Ficusmicrochlorin A—C, Two New Methoxy Lactone Chlorins and an Anhydride Chlorin from the Leaves of *Ficus microcarpa*

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Two new methoxy lactone chlorins ficusmicrochlorin A (1) and ficusmicrochlorin B (2), and one new anhydride chlorin ficusmicrochlorin C (3), along with eight known pheophytins were isolated from the leaves of *Ficus microcarpa*. Their structures were determined by the extensive 1D- and 2D-NMR techniques. New pheophytin compound was rarely obtained from natural sources. In the past ten years, only three new natural pheophytins were characterized.

Key words Chinese herb; *Ficus microcarpa*; Moraceae; chlorin; ficusmicrochlorin

Ficus microcarpa Linn. f. belongs to the family Moraceae and is widely distributed as an ornamental tree in Taiwan. Over 850 species of *Ficus* have been found in tropic and subtropic areas. In Taiwan, twenty-one endemic and twenty transplanted species have been reported. The strong vitality of this plant with the unique aerial roots and antiplatelet activity promoted us to investigate the phytochemical constituents present in different tissues of this plant. As part of our program aimed at the discovery of the secondary metabolites from *Ficus microcarpa*, two new isoflavones besides 28 compounds were identified from the bark,[1,2] nine new containing lignans, one γ-lactone, one monoterpene, and phenols were isolated from the heartwood.[3—5] 33 new triterpenes (including 16 taraxastanes, 7 ursanes, 5 oleananes, 4 lupanes, and 1 lanostane) and two novel spirotocopheroids were elucidated from the unique aerial roots,[6—12] and in which pentacyclic triterpenes possessing a carboxylic acid functionality at C-28 showed significant *in vitro* cytotoxic activity against some cancer cell lines.[13] Additionally, four new water-soluble constituents were also isolated from the aerial root.[14] For the purpose of characterization of the interesting compounds from *Ficus microcarpa*, the further chemical study of its leaves part was carried out by us.

Previously, Higa *et al.* have investigated on the constituents from the leaves of *Ficus microcarpa*, and six known compounds were identified.[15] We have also reported the isolation and structure elucidation of two novel triterpenes together with nine known triterpenes from the ethyl acetate soluble fraction of the methanol extract of *Ficus microcarpa* leaves.[16] In our continuing investigation on the same extract, we further isolated three new chlorin compounds, 15^1(*S*)-methoxypurpurin-7-lactone-15^1-methoxy-15^2,17^3-dimethyl ester (**1**), 15^1(*S*)-methoxypurpurin-7-lactone-15^1-methoxy-15^2-methyl-17^3-phytyl ester (**2**), and 7-oxoaristophyll-C (**3**), as well as eight known pheophytin compounds, aristophyll-C (**4**), pyropheophytin a (**5**), 13^2(*S*)-pheophyton a (**6**), 13^2(*R*)-pheophyton a (**7**), 13^2(*R*)-hydroxypheophytin a (**8**), 13^2(*S*)-hydroxypheophytin a (**9**), 13^2(*R*)-hydroxypheophyton a (**10**), 13^2(*S*)-hydroxypheophyton a (**11**) (Fig. 1). In this paper, we describe the isolation and structure elucidation of the new compounds **1**—**3**.

Chlorins are the most ubiquitous of all natural pigments and have a long-established history of utilization in being traditional medicine and therapeutic propose.[17] Their special structures, which are composed of cyclic tetrapyrroles, exhibit as photosensitizers for use in photodynamic therapy (PDT), a physical treatment for cancer with combination of

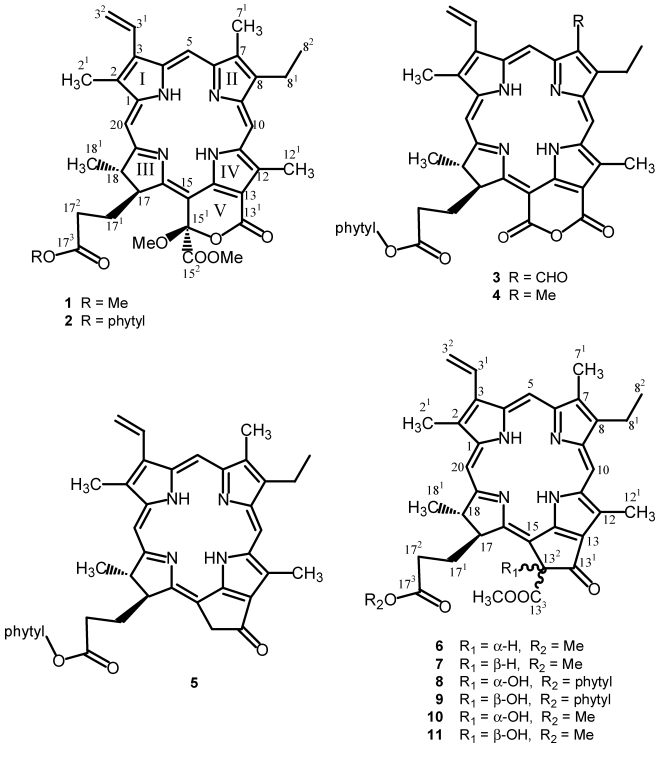

Fig. 1. Structures of Compounds **1**—**11** from *Ficus microcarpa*

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photosensitizers and light.[18,19] Moreover, chlorins integrating with different transition metal ions present photo-independent cytotoxic activity, which broaden its potential for anticancer drug development. Recent studies demonstrate that some natural chlorins reveal bioactivities for anti-herpes simplex virus type 1 (HSV-1) and anti-proliferation in 3T3-L1 adipocytes.[20,21] These results take our interest in isolating new natural chlorin compounds from natural sources assumed to contain abundant chlorin derivatives.

Results and Discussion

Compounds **1**—**11** were isolated from the ethyl acetate soluble fraction of methanol extract of *F. micorcarpa* leaves. In order to monitor the chlorin pigments, TLC examinations were proceeded with every subfractions after normal phase column chromatography gradient with non-polar to high-polar solvent (*n*-hexane/ethyl acetate to ethyl acetate/methanol). Then, these subfractions showed similar TLC results were combined, and subjected to continual HPLC purification.

Compound **1** was isolated as a dark-green solid. The high resolution fast atom bombardment mass spectrum (HR-FAB-MS) of **1** showed a protonated molecular ion at *m/z* 653.2988, which corresponded to the molecular formula, $C_{37}H_{40}O_7N_4$. The IR spectrum showed the presence of amino (3337 cm^{-1}) and ester carbonyl (1736 cm^{-1}) functionalities. The UV–vis spectrum showed the maxima absorptions at 399 and 667 nm, which indicated that compound **1** belonged to the pheophytin a type chlorophyll.[22] The ^{1}H-NMR (Table 1) spectrum of compound **1** exhibited signals for three aromatic methine groups [δ_H 9.76, s, (H-10), 9.52, s, (H-5) and 8.74, s (H-20)], one conjugated vinyl group [δ_H 7.97, dd, J=17.8, 11.8 Hz, (H-3^1), 6.31, d, J=17.8 Hz, (H_a-3^2) and 6.16, d, J=11.8 Hz, (H_b-3^2)], three aromatic methyl groups [δ_H 3.90, s, (CH_3-12^1), 3.42, s, (CH_3-2^1) and 3.23, s (CH_3-7^1)], two methyl ester groups [δ_H 3.59, s, (OCH_3-15^2) and 3.52, s, (OCH_3-17^3)], one ethyl group [δ_H 3.71, q, J=7.8 Hz, (CH_2-8^1) and 1.68, t, J=7.8 Hz, (CH_3-8^2)], two amino group protons [δ_H −1.10, br s (N–H) and −1.37, br s, (N–H)], one methoxy group [δ_H 3.91, s , (OCH_3-15^1)], and one downfield shifted methyl group [δ_H 1.59, d, J=7.7 Hz, (CH_3-18^1)]. The ^{1}H-NMR data of (Table 1) spectrum of compound **1** showed close resemblance with those of 13^2(*S*)-pheophyton a (**6**) and 13^2(*R*)-pheophyton a (**7**).[23] However, compound **1** possessed the ^{1}H-NMR signals for six methyl groups with chemical shift between δ_H 3—4 ppm [δ_H 3.91, s, (OCH_3-15^1), 3.90, s,

Table 1. ^{1}H-NMR Data for Compounds **1**—**3** (500 MHz in $CDCl_3$), δ in ppm, J in Hz

Position	1	2	3
2^1	3.42 s	3.42 s	3.27 s
3^1	7.97 dd (17.8, 11.8)	7.98 dd (17.8, 11.5)	7.84 dd (17.5, 11.5)
3^2	6.31 d (17.8 H_a)	6.32 d (17.8, H_a)	6.35 d (17.5, H_a)
	6.16 d (11.8, H_b)	6.16 d (11.5, H_b)	6.21 d (11.5, H_b)
5	9.52 s	9.50 s	10.25 s
7^1	3.23 s	3.24 s	11.01 s
8^1	3.71 q (7.8)	3.70 q (7.6)	3.96 q (7.5)
8^2	1.68 t (7.8)	1.68 t (7.6)	1.78 t (7.5)
10	9.76 s	9.74 s	9.62 s
12^1	3.90 s	3.90 s	3.75 s
OMe-15^1	3.91 s	3.89 s	—
OMe-15^2	3.59 s	3.59 s	—
17	4.68 dd (9.2, 2.4)	4.66 br d (7.4)	5.12 br dd (9.4, 2.0)
17^1	2.48 m, 1.98 m	2.46 m, 1.97 m	2.45 m, 1.97 m
17^2	2.50 m	2.58 m	2.71 m
	2.21 m	2.17m	2.43 m
OMe-17^3	3.52 s	—	—
18	4.44 q (7.7)	4.45 q (7.2)	4.33 q, (7.0)
18^1	1.59 d (7.7)	1.58 d (7.2)	1.71 q, (7.0)
20	8.74 s	8.71 s	8.45 s
NH	−1.10 br s	−1.10 br s	0.72 br s
	−1.37 br s	−1.37 br s	0.28 br s
P1	—	4.43 d (6.9)	4.50 dd (12.6, 6.6)
			4.48 dd (12.6, 6.6)
P2	—	5.14 t (6.9)	5.21 t (6.6)
P4	—	1.87 m	1.93 m
P15	—	1.48 m	1.46 m
P16	—	0.83 d (6.6)	0.82 d (6.4)
P17	—	0.83 d (6.6)	0.82 d (6.4)
P18	—	0.78[a] d (6.6)	0.78[a] d (6.4)
P19	—	0.74[a] d (6.6)	0.74[a] d (6.4)
P20	—	1.55 br s	1.60 br s

a) Values may be interchanged. P: phytyl moiety.

Table 2. ^{13}C-NMR Data for Compounds **1**—**3** (100 MHz in $CDCl_3$)

Position	1	2	3
1	141.3 s	141.3 s	146.0 s
2	131.6 s	131.6[a] s	132.2 s
2^1	12.1 q	12.1 q	11.9 q
3	136.3[a] s	136.3[b] s	139.4 s
3^1	128.9 d	129.0 d	127.8 d
3^2	122.7 t	122.7 t	124.6 t
4	136.2[a] s	136.2[b] s	137.5 s
5	99.5 d	99.5 d	107.3 d
6	155.8 s	155.8 s	152.1 s
7	136.0[a] s	136.1[b] s	132.9 s
7^1	11.2 q	11.2 q	187.3 d
8	145.5 s	145.5 s	146.3 s
8^1	19.5 t	19.5 t	18.9 t
8^2	17.5 q	17.5 q	19.4 q
9	149.7 s	149.4 s	159.8 s
10	104.1 d	104.1 d	110.0 d
11	138.6 s	138.8 s	143.1 s
12	131.4 s	131.4[a] s	131.6 s
12^1	12.4 q	12.4 q	12.5 q
13	111.8 s	111.9 s	112.3 s
13^1	161.2 s	161.2 s	163.5 s
14	135.1 s	135.2 s	140.9 s
15	100.6 s	100.7 s	92.9 s
15^1	106.3 s	106.3 s	158.8 s
OMe-15^1	53.0 q	53.0 q	—
15^2	168.7 s	168.7 s	—
OMe-15^2	53.2 q	52.9 q	—
16	167.5 s	167.5 s	179.9 s
17	53.7 d	53.9 d	55.1 d
17^1	32.2 t	32.2 t	31.2 t
17^2	32.2 t	32.5 t	32.7 t
17^3	173.7 s	173.2 s	173.1 s
OMe-17^3	51.5 q	—	—
18	50.3 d	50.3 d	49.2 d
18^1	22.5 q	22.5 q	23.8 q
19	171.8 s	171.9 s	178.8 s
20	93.9 d	94.0 d	95.3 d
Phytyl carbons	—	142.6 s, 117.9 d, 61.3 t, 39.8 t, 39.3 t, 37.4 t, 37.3 t, 37.2 t, 36.6 t, 32.5 d, 32.2 d, 28.0 d, 25.0 t, 24.8 t, 24.4 t, 22.6[c] q, 22.7[c] q, 19.6[d] q, 19.7[d] q, 16.2 q	142.8 s, 117.8 d, 61.5 t, 39.8 t, 39.3 t, 37.4 t, 37.3 t, 37.2 t, 36.6 t, 32.7 d, 32.7 d, 25.0 t, 27.9 d, 24.7 t, 24.4 t, 22.7[a] q, 22.6[a] q, 19.7[b] q, 19.6[b] q, 16.2 q

a—*d*) Values may be interchanged.

(CH_3-12[1]), 3.59, s, (OCH_3-15[2]), 3.52, s, (OCH_3-17[3]), 3.42, s, (CH_3-2[1]) and 3.23, s (CH_3-7[1])], which hinted that the structure of **1** exhibited one more methoxy group [δ_H 3.91, s, (OCH_3-15[1])] than compounds **6** and **7**. Besides, the ^{1}H-NMR spectrum of the CH-13[2] methine group in **6** (δ_H 6.21, s) and **7** (δ_H 6.26, s) did not exist in compound **1**.[23] In the ^{13}C-NMR (Table 2) and distortionless enhancement by polarization transfer (DEPT) spectra of **1**, most signals were quite similar to 13[2](*S*)-pheophyton a (**6**) and 13[2](*R*)-pheophyton a (**7**). However, one conjugated ester group [δ_C 161.2, (C-13[1])], one dioxygenated carbon [δ_C 106.1, (C-15[1])], and one more methoxy carbon [δ_C 53.0, (OCH_3-15[1])] emerged in **1** instead of the conjugated cyclopentenone group signal in **6** and **7** [δ_C *ca.* 198, (C-13[1])].[23] By comparing the ^{1}H- and ^{13}C-NMR data of **1** with those of **6** and **7** suggested that **1** exhibited identical tetrapyrrole moiety structure as **6** and **7**, and the only difference being the structure of V-ring. The heteronuclear multiple bond coherence (HMBC) experiment data (Fig. 2) were employed to assign the V-ring structure of **1**. Figure 2 showed the HMBC correlation between O$C\underline{H}_3$-15[1] and C-15[1], which proved that this additional methoxy group attached to the dioxygenated carbon (C-15[1]). According to the above HMBC and ^{13}C-NMR informations, the structure of the V-ring of **1** was constructed to be a conjugated δ-lactone with an acetal group instead of a conjugated cyclopentenone in pheophytin a. The stereochemistry at O$C\underline{H}_3$-15[1] of compound **1** was deduced by nuclear Overhauser enhancement exchange spectroscopy (NOESY) experiment (Fig. 3). The 15[1]-methoxy group was in β orientation, which was confirmed by the significant NOE correlation between O$C\underline{H}_3$-15[1] and H-17[1]. Based on the above evidence, compound **1** was elucidated as 15[1](*S*)-purpurin 7-lactone-15[1]-methoxy-15[2], 17[3]-dimethyl ester, and was named as ficusmicrochlorin A. Compound **1** has been synthesized by Fischer *et al.* through allomerization reaction.[24,25] It was conducted by direct addition of singlet oxygen to the C-13[2] radical of pheophyton a (**6**), then the cyclopentenone was opened and reconstructed into a δ-lactone producing compound **1** and its C-15[1] diastereomer. No spectral data could be consulted because of inexistence of spectrometer in that age. Compound **1** has not previously found as a naturally occurring substance, and its spectral data were described here for the first time.

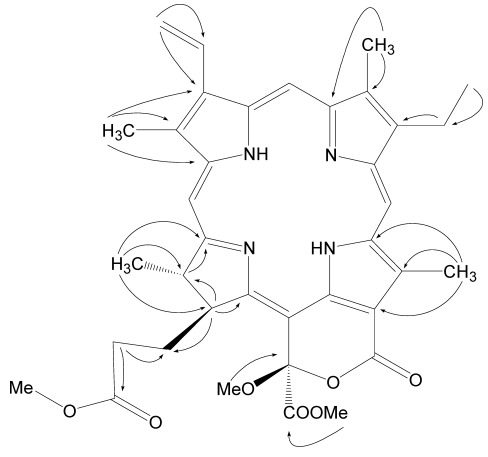

Fig. 2. Selected HMBC Correlations of Compound **1**

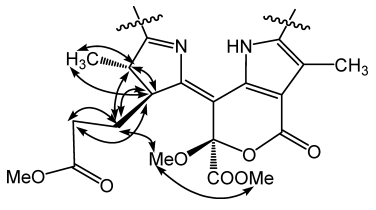

Fig. 3. Selected NOESY Correlations of Compound **1**

Compound **2** was obtained as a dark-green solid, analyzed for $C_{56}H_{76}O_7N_4$ by HR-FAB-MS data. The UV–vis (λ_{max} 400, 667 nm) and IR (3337, 1743 cm^{-1}) spectra of compound **2** suggested that it contains identical functionalities as **1**. In addition, its ^{1}H- and ^{13}C-NMR (Tables 1, 2) spectra exhibited almost identical signals as **1** besides phytyl ester instead of methyl ester at C-17[3]. Moreover, the HMBC correlation between O$C\underline{H}_3$-15[1]/C-15[1] and the NOESY correlation between O$C\underline{H}_3$-15[1]/H-17[1] in the HMBC and NOESY spectra, respectively, of **2** proved that the V-ring structure of compounds **1** and **2** were exactly identical. On the basis of above evidence, compound **2** was assigned as 15[1](*S*)-purpurin 7-lactone-15[1]-methoxy-15[2]-methyl-17[3]-phytyl ester, namely ficusmicrochlorin B.

Ficusmicrochlorin C (**3**) was obtained as a dark-green solid. The molecular formula was determined as $C_{53}H_{68}O_6N_4$ by HR-FAB-MS. The IR spectrum displayed absorption bands for amino (3353 cm^{-1}) and carbonyl (1749 cm^{-1}) functionalities. The UV–vis spectrum showed maxima absorptions at 432 and 674 nm, which indicated that compound **3** belonged to the pheophytin b type chlorophyll.[22] Most ^{1}H- and ^{13}C-NMR (Tables 1, 2) signals of compound **3** showed similar characteristics with the known compound, aristophyll-C (**4**),[26] such as one down-field methine group [δ_H 5.12 (br dd, J=9.4, 2.0 Hz, H-17)] which was deshielded by a conjugated anhydride group [δ_C 163.5 (C-13[1]), 158.8 (C-15[1])]. However, it also revealed the pheophytin b-type characteristics [δ_H 11.01 (s, H-7[1]), 10.25 (s, H-5); δ_C 187.3 (C-7[1])] and two aromatic methyl groups [δ_H 3.75 (s, H_3-12[1]), 3.27 (s, H_3-2[1])], which were quite different against **4**. These above spectral evidence apparently indicated that compound **3** was a 7-oxo derivative of aristophyll-C (**4**). Consequently, compound **3** was elucidated as 7-oxoaristophyll-C, and named as ficusmicrochlorin C. The two carbonyl carbons, C-13[1] and C-15[1], of conjugated anhydride group were assigned as δ_C 163.5 and 158.8 individually by comparing the ^{13}C-NMR data of **3** with compounds **1** and **2**. Both C-16 (δ_C 179.9) and C-19 (δ_C 178.8) carbons of **3** shifted to more down-field region than common pheophytin b type com-

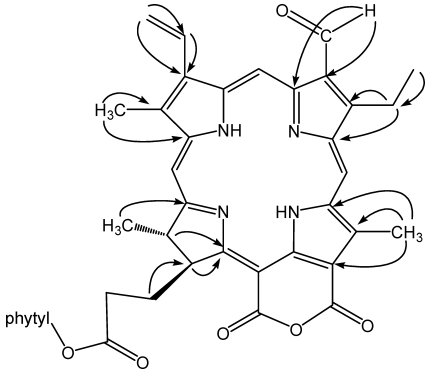

Fig. 4. Selected HMBC Correlations of **3**

pounds (C-16, δ_C *ca.* 165; C-19, δ_C *ca.* 175) because of the resonance effect from C-15[1] carbonyl functionality. Also, the locations of C-16 and C-19 were confirmed by HMBC correlations between H-18/C-16 and H_3-18[1]/C-19 (Fig. 4).

Moreover, eight known pheophytin compounds were also isolated from the leaves of *Ficus microcarpa*. These were characterized as aristophyll-C (**4**),[26] pyropheophytin a (**5**),[27] 13^2(*S*)-pheophyton a (**6**),[23] 13^2(*R*)-pheophyton a (**7**),[23] 13^2(*R*)-hydroxypheophytin a (**8**),[28] 13^2(*S*)-hydroxypheophytin a (**9**),[28] 13^2(*R*)-hydroxypheophyton a (**10**),[23] 13^2(*S*)-hydroxypheophyton a (**11**).[23] The structures of these compounds were elucidated by comparison of their IR, MS and NMR data with literature values.

Although δ-lactone and anhydride functionalities on V-ring of chrolins could be obtained by chemical reaction between chlorins and triplet oxygen through free radical allomerization reaction, compounds **1**—**3** were produced as plant metabolite or during methanol extraction process is still indefinite.[25,29] If these compounds have been cumulated by plant, their biosynthesis pathways should be revealed by discovering more new chlorins as intermediates and proceeding labeling experiments. Moreover, further bioactivity investigations are necessary for these chlorin compounds, and how the structural variation influences the bioactivity.

Experimental

General Experimental Procedures Extracts were purified by using silica gel column chromatography (CC) (Merck 70—230 and 230—400 mesh, ASTM, Darmstade, Germany) and HPLC (LDC Analytical-III; LiChrosorb Si60 column (250 mm×10 mm, 5 μm)). Melting points were determined by the Yanagimoto micro-melting-point apparatus (MP500D, Yanagimoto Co., Kyoto, Japan) and are uncorrected. FAB-MS and HR-FAB-MS were obtained from JEOL JMS-HX 300 mass spectrometer; in *m/z* (rel. %). The UV–vis and IR spectra were measured on Heλios β UV–vis spectrophotometer and Perkin-Elmer 983-G spectrophotometer, respectively. ^{1}H-, ^{13}C-, DEPT and 2D-NMR spectra were recorded on a Bruker DMX-400 and a DMX-500 spectrometer. Optical rotations were measured on a Jasco DIP-180 digital polarimeter.

Plant Material The leaves of *Ficus microcarpa* Linn. f. were collected on the campus of National Taiwan University, Taipei, Taiwan, in 2000. Identification of the voucher specimens was done by Mr. Muh-Tsuen Gun (retired), Department of Botany, National Taiwan University. A voucher specimen (No. 038671) has been deposited at the Herbarium of the Department of Botany, National Taiwan University, Taipei, Taiwan.

Extraction and Isolation The dried leaves of *Ficus microcarpa* Linn. f. (7.1 kg) were extracted with MeOH (80 l) at room temperature (10 d×3). The combined extracts were evaporated in vacuum to yield a residue, which was suspended in H_2O to bring the total volume to 1 l. Then, this phase was extracted with ethyl acetate (1 l×3) and the combined ethyl acetate phase was evaporated under reduced pressure to obtain ethyl acetate extract (345 g). The ethyl acetate extract was repeatedly purified by CC (silica gel, *n*-hexane/ethyl acetate) and HPLC ((LiChrosorb Si60), *n*-hexane/ethyl acetate and dichloromethane/ethyl acetate). Pyropheophytin a (**5**, 12 mg) was eluted with 20% ethyl acetate in *n*-hexane. Ficusmicrochlorin B (**2**, 17 mg), aristophyll-C (**4**, 45 mg), 13^2(*R*)-hydroxypheophytin a (**8**, 535 mg), and 13^2(*S*)-hydroxypheophytin a (**9**, 3.2 g) were eluted with 30% ethyl acetate in *n*-hexane. Ficusmicrochlorin A (**1**, 12 mg), ficusmicrochlorin C (**3**, 6 mg), 13^2(*S*)-pheophyton a (**6**, 4 mg), 13^2(*R*)-pheophyton a (**7**, 26 mg), 13^2(*R*)-hydroxypheophyton a (**10**, 6 mg), 13^2(*S*)-hydroxypheophyton a (**11**, 22 mg) were eluted with 50% ethyl acetate in *n*-hexane.

Ficusmicrochlorin A (**1**): Dark green amorphous solid, mp 234—236 °C; $[\alpha]_D^{23}$ +17.2 (*c*=0.19, $CHCl_3$); UV–vis (MeOH) λ_{max} (log ε) nm: 225 (sh, 4.25), 276 (4.05), 318 (sh, 4.07), 365 (sh, 4.49), 399 (4.84), 499 (3.77), 530 (3.71), 612 (3.57), 667 (4.29); IR (KBr) cm^{-1}: 3337, 1736, 1601, 1524, 1250, 1201, 1083, 1038, 714; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; FAB-MS *m/z*: 653 $[M+H]^+$ (85), 652 (55), 593 (94), 565 (91), 503 (100), 491 (50), 473 (27), 420 (21), 289 (18); HR-FAB-MS *m/z*: 653.2988 (Calcd for $C_{37}H_{40}O_7N_4$, 653.2984).

Ficusmicrochlorin B (**2**): Dark green amorphous solid, mp 228—230 °C; $[\alpha]_D^{23}$ +30.9 (*c*=0.25, $CHCl_3$); UV–vis (MeOH) λ_{max} (log ε) nm: 226 (sh, 4.26), 277 (3.97), 372 (sh, 4.20), 400 (4.46), 499 (3.44), 530 (3.35), 610 (3.20), 667 (3.91); IR (KBr) cm^{-1}: 3337, 1743, 1603, 1521, 1300, 1163, 1081, 1008, 783, 673; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; FAB-MS *m/z*: 917 $[M+H]^+$ (81), 916 (66), 857 (68), 579 (20), 503 (47), 491 (34), 463; HR-FAB-MS *m/z*: 917.5810 (Calcd for $C_{56}H_{76}O_7N_4$, 917.5807).

Ficusmicrochlorin C (**3**): Dark green amorphous solid, mp 217—220 °C; $[\alpha]_D^{23}$ +88.5 (*c*=0.1, $CHCl_3$); UV–vis (MeOH) λ_{max} (log ε) nm: 217 (sh, 4.88), 269 (3.70), 335 (4.59), 357 (4.59), 414 (sh, 4.98), 432 (5.11), 519 (4.02), 556 (3.99), 627 (3.84), 674 (4.37); IR (KBr) cm^{-1}: 3353, 2741, 1749, 1730, 1721, 1661, 1607, 1533, 1313, 1162, 1054, 1017, 995, 724; ^{1}H- and ^{13}C-NMR data, see Tables 1 and 2; FAB-MS *m/z*: 857 ($[M+H]^+$, 100), 856 (87); HR-FAB-MS *m/z*: 857.5235 (Calcd for $C_{53}H_{68}O_6N_4$, 857.5230).

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