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Reaction of Astaxanthin with Peroxynitrite

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The *in vitro* reactivities of astaxanthin toward peroxynitrite were investigated and the reaction products after scavenging with peroxynitrite were analyzed in order to determine the complete mechanism of this reaction. A series of carotenoids, 13-apo-astaxanthinone (1), 12′-apo-15′-nitroastaxanthinal (2), 12′-apo-astaxanthinal (3), 10′-apo-astaxanthinal (4), 9-*cis*-14′-s-*cis*-15′-nitroastaxanthin (5), 14′-s-*cis*-15′-nitroastaxanthin (6), 13-*cis*-14′-s-*cis*-15′-nitroastaxanthin (7), 10′-s-*cis*-11′-*cis*-11′-nitroastaxanthin (8), 13,15,13′-tri-*cis*-15′-nitroastaxanthin (9), 9-*cis*-astaxanthin (10), and 13-*cis*-astaxanthin (11), were isolated from the reaction products of carotenoids with peroxynitrite. Our previous studies achieved for the first time the isolation of nitro derivatives from the reaction of astaxanthin with peroxynitrite. Here we identify the major remaining reaction products of this reaction and investigate the stabilities of the nitro astaxanthins.

Key words: astaxanthin; carotenoids; peroxynitrite; antioxidative compounds

Peroxynitrite, a reaction product of superoxide and nitric oxide, is a strong oxidant and has potential to induce several types of biological damage. It is produced by macrophages and neutrophils and can induce DNA strand scission, protein modification by nitration and hydroxylation, and membrane lipid peroxydation in the LDL.[1,2)]

Carotenoids might play an important role *in vivo* in scavenging peroxynitrite, particularly inside the LDL and in the hydrophobic environment. We have reported new modes of reaction exhibited by peroxynitrite, including reaction of peroxynitrite by lycopene and retinol *in vitro*[3,4)] and the consecutive reaction products and mechanisms involved. Astaxanthin found as a common pigment in algae, crustaceans, fish, and birds, has been reported to be more effective than β-carotene or lycopene in preventing lipid peroxidation in solution and in various biomembrane systems.[5)] Furthermore, it possesses a different structure than lycopene, containing ionone rings and number of oxygen atoms, and may yield new reaction products. Considering this, we have concentrated our research on the reactivity of astaxanthin with peroxynitrite and its reaction products. Our recent studies on astaxanthin and β-carotene showed that these carotenoids might undergo nitration reactions to form nitro derivatives.[6)] In this study, we investigated all major reaction products of reaction of astaxanthin with peroxynitrite.

Materials and Methods

Positive-ion FAB-MS spectra were recorded on a JEOL JMX HX 101A mass spectrometer (JEOL, Tokyo) using *m*-nitrobenzyl alcohol as a matrix. ^{1}H (500 MHz) and ^{13}C NMR (125 MHz) were recorded on a Varian Unity Inova 500 spectrometer in $CDCl_3$ using TMS as an internal standard. 2D NMR (COSY, NOESY, HSQC, and HMBC) were acquired using standard pulse program Varian 6.1 A. The HPLC conditions were C30, Develosil C30-UG-5 Φ200 × 250 mm, 82% MeCN, column; C30, Develosil C30-UG-5 Φ4.6 × 250 mm, 82 or 75% MeCN, flow rate, 1 ml/min, column temperature, 40 °C; detection range, 200–600 nm. Standard astaxanthin was purchased from Sigma-Aldrich Japan (Shinagawa, Japan).

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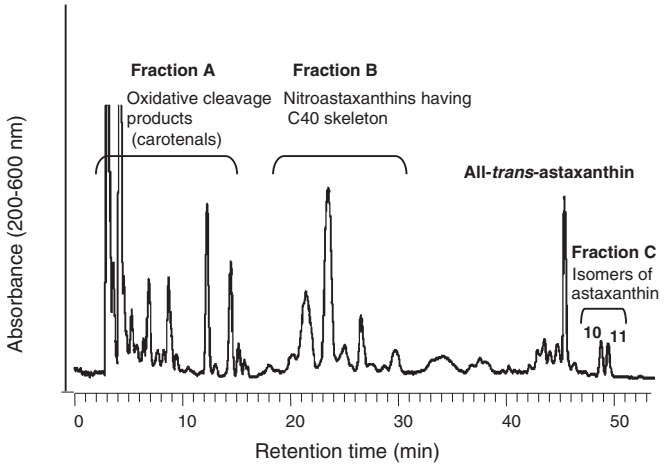


Fig. 1. HPLC Chromatogram of Peroxynitrite Reaction of Astaxanthin.
HPLC conditions: Develosil C30-UG-5 ϕ4.6 × 250 mm, MeCN:H_2O = 82:18; flow rate, 1 ml/min; column temperature, 40 °C; detection range, 200–600 nm.

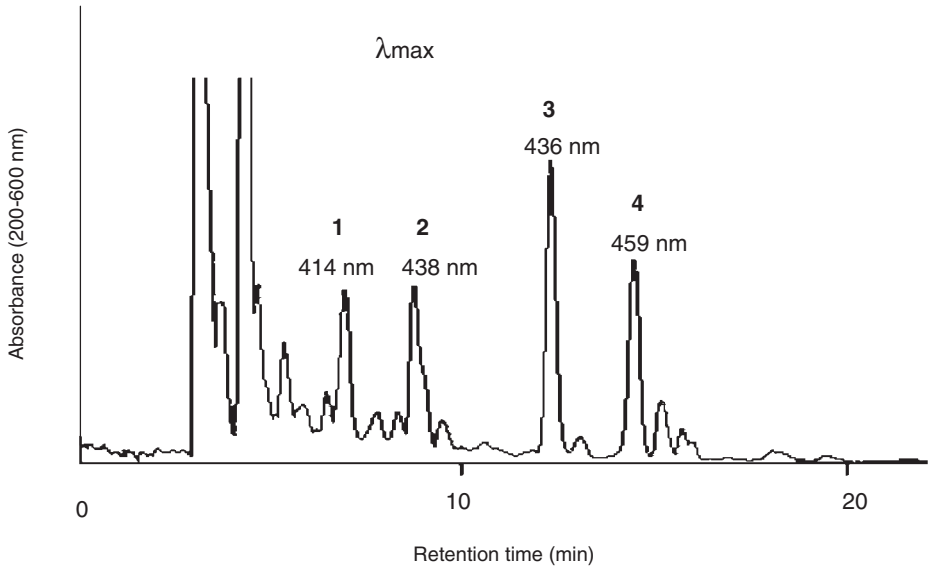


Fig. 2. Chromatographic Purification of Fraction A.
HPLC conditions: Develosil C30-UG-5 ϕ4.6 × 250 mm, MeCN:H_2O = 75:25; flow rate, 1 ml/min; column temperature, 40 °C; detection range, 200–600 nm.

Other reagents were purchased from Wako Pure Chemical Industries (Osaka, Japan). Peroxynitrite was prepared according to a method described in the literature.[7] All-*trans*-astaxanthin was reacted with peroxynitrite, and the reaction products were analyzed by HPLC. The reaction procedure was performed following a procedure described in our previous studies.[6]

Results and Discussion

On HPLC analysis, three main groups of reaction products were observed, fraction A (*Rt* 0–16 min), fraction B (*Rt* 16–32 min), and fraction C (*Rt* 48–52 min), as shown in Fig. 1. The peaks in fraction A were observed to be of lower λ_{max} than fraction B or C, indicating the carotenal class of the compounds. The fraction B compounds contained mainly nitroastaxanthins having the C-40 skeleton. The compounds in fraction C were 9-*cis*-astaxanthin and 13-*cis*-astaxanthin identified by comparison of the absorption spectra and HPLC retention times with the published values.[8]

In this study, on further separation of fractions A, B, and C, altogether 11 reaction products were identified as 13-apoastaxanthinone (**1**), 12′-apo-15′-nitro-astaxanthinal (**2**), 12′-apoastaxanthinal (**3**), 10′-apoastaxanthinal (**4**) from the fraction A (Figs. 1, 2 and 3), 9-*cis*-14′-s-*cis*-15′-nitroastaxanthin (**5**), 14′-s-*cis*-15′-nitroastaxanthin (**6**),[6] 13-*cis*-14′-s-*cis*-15′-nitroastaxanthin (**7**), 10′-s-*cis*-11′-*cis*-11′-nitroastaxanthin (**8**),[6] 13,15,13′-tri-*cis*-15′-nitroastaxanthin (**9**) from the fraction B (Figs. 1, 4

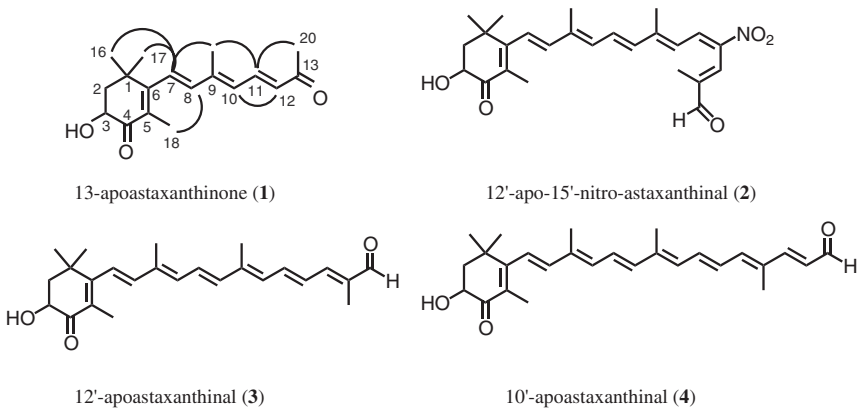


Fig. 3. Structures of the Compounds Isolated from Fraction A. (∩NOE)

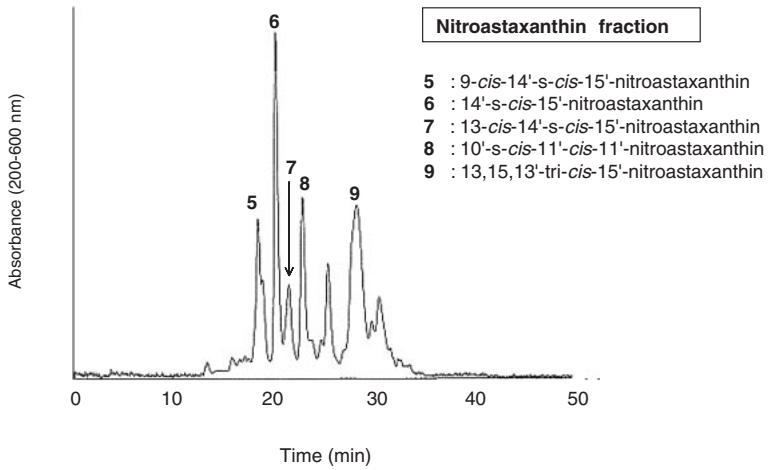


Fig. 4. Chromatographic Purification of Fraction B.
HPLC conditions: Develosil C30-UG-5 ϕ4.6 × 250 mm, MeCN:H_2O = 75:25; flow rate, 1 ml/min; column temperature, 40 °C; detection range, 200–600 nm.

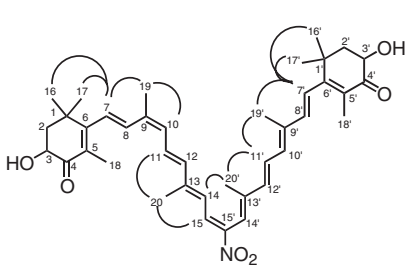
and 5) and 9-*cis*-astaxanthin (**10**), and 13-*cis*-astaxanthin (**11**) from fraction C (Fig. 1) by MS, UV-VIS, ^{1}H, and ^{13}C NMR data and by comparison with authentic samples. Among these, we report for the first time the identification of **1**, **2**, **3**, **4**, **5**, **7**, and **9** as major products of the reaction between astaxanthin and peroxynitrite. Detailed structural determination of all the new isolated compounds with their respective spectrometric analysis data explained below.

13-Apoastaxanthinone (**1**), yield 0.8 mg, UV-Vis λ_{max} (CH_3CN) nm 414, FAB MS m/z 288 (M^+). Compound **1** showed a molecular ion peak at m/z 288, compatible with the molecular formula of $C_{18}H_{24}O_3$. ^{1}H NMR data (Table 1), assigned by 2D NMR, were in agreement with the structure of 13-apoastaxanthinone.

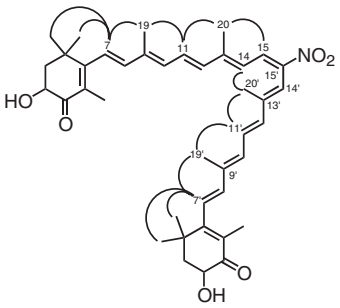
12′-Apo-15′-nitro-astaxanthinal (**2**), yield 3.3 mg, UV-Vis λ_{max} (CH_3CN) nm 438; high resolution (HR) FAB-MS calcd for $C_{25}H_{31}O_5N + H^+$ 426.2280: found 426.2271. Compound **2** showed the molecular formula of $C_{25}H_{31}O_5N$, compatible with the structure of nitro-substituted C_{25}-apoastaxanthinal. The ^{1}H NMR signal of 9.71 ppm (H-12′, s) indicated the presence of an aldehyde group in **2**. Disappearance of the H-15′ signal in **2** clearly indicated that the nitro group was replaced at the C-15′ position. The remarkable down field shift of H-15 (8.25 ppm), H-20 (2.18 ppm), and high field shift of H-20′ (1.72 ppm), H-14 (5.92 ppm) were in agreement with a 14′-s-*cis*-15′-nitro structure.[6] The remarkable down field shift of H-14′ (7.83 ppm) might have been due to the de-shielding effect of the aldehyde group at C-12′. Thus, the structure of **2** was determined to be 12′-apo-15′-nitro-astaxanthinal.

12′-Apoastaxanthinal (**3**), yield 1.6 mg, UV-Vis λ_{max} (CH_3CN) nm 436, FAB MS m/z 380 (M^+). Compound **3** was identified as 12′-apoastaxanthinal by comparison of FAB MS and ^{1}H NMR (Table 1) and ^{13}C NMR data (Table 2) with the published values.[9]

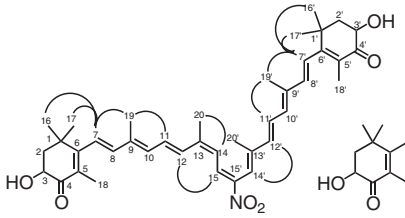
10′-Apoastaxanthinal (**4**), yield 2.0 mg, UV-Vis λ_{max} (CH_3CN) nm 459, FAB MS m/z 406 (M^+). Compound **4** showed a molecular ion peak at m/z 406 compatible with the molecular formula of $C_{27}H_{34}O_3$. ^{1}H NMR (Table 1) and ^{13}C NMR data (Table 2), assigned by 2D NMR, were in agreement with the structure of 10′-apoastaxanthinal.



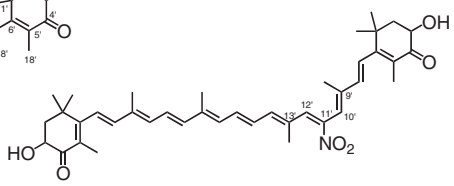
9-*cis*-14'-s-*cis*-15'-nitroastaxanthin (**5**)



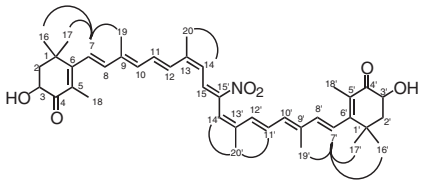
14'-s-*cis*-15'-nitroastaxanthin (**6**)



13-*cis*-14'-s-*cis*-15'-nitroastaxanthin (**7**)



10'-s-*cis*-11'-*cis*-11'-nitroastaxanthin (**8**)



13,15,13'-tri-*cis*-15'-nitroastaxanthin (**9**)

Fig. 5. Structures of the Compounds Isolated from Fraction B. (∩NOE)

Table 1. ^{1}H NMR Spectral Data for Apo-Astaxanthins

Number	1	2	3	4
	^{1}H δ (ppm)	^{1}H δ (ppm)	^{1}H δ (ppm)	^{1}H δ (ppm)
2α	2.16 (1H, dd, J = 13.0, 6.0)	2.16 (1H, dd, J = 13.0, 6.0)	2.16 (1H, dd, J = 13.0, 6.0)	2.16 (1H, dd, J = 13.0, 6.0)
2β	1.82 (1H, t, J = 13.0)	1.82 (1H, t, J = 13.0)	1.82 (1H, t, J = 13.0)	1.82 (1H, t, J = 13.0)
3	4.33 (1H, dd, J = 13.0, 6.0)	4.34 (1H, dd, J = 13.0, 6.0)	4.33 (1H, dd, J = 13.0, 6.0)	4.33 (1H, dd, J = 13.0, 6.0)
7	6.31 (1H, d, J = 15.0)	6.37 (1H, d, J = 16.0)	6.26 (1H, d, J = 16.0)	6.24 (1H, d, J = 15.0)
8	6.40 (1H, d, J = 15.0)	6.40 (1H, d, J = 16.0)	6.42 (1H, d, J = 16.0)	6.42 (1H, d, J = 15.0)
10	6.32 (1H, d, J = 11.5)	6.30 (1H, d, J = 11.0)	6.31 (1H, d, J = 11.0)	6.31 (1H, d, J = 11.0)
11	7.23 (1H, dd, J = 15.5, 11.5)	7.03 (1H, dd, J = 15.0, 11.0)	6.78 (1H, dd, J = 15.5, 11.0)	6.74 (1H, dd, J = 15.0, 11.0)
12	7.81 (1H, d, J = 15.5)	6.48 (1H, d, J = 15.0)	6.46 (1H, d, J = 15.5)	6.46 (1H, d, J = 15.0)
14		5.92 (1H, d, J = 13.0)	6.35 (1H, d, J = 11.5)	6.65 (1H, d, J = 12.0)
15		8.25 (1H, d, J = 13.0)	7.03 (1H, dd, J = 15.5, 11.5)	6.86 (1H, dd, J = 12.0, 12.0)
16	1.20 (3H, s)	1.20 (3H, s)	1.21 (3H, s)	1.21 (3H, s)
17	1.32 (3H, s)	1.32 (3H, s)	1.33 (3H, s)	1.32 (3H, s)
18	1.92 (3H, s)	1.93 (3H, s)	1.94 (3H, s)	1.94 (3H, s)
19	2.07 (3H, s)	2.07 (3H, s)	2.03 (3H, s)	2.02 (3H, s)
20	2.45 (3H, s)	2.18 (3H, s)	2.00 (3H, s)	2.03 (3H, s)
10′				9.60 (1H, d, J = 9.0)
11′				6.21 (1H, dd, J = 15.0, 9.0)
12′		9.71 (1H, s)	9.47 (1H, s)	7.16 (1H, d, J = 15.0)
14′		7.83 (1H, s)	6.96 (1H, d, J = 12.5)	6.34 (1H, d, J = 12.0)
15′			6.73 (1H, dd, J = 15.5, 12.5)	6.63 (1H, dd, J = 12.0, 12.0)
20′		1.72 (3H, s)	1.89 (3H, s)	1.98 (3H, s)

Table 2. ^{13}C NMR Spectral Data for Apo-Astaxanthins

Number	3	4
	δ (ppm)	δ (ppm)
1	36.8	36.8
2	45.4	45.4
3	69.2	69.2
4	200.4	200.4
5	127.0	127.0
6	162.0	162.1
7	124.2	126.0
8	141.9	142.1
9	137.4	134.3
10	134.5	135.6
11	126.8	123.9
12	141.1	139.2
13	138.8	139.4
14	137.3	142.1
15	132.2	132.9
16	26.1	26.1
17	30.7	30.7
18	14.0	14.0
19	12.7	12.9
20	13.0	12.8
10′		193.7
11′		127.4
12′	194.4	156.3
13′	136.1	134.8
14′	148.5	134.9
15′	128.2	129.3
20′	9.6	12.6

9-*cis*-14′-s-*cis*-15′-nitroastaxanthin (**5**), yield 0.5 mg, UV-Vis λ_{max} (Et_2O) nm 275, 374, 460. Compound **5** showed a molecular ion peak at m/z 641 compatible with the molecular formula of $C_{40}H_{51}O_6N$.

13-*cis*-14′-s-*cis*-15′-Nitroastaxanthin (**7**), yield 1.5 mg, UV-Vis λ_{max} (Et_2O) nm 366, 462. Compound **7** showed a molecular ion peak at m/z 641 compatible with the molecular formula of $C_{40}H_{51}O_6N$.

13,15,13′-tri-*cis*-15′-Nitroastaxanthin (**9**), yield 3.0 mg, UV-Vis λ_{max} (Et_2O) nm 364, 476; high resolution (HR) FAB-MS calcd for $C_{40}H_{51}O_6N + H^+$ 642.3795 found 642.3786. The ^1H NMR and ^{13}C NMR data for these compounds are compiled in Tables 3 and 4 respectively. Compounds **5**, **7**, and **9** possessed the same molecular formula as $C_{40}H_{51}O_6N$, compatible with the structure of nitroastaxanthin. Disappearance of the H-15′ signal as compared with astaxanthin clearly indicated substitution of the nitro group at C-15′ in **5**, **7**, and **9**. The stereochemistry of the compounds were determined by NOESY data. The NOEs between H-19 and H-10 and between H-20′ and H-14 indicated a 9-*cis*-14′-s-*cis* configuration for compound **5**, as shown in Fig. 5. Similarly, a 13-*cis*-14′-s-*cis* configuration for **7** is proposed based on the NOEs between H-20 and H-14 and between H-12 to H-15. A 13, 13′-*cis* configuration for **9** is proposed based on the NOEs between H-20′ and H-14 and between H-20 and H-14′. Furthermore, the high field shift of the H-15 signal (6.29 ppm) of **9**, as compared with **5** (8.06 ppm) and **7** (8.20 ppm), indicated that H-15 and the nitro group were located in the *E* configuration at the double bond of C15. Hence a 13, 15, 13′-tri-*cis* configuration is proposed for compound **9**.

The above results indicate that nitroastaxanthins were the major products of the reaction of astaxanthin with peroxynitrite. Furthermore, of the above isolated compounds, 12′-apo-15′-nitro-astaxanthinal (**2**) was observed to be an oxidation product containing a nitro group in its structure. This result suggests that the nitro carotenoids might have undergone a further reaction with peroxynitrite to yield compound **2**.

In order to check the validity of the above assumption as well as the stability of the nitroastaxanthins, we further treated the major high yield nitroastaxanthins *viz.*, **6**, **7**, **8**, and **9**, with peroxynitrite. Compound **5** was obtained in small quantity and hence was not a subject in this investigation. The reaction procedure was maintained as described in our previous report.[6)]

On reaction with peroxynitrite, compounds **7**, **8**, and **9** were further transformed into other products, such as oxidation products and *cis* isomers of nitroastaxanthin, when analyzed on the HPLC chromatogram. However, compound **6** did not react further with peroxynitrite, and a peak having a similar retention time and UV-*vis* λmax value was observed on HPLC, indicating the stability of **6** (Fig. 6). The new transformed reaction products resulting from the reactions of **7**, **8**, and **9** with peroxynitrite could not be purified for structural investigation due to a very small reaction yield and the complicated nature of the peaks. However, as shown in the Fig. 6, compound **6** emerged as the most stable and high yield major reaction product. In our continued research, we intend to clarify the structures of the new transformed compounds by performing the reactions in high initial amounts.

Conclusion

We investigated the *in vitro* reactivities of astaxanthin toward peroxynitrite, and analyzed the reaction products after scavenging with peroxynitrite in order to determine the complete mechanism of this reaction. A series of carotenoids, 13-apoastaxanthinone (**1**), 12′-apo-15′-nitro-astaxanthinal (**2**), 12′-apoastaxanthinal (**3**), 10′-apoastaxanthinal (**4**), 9-*cis*-14′-s-*cis*-15′-nitroastaxanthin (**5**), 14′-s-*cis*-15′-nitroastaxanthin (**6**), 13-*cis*-14′-s-*cis*-15′-nitroastaxanthin (**7**), 10′-s-*cis*-11′-*cis*-11′-nitroastaxanthin (**8**), 13,15,13′-tri-*cis*-15′-nitroastaxanthin (**9**), 9-*cis*-astaxanthin (**10**), and 13-*cis*-astaxanthin (**11**), were isolated from the reaction products of carotenoids with peroxynitrite. Among these, compounds **1**, **2**, **3**, **4**, **5**, **7**, and **9** were identified for the first time as major products of this reaction.

HPLC analysis revealed that the nitroastaxanthins (**7**, **8**, and **9**) on further reaction with peroxynitrite were transformed into oxidation products, but attachment of the nitro group to the oxidation products, as observed in compound **2** could not be confirmed due to very small

Table 3. ^{1}H NMR Spectral Data for Nitro-Astaxanthins

Number.	5	6	7	8	9
	δ (ppm)	δ (ppm)	δ (ppm)	δ (ppm)	δ (ppm)
2α	2.17* (1H)	2.16 (1H, dd, J = 13, 6)	2.16* (1H)	2.16 (1H, dd, J = 13, 6)	2.16 (1H, dd, J = 13, 6)
2β	1.83 (1H, t, J = 13)	1.82 (1H, t, J = 13)	1.82 (1H, t, J = 13)	1.82 (1H, t, J = 13)	1.82 (1H, t, J = 13)
3	4.33 (1H, dd, J = 13, 6)	4.33 (1H, dd, J = 13, 6)	4.34 (1H, dd, J = 13, 6)	4.33 (1H, dd, J = 13, 6)	4.33 (1H, dd, J = 13, 6)
7	6.32 (1H, d, J = 16)	6.27 (1H, d, J = 17)	6.31 (1H, d, J = 16)	6.26 (1H, d, J = 16)	6.25 (1H, d, J = 16)
8	6.92 (1H, d, J = 16)	6.42 (1H, d, J = 17)	6.43 (1H, d, J = 16)	6.42 (1H, d, J = 16)	6.41 (1H, d, J = 16)
10	6.23 (1H, d, J = 12)	6.28 (1H, d, J = 12)	6.28 (1H, d, J = 11)	6.30 (1H, d, J = 12)	6.29 (1H, d, J = 16)
11	6.97 (1H, dd, J = 15, 12)	6.94 (1H, dd, J = 15, 12)	6.94 (1H, dd, J = 15.5, 11)	6.77 (1H, dd, J = 15.5, 11)	6.74 (1H, dd, J = 15, 11)
12	6.40 (1H, d, J = 15)	6.47 (1H, d, J = 15)	7.06 (1H, d, J = 15.5)	6.45 (1H, d, J = 15)	6.43 (1H, d, J = 15)
14	5.99 (1H, d, J = 13)	6.00 (1H, d, J = 13)	5.88 (1H, d, J = 13)	6.33 (1H, d, J = 12)	6.51 (1H, d, J = 11)
15	8.06 (1H, d, J = 13)	8.06 (1H, d, J = 13)	8.20 (1H, d, J = 13)	6.89 (1H, dd, J = 13, 12)	6.29 (1H, d, J = 11)
16	1.34 (3H, s)	1.33 (3H, s)	1.33 (3H, s)	1.33 (3H, s)	1.32 (3H, s)
17	1.22 (3H, s)	1.21 (3H, s)	1.22 (3H, s)	1.21 (3H, s)	1.21 (3H, s)
18	1.97 (3H, s)	1.95 (3H, s)	1.95 (3H, s)	1.94 (3H, s)	1.95 (3H, s)
19	2.05 (3H, s)	2.02 (3H, s)	2.05 (3H, s)	2.04 (3H, s)	2.01 (3H, s)
20′	2.16 (3H, s)	2.18 (3H, s)	2.14 (3H, s)	2.02 (3H, s)	1.97 (3H, s)
2′α	2.19* (1H)	2.18 (1H, dd, J = 13, 6)	2.18* (1H)	2.18 (1H, dd, J = 13, 6)	2.16 (1H, dd, J = 13, 6)
2′β	1.85 (1H, t, J = 13)	1.83 (1H, t, J = 13)	1.83 (1H, t, J = 13)	1.84 (1H, t, J = 13)	1.82 (1H, t, J = 13)
3′	4.34 (1H, dd, J = 13, 6)	4.34 (1H, dd, J = 13, 6)	4.34 (1H, dd, J = 13, 6)	4.35 (1H, dd, J = 13, 6)	4.33 (1H, dd, J = 13, 6)
7′	6.29 (1H, d, J = 16)	6.30 (1H, d, J = 18)	6.28 (1H, d, J = 18)	6.35 (1H, d, J = 16)	6.30 (1H, d, J = 17)
8″	6.43 (1H, d, J = 16)	6.38 (1H, d, J = 18)	6.39 (1H, d, J = 18)	6.47 (1H, d, J = 16)	6.39 (1H, d, J = 17)
10′	6.31 (1H, d, J = 11)	6.30 (1H, d, J = 12)	6.31 (1H, d, J = 11)	6.41 (1H, s)	6.29 (1H, d, J = 11)
11′	6.79 (1H, dd, J = 15, 11)	6.79 (1H, dd, J = 15, 12)	6.79 (1H, dd, J = 15.5, 11)	—	6.89 (1H, dd, J = 15, 11)
12′	6.58 (1H, d, J = 15)	6.57 (1H, d, J = 15)	6.58 (1H, d, J = 15.5)	7.84 (1H, s)	6.32 (1H, d, J = 15)
14′	6.36 (1H, s)	6.36 (1H, s)	6.36 (1H, s)	6.78 (1H, d, J = 12)	6.50 (1H, s)
15′	—	—	—	6.63 (1H, dd, J = 13, 12)	—
16′	1.34 (3H, s)	1.31 (3H, s)	1.32 (3H, s)	1.32 (3H, s)	1.32 (3H, s)
17′	1.21 (3H, s)	1.20 (3H, s)	1.20 (3H, s)	1.20 (3H, s)	1.21 (3H, s)
18′	1.95 (3H, s)	1.92 (3H, s)	1.93 (3H, s)	1.94 (3H, s)	1.92 (3H, s)
19′	2.03 (3H, s)	2.04 (3H, s)	2.03 (3H, s)	1.76 (3H, s)	2.03 (3H, s)
20′	1.78 (3H, s)	1.76 (3H, s)	1.78 (3H, s)	1.89 (3H, s)	2.09 (3H, s)

*overlapped signal

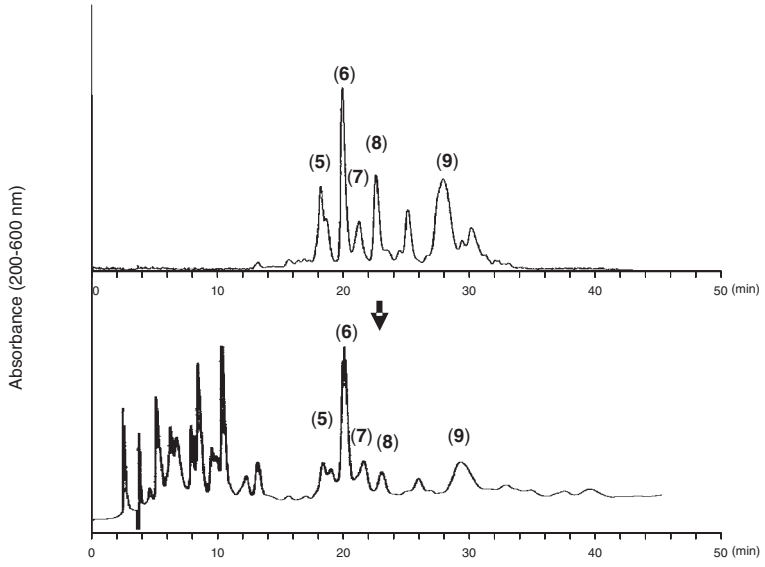


Fig. 6. HPLC Analysis of Nitroastaxanthins (Above) and Their Peroxynitrite Reaction Products (Below).
HPLC conditions: Develosil C30-UG-5 ϕ4.6 × 250 mm, MeCN:H_2O = 75:25; flow rate, 1 ml/min; column temperature, 40 °C; detection range, 200–600 nm.

yield of the corresponding reaction products. Compound **6** did not react further with peroxynitrite, and it emerged as the most stable nitroastaxanthin derivative of **6**, **7**, **8**, and **9**.

The above research results also confirm that 14′-s-*cis*-15′-nitroastaxanthin (**6**) was the most stable, high yield nitroastaxanthin of the five isolated nitroastaxanthins. Moreover, 14′-s-*cis*-15′-nitroastaxanthin (**6**), being sta-

Table 4. ^{13}C NMR Spectral Data for Nitro-Astaxanthins

Number	6	8	9
	δ (ppm)	δ (ppm)	δ (ppm)
1	36.8	36.8	36.8
2	45.4	45.3	45.3
3	69.2	69.2	69.2
4	200.4	200.5	200.4
5	127.1	126.6	126.9
6	161.9	162.3	162.1
7	124.5	124.1	124.0
8	141.7	142.0	142.1
9	136.5	140.5	135.9
10	133.8	134.7	134.7
11	129.6	126.5	126.4
12	138.1	139.4	138.9
13	148.8	135.9	140.3
14	126.7	132.8	128.1
15	130.1	135.9	132.1
16	26.1	26.1	26.1
17	30.7	30.7	30.7
18	14.0	14.0	14.0
19	12.7	12.7	12.6
20	13.6	13.1	12.8
1′	36.8	36.8	36.8
2′	45.4	45.3	45.3
3′	69.2	69.2	69.2
4′	200.4	200.5	200.4
5′	127.3	126.6	126.9
6′	161.8	162.3	162.1
7′	125.4	127.1	125.4
8′	141.5	139.0	141.4
9′	138.2	143.6	138.2
10′	133.8	122.9	134.0
11′	127.4	143.6	130.3
12′	137.7	140.2	129.1
13′	142.7	131.0	127.3
14′	120.0	144.3	120.1
15′	146.1	129.1	150.8
16′	26.1	26.0	26.1
17′	30.7	30.6	30.7
18′	14.0	13.9	14.0
19′	12.8	14.3	12.6
20′	15.2	14.7	13.1

ble should be able to metabolize in the body and may be considered the most stable major antioxidant derivative of astaxanthin *in vivo*. Thus, astaxanthin can be considered a potent antioxidant on reaction with peroxynitrite. We believe that these reactions are probably involved *in vivo*. It would be helpful to understand the *in vivo* reaction patterns of potent carotenoids like astaxanthin on reaction with peroxynitrite.

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