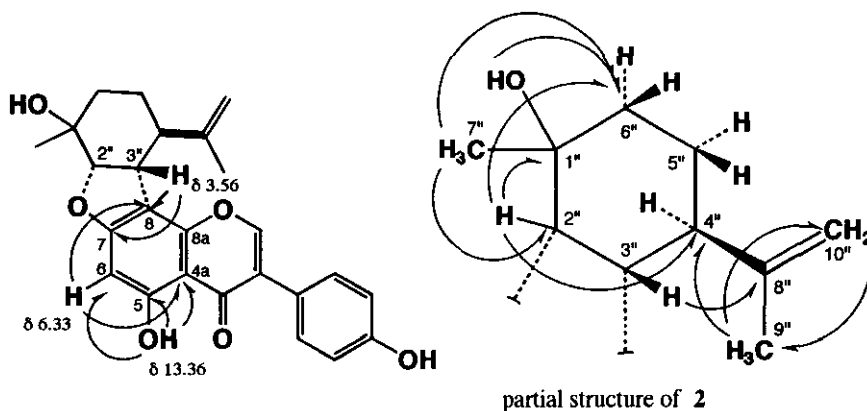


Figure 3 HMBC spectrum of **2** (δ in acetone- d_6)

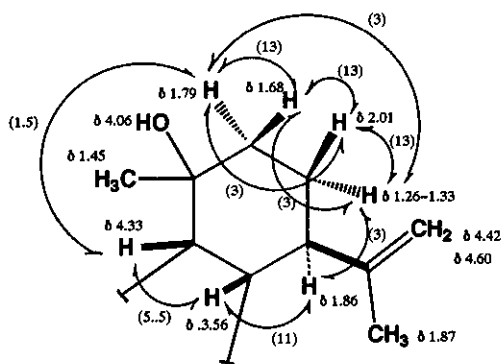


Figure 4 ^1H - ^1H COSY spectrum of **2** (monoterpene moiety) and coupling constants (Hz) (δ in acetone- d_6)

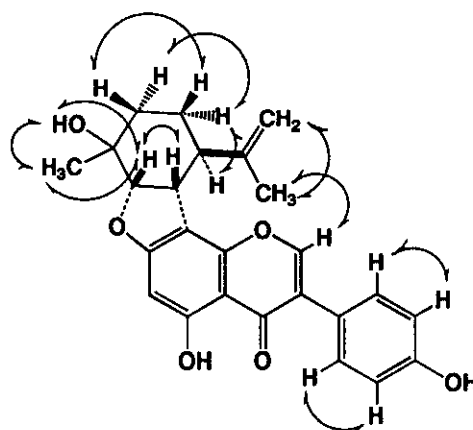


Figure 5 NOESY spectrum of **2** (measured in acetone- d_6)

^1H 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 ^1H - ^1H COSY spectrum as well as the 2D ^1H - ^{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 derivatives¹⁶ revealed that the structure of the $\text{C}_{10}\text{H}_{16}\text{O}$ 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 ^1H 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 follows¹⁸: 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 $\text{C}_{10}\text{H}_{16}\text{O}$ 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

Abbreviations: s = singlet, d = doublet, dd = double doublet, t = triplet, m = multiplet, br = broad, sh = shoulder, inf = 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. FeCl₃ test : positive (dark green). $[\alpha]_D^{23} + 34^\circ$ (*c* = 1.52, MeOH). EI-*m/s* : *m/z* (rel. int.) 404 (*M*⁺, 13%), 336 (73), 321 (100), 283 (25), 270 (15), 203 (9.5), 174 (15). HR-*m/s* : *m/z* 404.1574 (*M*⁺, C₂₅H₂₄O₅ requires 404.1624), *m/z* 336.0923 (C₂₀H₁₆O₅ requires 336.0997), *m/z* 321.0772 (C₁₉H₁₃O₅, requires 321.0763), 283.0574 (C₁₆H₁₁O₅, requires 283.0607). Ir ν_{\max}^{KBr} cm⁻¹ : 3600 - 3000 (br), 1680 (sh), 1650 (sh), 1640, 1610, 1500, 1420. Uv $\lambda_{\max}^{\text{MeOH}}$ 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. FeCl₃ test : positive (dark green). $[\alpha]_D^{23} - 173^\circ$ (*c* = 0.52, MeOH). EI-*m/s* : *m/z* (rel. int.) 420 (*M*⁺, 40%), 337 (55), 295 (100), 176 (16). HR-*m/s* : *m/z* 420.1602 (*M*⁺, C₂₅H₂₄O₆, requires 420.1573), *m/z* 337.0659 (C₁₉H₁₃O₆, requires 337.0712), *m/z* 295.0576 (C₁₇H₁₁O₅, requires 295.0607). Ir ν_{\max}^{KBr} cm⁻¹ : 3600 - 3000 (br), 1680 (sh), 1650, 1640, 1610, 1510, 1420. Uv $\lambda_{\max}^{\text{MeOH}}$ 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. FeCl₃ test : positive (dark green). EI-*m/s* : *m/z* 270 (*M*⁺). Uv $\lambda_{\max}^{\text{MeOH}}$ 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)

ACKNOWLEDGEMENT

We are grateful to Eisai Co., LTD., and P. T. Eisai Co. LTD., for their kind offer of facilities to collect the plant material. Authors' thanks are due to the members of Botanical Garden of Bogor, Indonesia, for identification of plant material.

REFERENCES

1. Part 26 in the series "Constituents of the Moraceae Plants". Part 25 in the series : Y.Hano, N. Itoh, A. Hanaoka, and Taro Nomura, *Heterocycles*, submitted.
2. Y. Hano, M. Aida, M. Shiina, T. Nomura, T. Kawai, H. Ohe, and K. Kagei, *Heterocycles*, 1989, **29**, 1447.
3. Y. Hano, M. Aida, and Taro Nomura, *J. Nat. Prod.*, 1990, **53**, 391.
4. Y. Hano, M. Aida, T. Nomura, and S. Ueda, *J. Chem. Soc., Chem. Commun.*, 1992, 1177.
5. M. Aida, K. Shinomiya, Y. Hano, and T. Nomura, *Heterocycles*, 1993, **36**, 575.
6. M. Aida, K. Shinomiya, K. Matsuzawa, Y. Hano, and T. Nomura, *Heterocycles*, 1994, **39**, 847.
7. K. Shinomiya, M. Aida, Y. Hano, and T. Nomura, *Phytochemistry*, in press.
8. Y. Hano, Y. Yamagami, M. Kobayashi, R. Isohata, and T. Nomura, *Heterocycles*, 1990, **31**, 877.
9. Y. Hano, R. Inami, and T. Nomura, *Heterocycles*, 1990, **31**, 1345.
10. Y. Hano, R. Inami, and T. Nomura, *Heterocycles*, 1993, **35**, 1341.
11. Y. Hano, P. Mitsui, and T. Nomura, *Heterocycles*, 1990, **30**, 1023.
12. Y. Hano, P. Mitsui, and T. Nomura, *Heterocycles*, 1990, **31**, 1315.
13. Y. Hano, P. Mitsui, T. Nomura, T. Kawai, and Y. Yoshida, *J. Nat. Prod.*, 1991, **54**, 1049.
14. Y. Hano, N. Itoh, A. Hanaoka, Y. Itoh, and T. Nomura, *Heterocycles*, 1995, **41**, 191.
15. T. J. Mabry K.R. Markham, and M. B. Thomas, "The Systematic Identification of Flavonoids", Springer Verlag, New York, 1970.
16. F. W. Wehrli and T. Nishida, *Fortschr. Chem. Org. Naturst.*, 1978, **36**, 24.
17. T. Nomura, *Fortschr. Chem. Org. Naturst.*, 1988, **53**, 86.
18. G. B. Russell, H. Md. Sirat, and O. R. W. Sutherland, *Phytochemistry*, 1990, **29**, 1287.
19. I. B. de Alleluia, R. B. Fo, O. R. Gottlieb, E. G. Magalhães, and R. Margues, *Phytochemistry*, 1978, **17**, 517.
20. K. Ichino, H. Tanaka, and K. Ito, *Tetrahedron*, 1988, **44**, 3251.
21. K. Ichino, H. Tanaka, and K. Ito, *Chem. Pharm. Bull.*, 1989, **37**, 944.

Received, 23rd June, 1995