Chromene and Prenylated Xanthones from the Roots of *Cratoxylum formosum* ssp. *pruniflorum*

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Received November 18, 2009; accepted December 11, 2009; published online December 15, 2009

Two new xanthones, namely pruniflorone K (1) and L (2), have been isolated from the roots of *Cratoxylum formosum* ssp. *pruniflorum*, along with thirteen known xanthones (3—15). Their structures were mainly established using the spectroscopic methods. Only isolated compounds with sufficient amount were evaluated for antibacterial and antifungal activities.

Key words *Cratoxylum formosum* ssp. *pruniflorum*; xanthone; antibacterial activity

Xanthones are secondary metabolites commonly occurring in the higher plants. Recently, many isolated xanthones have been reported to possess several biological activities such as antibacterial,[1,2] anti-inflammatory[3] and cytotoxic activities.[4—6] In the previous work, we reported the isolation of xanthones from the plants of *Cratoxylum* genus, which were collected in Thailand. We found that several isolated xanthones showed the antibacterial,[7—9] antifungal[10] and cytotoxic activities.[7—9] Previous investigation of the roots of *Cratoxylum formosum* ssp. *pruniflorum* had resulted in nine new and nine known xanthones.[7] Herein, this paper describes further investigation of CH_2Cl_2 extract from the roots of the same plant which led to the isolation of two new xanthones (**1**, **2**) along with thirteen known xanthones (**3**—**15**). In addition, the antibacterial and antifungal activities were also evaluated.

Results and Discussion

The crude CH_2Cl_2 extract of the roots of *C. formosum* ssp. *pruniflorum* was subjected to chemical investigation leading to the isolation of two new xanthones, pruniflorone K (**1**) and L (**2**), together with thirteen known xanthones identified as cochinchinone A (**3**),[11] formoxanthone B (**4**),[8] 1,7-dihydroxy-8-methoxyxanthone (**5**),[12] cochinchinone I (**6**),[10] 1,3,7-trihydroxy-2,4-diisoprenylxanthone (**7**),[13] celebixanthone methyl ether (**8**),[14] vieillardiixanthone B (**9**),[15] macluraxanthone (**10**),[16] dulcisxanthone B (**11**),[17] norcowanin (**12**),[18] 5,9-dihydroxy-8-methoxy-2,2-dimethyl-7-(3-methylbut-2-enyl)-2*H*,6*H*-pyrano[3,2*b*]xanthone (**13**),[19] Garcinone B (**14**),[20] and brasilixanthone (**15**).[21] Their structures were elucidated by NMR analysis and comparison of their spectroscopic data with those reported in the literatures.

Pruniflorone K (**1**) was obtained as a yellow powder with a molecular ion peak at *m/z* 446.2092 $[M]^+$ in the HR-EI-MS, corresponding to $C_{28}H_{30}O_5$. The UV spectrum showed absorption bands of a xanthone (245, 260, 317, 367 nm),[13] while the IR spectrum exhibited the hydroxyl and conjugated carbonyl functionalities at ν_{max} 3338 and 1647 cm^{-1} respectively. The ^1H- and ^{13}C-NMR data of **1** (Table 1) were comparable to those of formoxanthone A previously isolated from the roots of *Cratoxylum formosum*.[8] The main difference was observed at C-2 and C-3, where the ^1H-NMR spectral data of **1** (Table 1) showed the signals of a chromene ring (δ 6.79, d, J=10.2 Hz, H-1′, δ 5.56, d, J=10.2 Hz, H-2′) bearing a methyl group (δ 1.45, s, CH_3-9′) and a six-carbon side chain of 4-methylpent-3-enyl group [δ 5.09 (br t, J=6.9 Hz, H-6′), 1.78 (m, H_2-4′), 2.12 (m, H_2-5′), 1.68 (s, CH_3-8′) and 1.45 (s, CH_3-10′)] instead of a geranyl moiety at C-2 and a free hydroxyl group at C-3 as in formoxanthone A. The loss of 4-methylpent-3-enyl moiety in EI-MS, *m/z* 363 ($[M]^+-83$), also supported the proposed structure. The location of the linear chromene ring was confirmed by heteronuclear multiple bond correlation (HMBC) (Table 1) in which the methine proton H-1′ (δ 6.79) was correlated with C-1 (δ 156.1), C-2 (δ 104.5) and C-3 (δ 158.4). From our previously proposed biosynthesis,[10] it could be suggested that compound **1** could be derived from formoxanthone A by epoxidation of a geranyl side chain followed by cyclization *via* a free hydroxyl at C-3. Further dehydration led to a linear chromene ring. Therefore, compound **1** was established to be pruniflorone K.

Pruniflorone L (**2**) was isolated as a pale yellow powder, with a molecular ion peak at *m/z* 462.2408 $[M]^+$ in the HR-EI-MS, corresponding to $C_{29}H_{34}O_5$. The UV spectrum showed absorption maxima at 245, 268, 317 and 388 nm. The IR spectrum exhibited the hydroxyl and conjugated carbonyl functionalities at ν_{max} 3421 and 1637 cm^{-1} respectively. The ^1H- and ^{13}C-NMR spectral data of **2** (Table 2) showed characteristic signals similar to those of cochinchinone A (**3**),[11] except that the hydroxyl group at C-3 of **3** was replaced by a methoxyl group in **2**, which appeared as a proton singlet signal at δ 3.74 whose HMBC correlation with C-3 (δ 163.8) confirmed its location. The ^3J HMBC correlations of the methylene protons at δ 3.34 (H_2-1′) of the isoprenyl group with C-1 (δ 158.8) and C-3 (δ 163.8) supported its location at C-2, whereas the ^3J HMBC correlations of the methylene protons (H_2-1″) of the geranyl group with C-3 (δ 163.8) and C-4a (δ 153.3) supported its location at C-4. Compound **2**, an *O*-methylated analogue of **3**, was therefore assigned as 1,7-dihydroxy-3-methoxy-2-isoprenyl-4-

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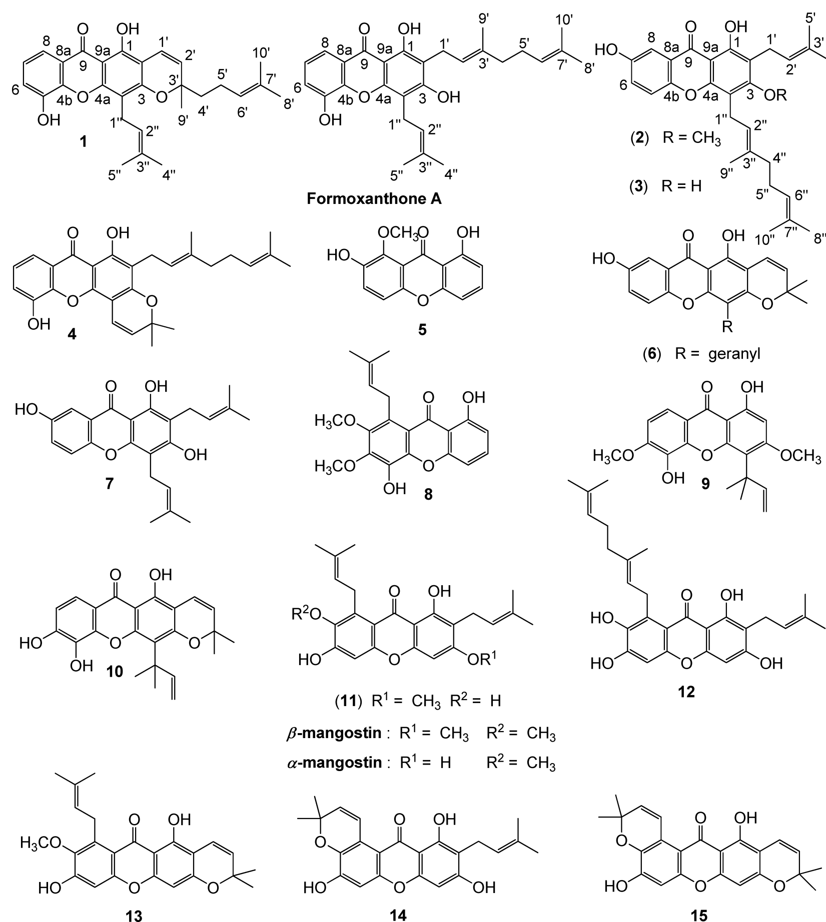

Chart 1. Structures of Compounds **1**—**15**, Formoxanthone A, β-Mangostin and α-Mangostin

Table 1. NMR Data of Compound **1** and Formoxanthone A in $CDCl_3$

No.	**1**			Formoxanthone A	
	δ_H (J in Hz)	δ_C	HMBC	δ_H (J in Hz)	δ_C
1-OH	13.08, s	156.1	1, 2, 3, 9a	13.20, s	158.6
2		104.5			109.0
3		158.4		6.51, br s	161.0
4		106.7			105.7
5	5.73, br s	144.5	5, 6	5.86, br s	144.4
6	7.30, dd, 7.8, 1.8	119.8	8, 4b	7.29, dd, 7.8, 0.9	119.8
7	7.23, t, 7.8	124.0	5, 8a	7.22, t	123.8
8	7.75, dd, 7.5, 1.8	116.8	8, 9, 4b	7.75, dd, 7.8, 0.9	116.9
9		181.5			181.1
4a		154.7			152.5
4b		144.4			144.3
8a		121.0			120.9
9a		103.3			103.3
1′	6.79, d, 10.2	116.2	1, 2, 3, 3′	3.50, d, 7.2	21.6
2′	5.56, d, 10.2	126.2	2, 3′, 4′, 9′	5.29, br t, 7.2	121.1
3′		80.9			140.1
4′	1.78, m	41.8	2′, 3′, 5′, 9′	2.11, m	39.7
5′	2.12, m	22.7	4′, 6′, 7′	2.11, m	26.3
6′	5.09, br t, 6.9	123.7	5′, 8′, 10′	5.06, m	123.7
7′		131.9			132.2
8′	1.68, s	25.6	6′, 7′	1.60, s	17.7
9′	1.45, s	27.2	2′, 3′, 4′	1.85, s	16.3
10′	1.45, s	17.6	6′, 7′	1.68, s	25.7
1″	3.50, d, 6.9	21.7	3, 4, 2″, 3″, 4a	3.54, d, 6.9	22.0
2″	5.23, br t, 6.9	122.7	1″, 5″	5.26, br t, 6.9	122.4
3″		131.7			133.1
4″	1.72, s	25.5	2″, 3″	1.74, s	25.6
5″	1.84, s	17.9	2″, 3″	1.86, s	17.9

Table 2. NMR Data of Compounds **2** and **3** in $CDCl_3$

No.	**2**			**3**	
	δ_H (J in Hz)	δ_C	HMBC	δ_H (J in Hz)	δ_C
1-OH	12.78, s	158.8	1, 2, 3, 9, 9a	12.79, s	158.2
2		116.9			109.2
3		163.8		6.44, br s	161.1
4		113.0			105.0
5	7.28, d, 8.7	119.2	6, 7, 9, 4b, 8a	7.03, d, 9.0	118.7
6	7.50, dd, 1.8, 8.7	124.4	7, 8, 5a, 8a	7.07, br s, 9.0	124.7
7	6.55, br s	150.7		7.82, br s	150.1
8	7.54, d, 1.8	109.0	6, 7, 9, 4b	7.44, br s	108.7
9		181.1			180.9
4a		153.3			152.9
4b		152.3			152.6
8a		120.7			120.2
9a		105.8			103.0
1′	3.34, d, 6.6	22.6	1, 2, 3, 2′, 3′	3.32, d, 6.9	21.8
2′	5.20, br t, 6.6	122.6	2, 1′, 4′, 5′	5.20, br t, 6.9	121.6
3′		131.9			134.8
4′	1.63, s	25.7	2′, 3′, 5′	1.55, s	25.6
5′	1.74, s	17.9	2′, 3′, 4′	1.75, s	17.9
1″	3.46, d, 6.6	22.7	3, 4, 2″, 3″, 9″, 4a	3.40, d, 6.9	21.6
2″	5.19, br t, 6.6	122.8	4, 1″, 4″, 9″	5.16, br t, 6.9	121.6
3″		135.3			137.9
4″	1.94, m	39.6	2″, 3″, 6″	1.99, m	39.7
5″	1.98, m	26.6	3″, 6″, 7	1.97, m	26.4
6″	4.95, br t, 6.6	124.1	4″, 5″, 8″, 10″	4.96, br t, 7.2	123.9
7″		131.4			131.8
8″	1.50, s	25.6	6″, 7″	1.67, s	25.8
9″	1.80, s	16.3	2″, 3″	1.77, s	16.2
10″	1.46, s	17.6	6″, 7″	1.48, s	17.6
3-OCH_3	3.74, s	61.9	3	—	—

Table 3. Antimicrobial Activity (MIC, μg/ml) of Compounds **1**—**3**, **5** and **9**—**15**

No.	Antibacterial activity								Antifungal activity
	Gram-positive bacteria[a]					Gram-negative bacteria[b]			
	B. subtilis	*S. aureus*	*E. faecalis*	MRSA	VRE	*S. typhi*	*S. sonei*	*P. aeruginosa*	*C. albicans*[c]
1	300	>300	>300	>300	>300	>300	>300	>300	>300
2	>300	>300	>300	>300	>300	>300	>300	>300	>300
3[d]	150	150	150	9.37	150	>150	>150	4.7	75
5	37.5	37.5	300	75	300	>300	>300	300	300
9	300	300	300	300	300	>300	>300	>300	>300
10[d]	18.75	37.5	37.5	37.5	37.5	>300	>300	37.5	4.7[d]
11	18.75	18.75	300	9.37	9.37	18.75	>300	300	>300
12	4.7	9.37	9.37	18.75	75	>300	>300	300	300
13	18.75	18.75	75	37.5	75	>300	>300	300	300
14	75	75	75	150	75	>300	>300	>300	>300
10+**14** (1 : 1)	4.7	2.4	9.37	2.4	4.7	>300	>300	>300	300
15	300	300	>300	300	300	>300	>300	>300	>300

a) *Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis* TISTR 459, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, vancomycin-resistant *Enterococcus faecalis* (VRE) ATCC 51299. *b*) *Salmonella typhi*, *Shigella sonei* and *Pseudomonas aeruginosa*. *c*) *Candida albicans*. *d*) It has been previously reported by Boonnak *et al.*[10]

geranylxanthone and named as pruniflorone L.

Only isolated compounds with sufficient amount were further evaluated for their antibacterial activities against both Gram-positive bacteria (*i.e. Bacillus subtilis*, *Staphylococcus aureus*, *Enterococcus faecalis* TISTR 459, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 43300, vancomycin-resistant *Enterococcus faecalis* (VRE) ATCC 51299) and Gram-negative bacteria (*i.e. Salmonella typhi*, *Shigella sonei* and *Pseudomonas aeruginosa*). Antifungal activity was also evaluated. From the antimicrobial activity results shown in Table 3, compound **11** (dulcisxanthone B) showed moderate activity against both MRSA and VRE with the same minimum inhibitory concentration (MIC) values of 9.37 μg/ml. By comparing these results with those of β-mangostin (MRSA: 75, VRE: 150 μg/ml) and α-mangostin (MRSA, VRE: 9.37 μg/ml) which were previously reported,[10] it could be suggested that one *O*-methylation either at C-3 (**11**) or C-7 (α-mangostin) of 1,3,6,7-tetraoxygenated xanthone with isoprenyl side chain at C-2 and C-8 was essential for antibacterial activity especially against Gram-positive bacteria. The antibacterial activity against Gram-positive bacteria of β-mangostin whose structure contained two *O*-methylated groups at C-3 and C-7 was decreased drastically (MIC $\geq$75 μg/ml).

Previously we have proposed that 1,3,7-trihydroxy xanthone with the isoprenyl or geranyl side chain at C-2 and C-4 is essential for its antibacterial activity against *P. aeruginosa*,[10] we can further added that the hydroxyl group at C-3 is also essential for the activity as compared pruniflorone L (**2**, MIC >300 μg/ml) with cochinchinone A (**3**, MIC 4.7 μg/ml). During the chromatographic separation, we have observed that compounds **10** and **14** came as a mixture. Therefore, these two compounds were tested as a mixture for anti-microbial activity whose result was good. This prompted us to purify the mixture of which was successfully separated by reversed phase RP-18 CC eluting with MeOH. We then tested each of the compounds **10**, **14** and their 1 : 1 mixture. Interestingly, the 1 : 1 mixture of compounds **10** and **14** significantly increased the antibacterial activities against *B. subtilis*, *S. aureus*, *E. faecalis*, MRSA and VRE compared with the pure forms (**10**, **14**). From this result, it may be possible that a 1 : 1 mixture of compounds **10** and **14** showed synergism effect for antibacterial activity against all Gram-positive bacteria tested. Compounds **1**, **9** and **15** were inactive against all micro-organism tested.

Experimental

General Procedures Melting points were determined on the Fisher–John melting point apparatus. Optical rotations were measured on a JASCO P-1020 digital polarimeter. UV and IR spectra were recorded on SPECORD S 100 (Analytikjena) and Perkin-Elmer FTS FT-IR spectrophotometer, respectively. The ^{1}H- and ^{13}C-NMR spectra were recorded on a 300 MHz Bruker FTNMR Ultra Shield^{TM} spectrometers in $CDCl_3$ with TMS as the internal standard. Chemical shifts are reported in δ (ppm) and coupling constants (*J*) are expressed in hertz. EI and HR-EI mass spectra were measured on a Kratos MS 25 RFA spectrometer. Quick column chromatography (QCC) and column chromatography (CC) were carried out on silica gel 60 F_{254} (Merck) and silica gel 100 (Merck), respectively.

Plant Material Roots of *C. formosum* ssp. *pruniflorum* were collected in May 2004 from Nong Khai Province, northeastern part of Thailand. Identification was made by Prof. Puangpen Sirirugsa, Department of Biology, Faculty of Science, Prince of Songkla University and a specimen (No. 0012677) was deposited at Prince of Songkla University Herbarium.

Isolation and Extraction The air-dried roots of *C. formosum* ssp. *pruniflorum* (5.00 kg) was extracted with CH_2Cl_2 (2×20 l, for a week) at room temperature and was further evaporated under reduced pressure to afford a deep green crude CH_2Cl_2 extract (58.87 g), which was subjected to QCC on silica gel using n-hexane as a first eluent and then increasing the polarity with acetone to give 12 fractions (FR1—FR12). Fractions FR6 and FR7 were separated by QCC eluting with a gradient of acetone–*n*-hexane to give 10 subfractions (FR6A—FR6J). Subfraction FR6B was separated by QCC and eluted with a gradient of acetone–*n*-hexane to give 11 subfractions (FR6B1—FR6B11). Subfractions FR6B6 and FR6B7 were separated by CC and eluted with 30% CH_2Cl_2–*n*-hexane to give 9 subfractions (FR6B6A—FR6B6I) and **1** (5.7 mg). Subfraction FR6B6E was further purified by CC on silica gel C-18 and eluted with MeOH to furnish **13** (7.0 mg). Subfractions FR6B8 and FR6B9 were separated by CC and eluted with 30% CH_2Cl_2–*n*-hexane to give 10 subfractions (FR6B8A—FR6B8J) and **4** (1.5 mg). Subfraction FR6H was further separated by QCC eluting with a gradient of acetone–*n*-hexane to give 11 subfractions (FR6H1—FR6H11), **2** (9.7 mg), **3** (80.7 mg) and **7** (150.2 mg). Subfraction FR6H5 was further purified by CC using 10% acetone–*n*-hexane as a mobile phase to give **6** (5.6 mg). Fractions FR8—FR11 was separated by QCC eluting with 30% EtOAc–*n*-hexane to give 8 subfractions (FR8A—FR8H). Subfractions FR8E and FR8F were separated by QCC and eluted with 30% EtOAc–*n*-hexane to obtain 20 sub-

fractions (FR8E1—FR8E20). Subfraction FR8E10—FR8E12 were separated by QCC and eluted with a gradient of CH_2Cl_2–*n*-hexane to give 12 subfractions (FR8E10A—FR8E10L). Subfraction FR8E10B was further purified by CC and eluted with 5% acetone–*n*-hexane to give **15** (4.5 mg). Subfraction FR8E10D was separated by CC eluting with 10% acetone–*n*-hexane to give 8 subfractions (FR8E10D1—FR8E10D8). Subfraction FR8E10D5 was further purified by CC and eluted with a gradient of CH_2Cl_2–*n*-hexane to give **8** (15.3 mg) and **9** (3.5 mg). Subfraction FR8E10E was separated by CC and eluted with a gradient of acetone–*n*-hexane to give 7 subfractions (FR8E10E1—FR8E10E7) and **12** (2.5 mg). Subfraction FR8E10F was separated by CC and eluted with a gradient of acetone–*n*-hexane to give 8 subfractions (FR8E10F1—FR8E10F8). Subfraction FR8E10F6 was further separated by CC and eluted with 60% $CHCl_3$–*n*-hexane to give 4 subfractions (FR8E10F6A—FR8E10F6D) and a mixture of compounds **10** and **14** (35.5 mg) which was further purified by CC on reversed-phase silica gel C-18 eluting with MeOH to give **10** (21.2 mg) and **14** (12.0 mg). Subfraction FR8E8 was separated by CC eluting with acetone–*n*-hexane to give **5** (7.5 mg). Subfraction FR8E9—FR8E11 were separated by CC and eluted with a gradient of acetone-n-hexane to give **11** (15.6 mg).

Pruniflorone K (**1**): Yellow viscous oil; $[\alpha]_D^{28}$ -18.2 (c=0.285, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 245 (4.63), 260 (4.56), 317 (4.37), 367 (3.73) nm; IR (KBr) ν_{max} 3338, 1647, 1617 cm^{-1}; HR-EI-MS *m/z*: $[M]^+$ 446.2092 (Calcd for $C_{28}H_{30}O_5$: 446.2093). EI-MS *m/z* (rel. int.): 446 $[M]^+$ (13), 363 (100), 295 (8), 149 (14), 83 (8), 69 (13). ^1H- ($CDCl_3$, 300 MHz) and ^{13}C- ($CDCl_3$, 75 MHz) NMR, see Table 1.

Pruniflorone L (**2**): Pale yellow powder, mp 259—260 °C; UV ($CHCl_3$) λ_{max} (log ε) 245 (4.05), 268 (4.19), 317 (3.81), 388 (3.39) nm; IR (KBr) ν_{max} 3421, 1637 cm^{-1}; HR-EI-MS *m/z*: $[M]^+$ 462.2408 (Calcd for $C_{29}H_{34}O_5$: 462.2406). EI-MS *m/z* (rel. int.): 462 $[M]^+$ (100), 419 (83), 407 (74), 393 (29), 337 (100), 323 (16), 305 (23) 369 (22), 137 (10), 69 (16). ^1H- ($CDCl_3$, 300 MHz) and ^{13}C- ($CDCl_3$, 75 MHz) NMR, see Table 2.

Antimicrobial Activity. Antibacterial Assay Only isolated compounds with sufficient amount from the roots of *C. formosum* ssp. *pruniflorum* were tested against both Gram-positive and Gram-negative bacteria (*i.e. Bacillus subtilis*, *Staphylococcus aureus* TISTR517, *Enterococcus faecalis* TISTR459, methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC43300, vancomycin-resistant *Enterococcus faecalis* (VRE) ATCC 51299, *Streptococcus faecalis*, *Salmonella typhi*, *Shigella sonei* and *Pseudomonas aeruginosa*). The tested micro-organisms were obtained from the culture collections, Department of Industrial Biotechnology and Department of Pharmacognosy and Botany, PSU, except for the TISTR and ATCC strains, which were obtained from Microbial Research Center (MIRCEN), Bangkok, Thailand. The antibacterial assay employed was the same as described in Boonsri *et al.*[8] Vancomycin, which was used as a standard, showed antibacterial activity of 75.0 μg/ml.

Antifungal Assay *Candida albicans* was obtained from Department of Pharmacognosy and Botany, PSU. The antifungal assay employed was the same as described in Boonsri *et al.*[8]

Acknowledgements We are greatly indebted to the Thailand Research Fund (TRF) for research grant (RSA5280033). Financial support from the Center of Excellence for Innovation in Chemistry (PERCH-CIC), Commission on Higher Education, Ministry of Education is gratefully acknowledged. NB thanks the Development and Promotion of Science and Technology Talents Project for the fellowship and the Graduate School, Prince of Songkla University for financial support. The Koshinocorporation Group, Japan is also acknowledged.

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