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Novel carotenoid pyropheophorbide A esters from abalone

Takashi Maoka [a,*], Tetsuji Etoh [b], Naoshige Akimoto [b], Hiroyuki Yasui [c]

[a] Research Institute for Production Development, 15 Shimogamo-morimoto-cho, Sakyo-ku, Kyoto 606-0805, Japan
[b] Graduate School of Pharmaceutical Sciences, Kyoto University, Yoshida-shimoadachi-cho, Sakyo-ku, Kyoto 606-8501, Japan
[c] Department of Analytical and Bioinorganic Chemistry, Kyoto Pharmaceutical University, Misasagi, Yamashina-ku, Kyoto 607-8414, Japan

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ABSTRACT

A series of carotenoid pyropheophorbide A esters, fucoxanthin pyropheophorbide A ester (**1**), halocynthiaxanthin 3′-acetate pyropheophorbide A ester (**2**), lutein pyropheophorbide A esters (**3**) and (**4**), and mutatoxanthin pyropheophorbide A ester (**5**), were isolated from the viscera of the abalone *Haliotis diversicolor aquatilis*. These structures were determined based on UV–vis, MS, and NMR spectroscopic data.

Since the first structural elucidation of β-carotene by Kuhn and Karrer in 1928–1930, about 750 naturally occurring carotenoids had been reported as of 2004.[1] Marine animals contain various carotenoids which show structural diversity.[2,3] Still interesting structural carotenoids can be found in marine animals.[2,3]

In the course of our studies on carotenoids in marine animals,[3] novel carotenoid pyropheophorbide A esters (Fig. 1) were isolated from the viscera of the abalone *Haliotis diversicolor aquatilis*. This Letter reports the isolation and structural elucidation of these compounds.

Viscera of abalone (100 g from 50 specimens) were extracted with Me_2CO. The Me_2CO extract was partitioned with hexane/Et_2O (1:1) and water. The hexane/Et_2O (1:1) layer was evaporated to dryness and chromatographed on silica gel using an increasing percentage of Et_2O in hexane. The fraction eluted with Et_2O/hexane (6:4) was subjected to HPLC on silica gel with Me_2CO/hexane (3:7) to yield a series of brown pigments: **1** (6.6 mg), **2** (1.0 mg), **3** (0.2 mg), **4** (0.2 mg), and **5** (0.2 mg).

Compound **1** showed absorption maxima at 226, 317, 411, 447, 469, 538, 608, and 666 nm.[4] This UV–vis spectrum resembled the additive UV–vis spectra of fucoxanthin[5] and pyropheophorbide A.[6] The molecular formula of **1** was determined as $C_{75}H_{90}O_8N_4$ by high-resolution (HR) FAB-MS.[4] The characteristic fragment ion at *m/z* 535.2719 corresponded to the pseudomolecularion (MH^+) of pyropheophorbide A ($C_{33}H_{35}O_3N_4$), suggesting the presence of a pyropheophorbide A moiety in **1** (Fig. 2). These data indicated that compound **1** consisted of fucoxanthin and pyropheophorbide A moieties. Both fucoxanthin and pyropheophorbide A structural moieties in **1** were characterized by the ^1H NMR and ^{13}C NMR spectra, including 2D NMR (COSY, ROESY, HSQC, and HMBC) experiments. NMR spectral data of **1**, assigned on the basis of the results of 2D NMR experiments and comparison with fucoxanthin[7] and pyropheophorbide A,[6] are compiled in Tables 1 and 2. NMR data revealed that the pyropheophorbide A and fucoxanthin moieties were linked with esterified bond. The ^1H NMR signal of H-3 (4.82 ppm) in the fucoxanthin moiety in **1**, which showed about 1 ppm downfield shift relative to the corresponding signal in fucoxanthin,[7] clearly indicated that the hydroxy group at C-3 in the fucoxanthin moiety was esterified with pyropheophorbide A. The key ROESY and HMBC correlations are shown in Figure 2. Therefore, the structure of brown pigment **1** was determined as fucoxanthin 3-pyropheophorbides A ester, as shown in Figure 1.

Compound **2** showed almost the same absorption spectrum as **1**.[8] The molecular formula of **2** was determined as $C_{75}H_{88}O_7N_4$ by HRFAB-MS.[8] The characteristic fragment ion at *m/z* 535 was also observed, indicating the presence of a pyropheophorbide A moiety. The ^1H NMR and ^{13}C NMR spectra (Tables 1 and 2) of **2**, assigned based on 2D NMR experiments, showed that compound **2** consisted of halocynthiaxanthin 3′-acetate[7] and pheophorbide A moieties with esterified linkage as shown in Figure 1. Therefore, the structure of **2** was determined as halocynthiaxanthin 3′-acetate pheophorbide A ester.

Both compounds **3** and **4** showed the same UV–vis spectra.[9,10] The molecular formula of both compounds was determined as $C_{73}H_{88}O_4N_4$ by HRFAB-MS.[9,10] The characteristic fragment ion at *m/z* 535 was also observed in both compounds. The ^1H NMR data (Tables 1 and 2) indicated the presence of lutein[7] and pheophorbide A moieties both in **3** and **4**. Detailed ^1H NMR analysis including decoupling, COSY and ROESY experiments revealed that the hydroxy group at C-3 at the β-end group of lutein was esterified with pheophorbide A in the case of **3**[11] and hydroxy group at the ε-end group in lutein was esterified with pheophorbide A in the case of **4**,[12] as shown in Figure 1.

Compound **5** showed absorption maxima at 226, 317, 411, 428, 457, 538, 608, and 666.[13] The molecular formula of **5** was determined as $C_{73}H_{88}O_5N_4$ by HRFAB-MS.[13] As well as in other compounds, the characteristic fragment ion at *m/z* 535 was also observed. The ^1H NMR (Tables 1 and 2) of **5** showed that compound

* Corresponding author. Tel.: +81 75 781 1107; fax: +81 75 781 1118.
E-mail address: maoka@mbox.kyoto-inet.or.jp (T. Maoka).

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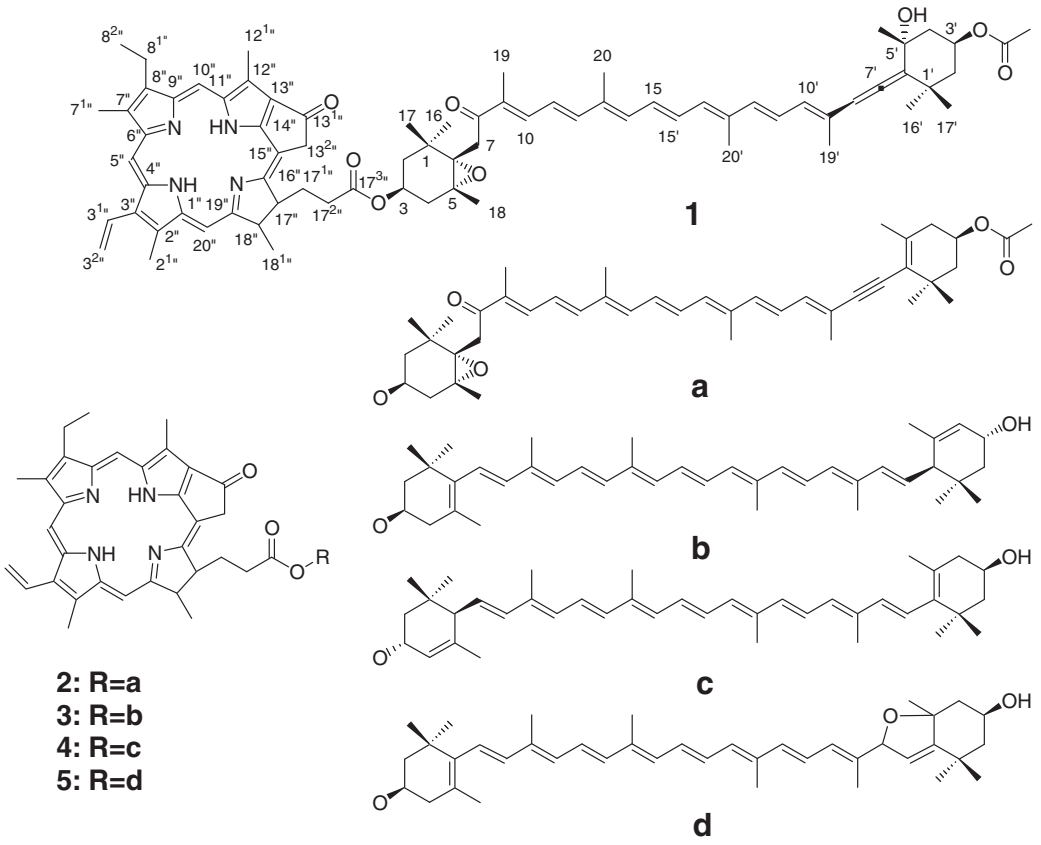

Figure 1. Structures of compounds **1**–**5**.

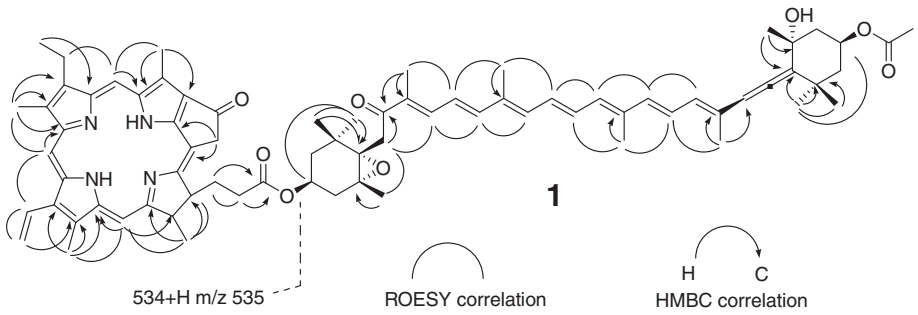

Figure 2. Key ROESY and HMBC correlations and the fragment ion of **1**.

Table 1
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of carotenoid parts of compounds **1** and **2** and ^{1}H NMR (500 MHz) of compounds **3**–**5** in $CDCl_3$

Position	**1**			**2**			**3**		**4**		**5**	
	δ 13C	δ^1H	mult. *J* (Hz)	δ 13C	δ^1H	mult. *J* (Hz)	δ^1H	mult. *J* (Hz)	δ^1H	mult. *J* (Hz)	δ^1H	mult. *J* (Hz)
1	35.1			35.1								
2	42.8	α 1.37	Overlapped	42.8	α 1.37	Overlapped	α 1.60	Overlapped	α 1.36	dd (14,7)	α 1.60	Overlapped
2		β 1.21	dd (13, 12)		β 1.21	dd (13, 12)	β 1.39	Overlapped	β 1.77	dd (14, 6)	β 1.39	Overlapped
3	68.0	4.82	m	68.0	4.82	m	5.00	m	5.26	m	5.00	m
4	37.6	α 2.27	Overlapped	37.6	α 2.27	Overlapped	α 2.53	Overlapped	5.34	br s	α 2.53	Overlapped
4		β 1.74	Overlapped		β 1.74	Overlapped	β 1.98	Overlapped			β 1.98	Overlapped
5	67.0			67.0								
6	65.8			65.8					2.34	d (10)		
7	40.6	2.56	d (17)	40.6	2.56	d (17)	6.03	d (18)	5.38	dd (15, 10)	6.03	d (18)
7		3.60	d (17)		3.60	d (17)						
8	197.5			197.5			6.09	d (18)	6.09	d (15)	6.09	d (18)
9	134.4			134.4								
10	139.0	7.10	d (11.5)	139.0	7.10	d (11.5)	6.14	d (11)	6.14	d (10)	6.14	d (11)
11	125.6	6.45	dd (15, 11.5)	125.6	6.45	dd (15, 11.5)	~6.62	Overlapped	~6.62	Overlapped	~6.62	Overlapped

(*continued on next page*)

Table 1 (*continued*)

Position	**1**			**2**			**3**		**4**		**5**	
	δ 13C	δ^1H	mult. *J* (Hz)	δ 13C	δ^1H	mult. *J* (Hz)	δ^1H	mult. *J* (Hz)	δ^1H	mult. *J* (Hz)	δ^1H	mult. *J* (Hz)
12	149.0	6.63	d (15)	149.0	6.63	d (15)	6.36	d (15)	6.37	d (15)	6.36	d (15)
13	136.1			136.1								
14	136.6	6.40	d (11)	136.6	6.40	d (11)	6.25m		6.25	m	6.25m	
15	129.4	6.60	dd (14, 11)	129.4	6.60	dd (14, 11)	~6.62	Overlapped	~6.62	Overlapped	~6.62	Overlapped
16	24.6	0.99	s	24.6	0.99	s	1.03	s	1.07	s	1.03	s
17	27.6	0.83	s	27.6	0.83	s	0.96	s	0.80	s	0.96	s
18	21.4	1.14	s	21.4	1.14	s	1.65	s	1.60	s	1.65	s
19	11.7	1.89	s	11.7	1.89	s	1.94	s	1.88	s	1.94	s
20	13.0	1.98	s	13.0	1.98	s	1.96	s	1.96	s	1.96	s
1′	35.7			36.1								
2′	45.2	α 1.99	Overlapped	42.3	α 1.83	ddd (12, 3, 1.5)	α 1.37	dd (14,7)	α 1.77	dd(12, 3)	α 1.51	Overlapped
2′		β 1.41	dd (12.5, 7)		β 1.57	dd (12, 12)	β 1.85	dd (14, 6)	β 1.48	dd (12, 12)	β 1.76	Overlapped
3′	67.9			68.0	5.04	m	4.25	m	4.00	m	4.25	m
4′	45.4	α 2.27	Overlapped	37.50	α 2.49	ddd(18,5,1.5)	5.55	br s	α 2.39	Overlapped	α 2.12	Overlapped
4′		β 1.51	dd (13, 12.5)		β 2.13	dd (18, 10)			β 2.04	dd (18, 10)	β 1.99	Overlapped
5′	72.7			137.0								
6′	117.5			124.3			2.41	d (10)				
7′	202.3			88.8			5.46	dd (15, 10)	6.10	d (16)	5.23	s
8′	103.3	6.05	s	98.7			6.14	d (15)	6.15	d (16)	5.17	s
9′	132.5			119.3								
10′	128.3	6.31	d (11.5)	135.1	6.45	d (11)	6.14	d (11)	6.15	d (11)	6.19	d (11)
11′	125.6	6.64	dd (15, 11.5)	124.3	6.52	dd (15, 11)	~6.62	Overlapped	~6.64	Overlapped	6.59	dd (15, 11)
12′	137.8	6.35	d (15)	138.0	6.37	d (15)	6.37	d (15)	6.36	d (15)		
13′	139.0			136.6								
14′	131.6	6.26	d (11)	135.5	6.29	m	6.25	m	6.25	m		
15′	132.1	6.74	dd (14, 11)	130.7	6.64	m	~6.62	Overlapped	~6.62	Overlapped		
16′	29.2	1.38	s	28.7	1.18	s	1.00	s	1.07	s	1.17	s
17′	32.1	1.07	s	30.2	1.20	s	0.85	s	1.07	s	1.33	s
18′	31.3	1.35	s	22.4	1.92	s	1.63	s	1.74	s	1.62	s
19′	14.0	1.81	s	13.0	2.01	s	1.92	s	1.97	s	1.71	s
20′	12.9	1.98	s	18.6	1.97	s	1.99	s	1.97	s	1.94	s
3-$COCH_3$	170.4			170.7								
3-$COCH_3$	20.8	2.04	s	21.6	2.04	s						

s: Singlet, d: doublet, t triplet, m: multiplet, br s: broad singlet.

Table 2
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of pyropheophorbide A part of compounds **1** and **2** and ^{1}H NMR (500 MHz) of compounds **3**–**5** in $CDCl_3$

Position	δ ^{13}C	δ^1H	mult. *J* (Hz)	Position	δ ^{13}C	δ^1H	mult. *J* (Hz)
1″	141.5			12″	128.5 or 130.5		
2″	132.4			12^{1}″	12.5	H_3 3.68	s
2^{1}″	12.7	H_3 3.42	s	13″	128.5 or 130.5		
3″	136.1or 137.8			13^{1}″	193.6		
3^{1}″	128.3	8.00	dd (17, 11.5)	13^{2}″	48.1	5.27	d (20)
3^{2}″	125.5	6.29	d (17)	13^{2}″		5.11	d (20)
3^{2}″		6.18	d (11.5)	14″	106.4 or 150.7		
4″	136.1 or 137.8			15″	106.4 or150.7		
5″	97.1	9.41	s	16″	160.4		
6″	137.1			17″	51.5	4.31	m
7″	155.2			17^{1}″	29.7	2.34	m
7^{1}″	12.1	H_3 3.26	s	17^{1}″		2.70	m
8″	144.9			17^{2}″	31.3	2.20	m
8^{1}″	19.5	H_3 3.71	q (7.5)	17^{2}″		2.48	m
8^{2}″	17.5	H_3 1.70	t (7.5)	17^{3}″	171.8		
9″	138.3			18″	49.9	4.50	m
10″	104.1	9.51	s	18^{1}″	23.2	1.81	d (6)
11″	139.0			19″	172.5		
				20″	93.0	8.57	s

s: Singlet, d: doublet, t: triplet, m: multiplet.

5 consisted of mutatoxanthin[7] and pheophorbide A moieties with esterified linkage as shown in Figure 1.

The major food sources of abalone are macroalgae such as brown and red algae, which contain fucoxanthin and lutein as major carotenoids, respectively.[14] Abalone accumulate fucoxanthin and lutein in the viscera from dietary algae. Halocynthiaxanthin 3′-acetate is a metabolite of fucoxanthin in shellfish.[2,3] Mutaoxanthin is derived from antherxanthin under acidic conditions.[15] Pyropheophorbide A is a metabolite of chlorophyll A in the viscera of abalone.[16] Compounds **1**–**5** might be formed from carotenoids and pyropheophorbide A by esterase in the abalone viscera. It is well-known that pyropheophorbide A is a photosensitizer and generates singlet oxygen ($^1O_2$) from ground-state molecular oxygen in the presence of light.[16] On the other hand, carotenoids are excellent quenchers of $^1O_2$ and prevent photooxidation.[17] Therefore, it is interesting that compounds acting as singlet oxygen generators and quenchers are linked with esterified bonds.

To investigate whether $^1O_2$ is generated from a porphyrin (pyropheophorbide A), and whether the compounds such as an $^1O_2$ quencher (fucoxanthin) and carotenoid pyropheophorbide A ester

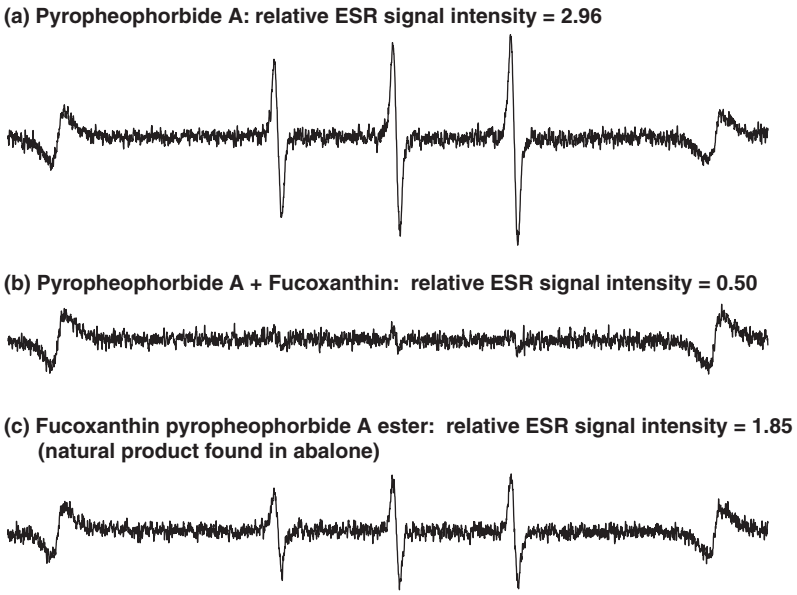

Figure 3. ESR spectra due to the singlet oxygen adducts of TMPD (4-oxo-TEMPO) derived from (a) pyropheophorbide A, (b) pyropheophorbide A and fucoxanthin, and (c) **1** at 30 s after UVA exposure.

(**1**) react with $^1O_2$ chemically, we examined the $^1O_2$ quenching activities of free and conjugated fucoxanthin in the chemical systems employing the ESR spin-trapping method.[18] $^1O_2$ was generated in the pyropheophorbide A solution under UVA irradiation. The signal intensity due to 4-oxo-TEMPO (2.96) decreased following additions of free (0.50) and conjugated (1.85) fucoxanthin in a structure-dependent manner, indicating that free fucoxanthin reacted chemically with $^1O_2$. However, fucoxanthin conjugated with pyropheophorbide A had a partial effect, as shown in Figure 3.

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4. *Compound* **1**: UV–vis (Et_2O) nm (ε) 226 (20,000), 317 (18,000), 411 (84,000), 447 (63,000), 469 shoulder (53,000), 538 (7000), 608 (5000), 666 (30,000); HRFAB-MS *m*/*z* 1175.6827 (MH^+) $C_{75}H_{91}O_8N_4$, calcd for 1175.6837, fragment ion at *m*/*z* 535.2719 $C_{33}H_{35}O_3N_4$, calcd for 535.2709; ^1H NMR and ^{13}C NMR (Tables 1 and 2); CD (Et_2O) nm ($\Delta\varepsilon$), 240 (−1.0), 265 (−0.8), 296 (−3.2), 320 (−0.2), 340 (−1.5).
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8. *Compound* **2**: UV–vis (Et_2O) nm (ε) 226 (20,000), 317 (18,000), 411 (84,000), 447 (63,000), 469 shoulder (53,000), 538 (7,000), 608 (5,000), 666 (30,000); HRFAB-MS *m*/*z* 1157.6709 (MH^+) $C_{75}H_{89}O_7N_4$, calcd for 1157.6731, fragment ion at *m*/*z* 535.2730 $C_{33}H_{35}O_3N_4$, calcd for 535.2709; ^1H NMR and ^{13}C NMR (Tables 1 and 2); CD (Et_2O) nm ($\Delta\varepsilon$),240 (−1.0), 265 (−0.8), 296 (−3.2), 320 (−0.2), 340 (−1.5).
9. *Compound* **3**: UV–vis (Et_2O) nm 226, 317, 411, 445, 474, 538, 608, 666; HRFAB-MS *m*/*z* 1085.6864 (MH^+) $C_{73}H_{89}O_4N_4$, calcd for 1085.6884, ^1H NMR (Tables 1 and 2).
10. *Compound* **4**: UV–vis (Et_2O) nm 226, 317, 411, 445, 474, 538, 608, 666; HRFAB-MS *m*/*z* 1085.6867 (MH^+) $C_{73}H_{89}O_4N_4$, calcd for 1085.6884, ^1H NMR (Tables 1 and 2).
11. The esterified oxy methine signal at C-3 in **3** was observed at 5.00 ppm, which was assigned by oxy methine signal at C-3 in the β-end group in lutein by COSY and ROESY experiments and showed about 1 ppm downfield shift relative to the corresponding signal in lutein.[7] This clearly indicated that the hydroxy group at C-3 in the β-end group in lutein moiety was esterified with pyropheophorbide A in the case of **3**. Key COSY correlations H-2α/H-3, H-2β/H-3, H-4α/H-3, H-4β/H-3; key ROESY correlations H-2α/H-3, H-4α/H-3, H-16/H-3.
12. The esterified oxy methine signal at C-3 in **4** was observed at 5.26 ppm, which was assigned by oxy methine signal at C-3 in the ε-end group in lutein by COSY and ROESY experiments and showed about 1 ppm downfield shift relative to the corresponding signal in lutein.[7] This clearly indicated that the hydroxy group at C-3 in the ε-end group in lutein moiety was esterified with pyropheophorbide A in the case of **4**. Key COSY correlations H-2α/H-3, H-2β/H-3, H-4/H-3; key ROESY correlations H-2α/H-3, H-16/H-3.
13. *Compound* **5**: UV–vis (Et_2O) nm 226, 317, 411, 428, 457, 538, 608, 666; HRFAB-MS *m*/*z* 1101.6848 (MH^+) $C_{73}H_{89}O_5N_4$, calcd for 1101.6833, ^1H NMR (Tables 1 and 2); key COSY correlations H-2α/H-3, H-2β/H-3, H-4α/H-3, H-4β/H-3; key ROESY correlations H-2α/H-3, H-4α/H-3, H-16/H-3. The esterified oxy methine signal at C-3 in **5** was observed at 5.00 ppm, which was assigned by oxy methine signal at C-3 in the β-end group in mutatoxanthin by COSY and ROESY experiments.
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18. In Figure 3, the reaction mixture contained 50 mM TMPD, (a) 62.5 μM pyropheophorbide A, (b) 62.5 μM pyropheophorbide A and 62.5 μM fucoxanthin, and (c) 62.5 μM **1** in a total volume of 0.2 mL of 50 mM phosphate buffer (pH 7.5) and ethanol (1:1, v/v).