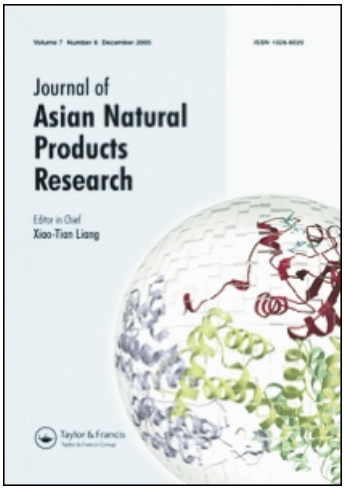


Journal of Asian Natural Products Research
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713454007

Water-soluble constituents from aerial roots of *Ficus microcarpa*

M. -A. Ouyang[a]; Y. -H. Kuo[b]
[a] Department of Bio-engineering and Technology, Huaqiao University, Quanzhou, Fujian, China [b] Department of Chemistry, Taiwan University, Taibei, Taiwan

To cite this Article Ouyang, M. -A. and Kuo, Y. -H.(2006) 'Water-soluble constituents from aerial roots of *Ficus microcarpa*', Journal of Asian Natural Products Research, 8: 7, 625 — 630
To link to this Article: DOI: 10.1080/10286020500208576
URL: http://dx.doi.org/10.1080/10286020500208576

Journal of Asian Natural Products Research, Vol. 8, No. 7, October–November 2006, 625–630

Water-soluble constituents from aerial roots of *Ficus microcarpa*

M.-A. OUYANG†* and Y.-H. KUO‡

†Department of Bio-engineering and Technology, Huaqiao University, Quanzhou, Fujian 362011, China
‡Department of Chemistry, Taiwan University, Taibei, Taiwan

(Received 8 December 2004; revised 22 March 2005; in final form 7 April 2005)

Three new water-soluble constituents [ficuscarpanoside B (**1**), (7*E*,9*Z*)-dihydrophaseic acid 3-*O*-β-D-glucopyranoside (**4**) and ficuscarpanic acid (**6**)] and the natural product 2,2′-dihydroxyl ether (**7**) have been isolated, together with three known compounds [(7*S*,8*R*)-syringoylglycerol (**2**), (7*S*,8*R*)-syringoylglycerol-7-*O*-β-D-glucopyranoside (**3**) and icariside D_2 (**5**)] from the aerial roots of *Ficus microcarpa*. Identification of their structures was achieved by 1D and 2D NMR experiments, including $^1H-^1H$ COSY, NOESY, HMQC and HMBC methods and FAB mass spectral data.

Keywords: *Ficus microcarpa*; Moraceae; Aerial roots; Phenolic glucosides; Sesquiterpenoid glucoside

1. Introduction

Ficus microcarpa, Moraceae, is a familiar plant in Southeast Asia. Twenty-four species of *Ficus* are used in Chinese herbal medicine. *F. microcarpa* is used for the treatment of rheumatism–arthralgia, diarrhea and acariasis, and as an antimalarial agent [1]. The antiplatelet activity, as well as the strong vitality of this plant, prompted us to determine its chemical constituents. Phytochemical studies of the plant have identified six triterpenoids from the leaves [2]. Two isoflavones [3], 28 known components [4] and six new compounds were previously isolated from the bark and heartwood [5,6]. Five cycloartanes were isolated from its aerial roots [7–10]. In this study, seven water-soluble compounds were isolated from the aerial roots of *F. microcarpa*. We report here the structure elucidation of these compounds. An important phytohormone (compound **4**), a derivative of abscisic acid (ABA), was isolated from the aerial roots. This compound was found to be a major constituent of the aerial roots. ABA performs several specific functions in plant growth and development, especially in primary seed dormancy [11].

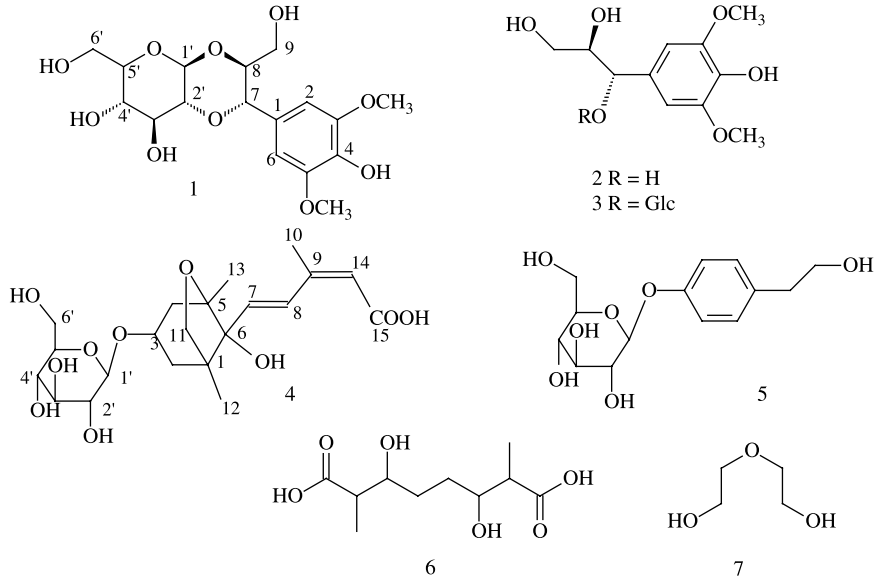
*Corresponding author. E-mail: maouyang@hqu.edu.cn

Journal of Asian Natural Products Research
ISSN 1028-6020 print/ISSN 1477-2213 online © 2006 Taylor & Francis
http://www.tandf.co.uk/journals
DOI: 10.1080/10286020500208576

2. Results and discussion

The water-soluble fraction of the methanol extract of the aerial roots of *F. microcarpa* was subjected to Sephadex LH-20, RP-18, and silica gel column chromatography to afford seven water-soluble compounds (**1**–**7**) (scheme 1). (7*S*,8*R*)-Syringoylglycerol (**2**), (7*S*,8*R*)-syringoylglycerol-7-*O*-β-D-glucopyranoside (**3**) [12,13] and icariside D_2 (**5**) [14] have already been isolated. Their structures were identified by comparing their spectral data with data reported in the literature.

Compounds **1**–**3** were derivatives of the parent syringoylglycerol. Compound **1** is a colorless powder, the elemental composition of which was determined to be $C_{17}H_{24}O_{10}$ by negative HR-FABMS, which showed a quasi-molecular peak at *m/z* 387.1287 representing six unsaturated moieties. The ^{13}C-NMR and DEPT spectra of **1** showed 17 carbons in the molecule, including two methoxys (δ_C 56.9 × 2), two methylenes (δ_C 62.1, 62.5), nine methines (δ_C 106.3 × 2, 99.7, 82.5, 80.9, 80.4, 79.8, 75.0, and 71.9), and four quaternary carbons (δ_C 149.2 × 2, 136.9, and 129.3). The chemical shifts at δ_C 82.5, 80.9, 80.4, 79.8, 75.0, and 71.9 suggested the presence of a sugar moiety and were identified as glucose by acid hydrolysis. Apart from the sugar moiety and two methoxy groups, **1** had another nine carbons—three oxygenated sp^3 carbons (one methylene and two methines) and six sp^2 carbons [two methines and four quaternary carbons (δ_C 106.3 × 2, 149.2 × 2, 136.9, and 129.3)]. Comparison of the NMR data between **1** and **3** revealed that they contained the same carbon numbers and the same functional moieties, such as glucose and a syringoylglycerol. Since the unsaturation due to the sugar and benzene was five, there should be an additional ring in **1**. Compound **3** is a normal glycoside, which has only one linkage position between the aglycone and the sugar. Compared with **3**, some chemical shifts of the oxygenated sp^3 carbons in **1** were changed, such as C-7 (δ_C 80.4), C-8 (δ_C 82.5), C-1′ (δ_C 99.7), C-2′ (δ_C 80.8), and C-3′ (δ_C 75.0). These changed carbons indicate an altered structure. In the HMBC spectrum, long-range correlations between H-2,6 [δ 6.72 (2H, s)] and C-4, C-1; H-7



Scheme 1. Structures 1–7.

[δ 4.44 (1H, d, $J = 9.5$ Hz)] and C-2, C-6, C-2′; H-2′ of Glc [δ 3.14 (1H, dd, $J = 9.6, 7.7$ Hz)] and C-7; and H-8 [δ 3.82 (1H, m)] and C-1, C-1′ (see figure 1) were observed. The above evidence indicates that C-1 of Glc links to C-8 of the aglycone and C-2 of Glc to C-7. NOESY indicated correlations between H-7 and H-2′ of Glc, between H-2, H-6 and anomeric proton H-1′ of Glc and H-8 and H-3′ of Glc, elucidating the relative configurations of H-7_β and H-8_α. Therefore, compound **1** was determined to be 2′,7-epoxy-syringoylglycerol 8-*O*-β-D-glucopyranoside (ficuscarpanoside B).

Compound **4** was isolated as a colorless powder. Its negative FAB-MS showed a quasi-molecular ion peak at *m/z* 443 $[M-H]^-$. The molecular formula was established as $C_{21}H_{32}O_{10}$ by HR-FABMS, representing six unsaturated moieties. Acid hydrolysis gave a mixture of an aglycone and glucose. The ^{1}H-NMR spectrum displayed three olefinic proton signals, at δ 7.92 (1H, d, $J = 16.0$ Hz), 6.46 (1H, d, $J = 16.0$ Hz), and 5.78 (1H, s), three methyl singlets, at δ 2.03, 1.17, and 0.94, and an anomeric proton signal at δ 4.37 (1H, d, $J = 7.8$ Hz). The ^{13}C-NMR spectrum revealed 21 carbons, including one carbonyl, two double bonds, three methyls, four methylenes, six methines, and three quaternary carbons (see table 1). From the reference data [15], compound **A** was given as (7*E*,9*E*)-dihydrophaseic acid 3-*O*-β-D-glucopyranoside. Comparison of the NMR data of **4** with those of **A** indicated that their structures were the same except for a difference in the $\Delta^{9,14}$ double bond; they are geometrical isomers (see figure 2). For the double bonds of **4**, the chemical shifts at δ 7.92 (1H, d, $J = 16.0$ Hz, H-8) and 6.46 (1H, d, $J = 16.0$ Hz, H-7) confirmed the presence of two *trans* olefinic protons. The NOESY spectrum indicated key correlations between H-10 (methyl signal at δ_H 2.06) and H-7 (δ_H 6.46), H-14 (δ_H 5.78), indicating the stereochemistry of an *E*/*Z* system for $\Delta^{7,8}$ and $\Delta^{9,14}$ of **4**. Therefore, compound **4** was determined to be (7*E*,9*Z*)-dihydrophaseic acid 3-*O*-β-D-glucopyranoside.

Compound **6** was obtained as colorless crystals exhibiting a quasi-molecular ion peak at *m/z* 235 $[M + H]^+$. The molecular formula was established as $C_{10}H_{18}O_6$ by HRFAB-MS. The ^{13}C-NMR spectrum showed ten carbons in the molecule, including two methyls (δ_C 13.9 × 2), two methylenes (δ_C 31.4 × 2), four methines (δ_C 74.4 × 2 and 47.6 × 2), and two quaternary carbons (δ_C 178.9 × 2). The chemical shifts at δ_C 178.9 and 74.4 confirmed the carboxyl and the carbon bearing oxygen. The ^{1}H-NMR spectrum revealed symmetric proton signals at δ 3.71 (2H, br. t, $J = 7.2$ Hz, H-3, H-6), 2.50 (2H, qui, $J = 7.2$ Hz, H-2, H-10), 1.81 (2H, dt, $J = 20.4, 7.2$ Hz, H_a-4, H_a-5), 1.38 (2H, m, H_b-4, H_b-5) and 1.13 (6H, d, $J = 6.8$ Hz, H-9, H-10). The ^{1}H- and ^{13}C-NMR data only displayed half-signals of the

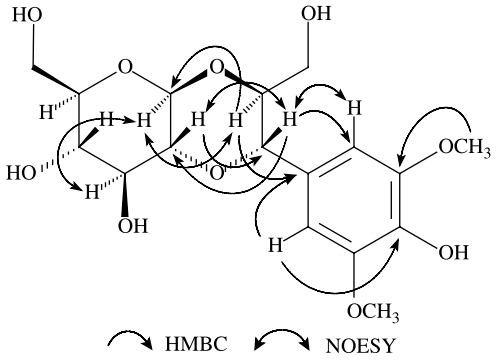


Figure 1. HMBC and NOESY correlations of compound **1**.

Table 1. ^{1}H- and ^{13}C-NMR data for compounds **1** and **4** (CD_3OD).

1	δ_H	δ_C	***4***	δ_H	δ_C
1		129.3 C	1		49.2 C
2,6	6.72 (2H, s)	106.3 CH	2	2.00 (1H, dd, $J = 13.6, 6.8$ Hz)	42.9 CH_2
				1.77 (1H, m)	
3,5		149.2 C	3	4.25 (1H, m)	74.0 CH
4		136.9 C	4	2.19 (1H, dd, $J = 14.0, 6.8$ Hz)	42.7 CH_2
				1.81 (1H, m)	
7	4.44 (1H, d, $J = 9.5$ Hz)	80.4 CH	5		87.4 C
8	3.82 (1H, m)	82.5 CH	6		83.2 C
9	3.39 (1H, br. d, $J = 11.3$ Hz)	62.1 CH_2	7	6.46 (1H, d, $J = 16.0$ Hz)	130.2 CH
	3.45 (1H, dd, $J = 11.3, 5.3$ Hz)				
1′	4.60 (1H, d, $J = 7.7$ Hz)	99.7 CH	8	7.92 (1H, d, $J = 16.0$ Hz)	132.8 CH
2′	3.14 (1H, dd, $J = 9.6, 7.7$ Hz)	80.8 CH	9		141.2 C
3′	3.59 (1H, t-like, $J = 9.6$ Hz)	75.0 CH	10	2.06 (3H, s)	20.8 CH_3
4′	3.40 (1H, overlap)	71.9 CH	11	3.80 (1H, d, $J = 7.5$ Hz)	77.1 CH_2
				3.75 (1H, d, $J = 7.5$ Hz)	
5′	3.49 (1H, m)	79.8 CH	12	0.94 (3H, s)	16.7 CH_3
6′	3.71 (1H, dd, $J = 11.7, 5.8$ Hz)	62.5 CH_2	13	1.17 (3H, s)	20.0 CH_3
	3.90 (1H, br. d, $J = 11.7$ Hz)				
3′,5′-OCH_3	3.85 (6H, s)	56.9 CH_3	14	5.78 (1H, s)	128.0 CH
			15		175.6 C
			1′	4.37 (1H, d, $J = 7.8$ Hz)	103.0 CH
			2′	3.15 (1H, dd, $J = 9.0, 7.8$ Hz)	75.0 CH
			3′	3.30 (1H, overlap)	77.8 CH
			4′	3.29 (1H, overlap)	71.5 CH
			5′	3.18 (1H, m)	77.7 CH
			6′	3.87 (1H, br. d, $J = 12.0$ Hz)	62.7 CH_2
				3.67 (1H, dd, $J = 12.0, 4.8$ Hz)	

molecule, indicating a symmetric structure. The methine signal at δ 2.50 (2H, qui, $J = 7.2$ Hz, C-2) suggested a vicinal position to be a carbonyl (δ_C 178.9, C-1). Because the spin-splitting of this C−H was a quintet peak (1:4:6:4:1), the other vicinal positions must link a methyl and another methane, bearing oxygen (see structures **1**–**7**). Thus, the structure of **6** was deduced as 2,7-dimethyl-3,6-dihydroxyl-1,8-di-octanoic acid (ficuscarpanic acid).

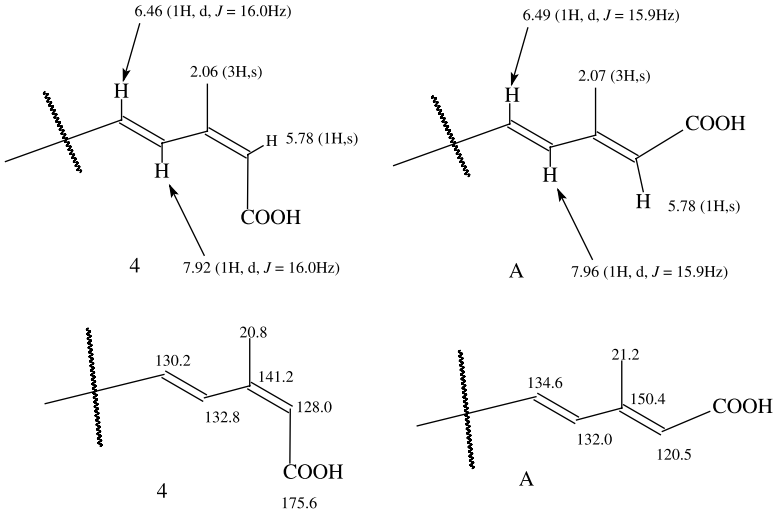


Figure 2. The partial structure of compounds **4** and **A**.

Compound **7** was first isolated from a natural source. It exhibited proton signals at δ 3.67 (4H, dd, $J = 5.6, 4.0$ Hz) and 3.56 (4H, dd, $J = 5.6, 4.0$ Hz) in the ^{1}H-NMR spectrum, and carbon signals at δ 73.6 (CH_2) and 62.4 (CH_2) in the ^{13}C-NMR spectrum, and a quasi-molecular ion peak at *m/z* 105 $[M-H]^-$ in FAB-MS. Thus, **7** was identified as 2,2′-dihydroxyl ether.

3. Experimental

3.1 General experimental procedures

Melting points were determined on a Yanaco MP-S3 apparatus and are uncorrected. UV spectra were recorded with a Backman DU-64 spectrometer. Optical rotations were measured with a Jasco DIP-180 digital polarimeter spectrophotometer. ^{1}H-, ^{13}C-, DEPT, ^{1}H–^{1}H COSY, NOESY, HMQC and HMBC NMR spectra were obtained using a Varian Unity Plus 400 instrument. FAB mass spectra were recorded on a Jeol JMS-HX 110 instrument. The chromatographic stationary phase used was RP-8 (40–60 μm, Merck), silica gel (160–200 mesh), Sephadex LH-20 (25–100 μm, Pharmacia Fine Chemicals) and MCI-gel CHP20P (75–150 μm, Mitsubish Chemical Industries). The following solvent systems were used: (a) $CHCl_3$–MeOH–H_2O (80:20:3), $CHCl_3$–MeOH–H_2O (70:30:5) and MeOH–H_2O (0–100%) for the glycosides; and (b) $CHCl_3$–MeOH–H_2O (7:3:1) lower-layer 9 ml + 1 ml HOAc for sugars. TLC spots were detected by spraying with 5% H_2SO_4 followed by heating. Sugars were detected by spraying with aniline–phthalate reagent.

3.2 Plant material

The aerial roots of *Ficus microcarpa* L. f. were collected from the campus of Taiwan University, Taipei, in 2002. The plant was identified by Professor M.T. Gun, Department of Botany, Taiwan University. A voucher specimen (No. 492330) has been deposited in the Department of Botany, National Taiwan University.

3.3 Extraction and isolation

Dry aerial roots of *Ficus microcaroa* (18 kg) were extracted (2 × 10 l) with MeOH at room temperature (7 days × 2). The extract was evaporated *in vacuo* to yield a residue that was dissolved in water and then filtered. The water-soluble fraction was passed through a D_{101} column and eluted with water and methanol. Evaporation of the methanol eluate yielded 75 g of a brown fraction (A). Fraction A was subjected to dry column chromatography (DCC) on silica gel (1.0 kg), and eluted with $CHCl_3$–MeOH–H_2O (10:2:0.2) to yield 13 fractions. Each fraction was purified by Sephadex LH-20, RP-8 gel column chromatography (solvent MeOH–H_2O, 10–70%) and finally repeatedly chromatographed on a silica gel column with $CHCl_3$–MeOH–H_2O (100:10:1–70:30:5) as eluent to yield **1** (48 mg), **2** (34 mg), **3** (42 mg), **4** (256 mg), **5** (24 mg), **6** (122 mg) and **7** (15 mg).

3.3.1 Ficuscarpanoside B (1). A colorless amorphous powder. $[\alpha]_D^{21} + 16$ (*c* 0.3, MeOH); mp 197–201°C; HRFABMS *m/z* 387.1287; UV λ_{MeOH} (nm) (log ϵ): 209 (3.92), 232 (3.45), 284 (3.24); FAB-MS *m/z* 387 $[M-H]^-$; ^{1}H-NMR and ^{13}C-NMR see table 1 (calcd for $C_{17}H_{23}O_{10}$, 387.1291).

3.3.2 (*7E,9Z*)-Dihydrophaseic acid 3-*O*-β-D-glucopyranoside (4). A colorless amorphous powder. $[\alpha]_D^{21}$ − 27(*c* 0.66, MeOH); FAB-MS *m/z* 443 $[M-H]^-$, 281 $[M-H-162]^-$; HR-FAB-MS *m/z* 443.1914 $[M-H]^-$; ^{1}H- and ^{13}C-NMR see table 1 (calcd for $C_{21}H_{31}O_{10}$, 443.1917).

3.3.3 Ficuscarpanic acid (6). Colorless crystals. FAB-MS *m/z* 235 $[M+H]^+$; HRFAB-MS *m/z* 235.1178; ^{1}H-NMR δ 3.71 (2H, br. t, $J = 7.2$ Hz), 2.50 (2H, qui, $J = 7.2$ Hz), 1.81 (2H, dt, $J = 20.4$, 7.2 Hz), 1.38 (2H, m), 1.13 (6H, d, $J = 6.8$ Hz); ^{13}C-NMR δ 178.9 (2 × C), 74.4 (2 × CH), 47.6 (2 × CH), 31.4 (2 × CH_2), 13.9 (2 × CH_3) (calcd for $C_{10}H_{17}O_6$, 235.1182).

3.3.4 2,2′-Dihydroxyl ether (7). Amorphous powder. $C_4H_{10}O_3$; FAB-MS *m/z* 105 $[M-H]^-$; ^{1}H-NMR δ 3.67 (4H, dd, $J = 5.6$, 4.0 Hz), 3.56 (4H, dd, $J = 5.6$, 4.0 Hz); ^{13}C-NMR δ 73.6 (CH_2), 62.4 (CH_2).

3.3.5 Acid hydrolysis. A solution of each compound (10 mg) was heated at 100°C in 2 M aqueous CF_3COOH (5 ml) and refluxed on a water bath for 3 h. After this period, the reaction mixture was diluted with H_2O (15 ml) and extracted with CH_2Cl_2 (3 × 5 ml). The combined CH_2Cl_2 extracts were washed with H_2O and then evaporated to dryness *in vacuo*. After evaporation of the aqueous layer with MeOH until neutral to dryness, the sugars were analysed by comparison with authentic samples (solvent system b) using silica gel HPTLC.

Acknowledgements

We thank the staff of the analytical group of the Department of Chemistry, Taiwan University, for measuring the NMR and FAB-MS spectra. This research was supported by the Chinese National Science Fund (research project 20272015).

References

[1] Jiang-Su Medicinal College, *A Dictionary of Traditional Chinese Medicine*, pp. 2528–2529, Shanghai Science and Technology Publishing House, Shanghai (1979).
[2] M. Higa, S. Yogi, K. Hokama. *Bull. Coll. Sci. Univ. Ryukyus*, **13**, 75 (1987).
[3] Y.C. Li, Y.H. Kuo. *J. Nat. Prod.*, **60**, 292 (1997).
[4] Y.H. Kuo, Y.C. Li. *J. Chin. Chem. Soc.*, **44**, 321 (1997).
[5] Y.C. Li, Y.H. Kuo. *Phytochemistry*, **49**, 2417 (1998).
[6] Y.H. Kuo, Y.C. Li. *Chem. pharm. Bull.*, **47**, 299 (1999).
[7] Y.H. Kuo, Y.M. Chiang. *Chem. pharm. Bull.*, **47**, 498 (1999).
[8] Y.M. Chiang, Y.H. Kuo. *J. nat. Prod.*, **63**, 898 (2000).
[9] Y.H. Kuo, Y.M. Chiang. *Chem. pharm. Bull.*, **48**, 593 (2000).
[10] Y.M. Chiang, J.K. Su, Y.H. Liu, Y.H. Kuo. *Chem. pharm. Bull.*, **49**, 581 (2001).
[11] L.B. Taylor, A.B. Burbidge, A.J. Thompson. *J. exp. Bot.*, **51**, 1563 (2000).
[12] H. Otsuka, M. Takeuchi, Y. Inoshiri, T. Sato, K. Yamasaki. *Phytochemistry*, **28**, 883 (1989).
[13] M. Sugiyama, M. Kikuchi. *Chem. pharm. Bull.*, **40**, 325 (1992).
[14] T. Miyase, A. Ueno, N. Takizawa, H. Kobayashi, H. Oguchi. *Phytochemistry*, **28**, 3483 (1989).
[15] Y. Champavier, G. Comte, J. Vercauteren, D.P. Allais, A.J. Chuli. *Phytochemistry*, **50**, 1219 (1999).