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Structural Studies of Natural Carotenoids by Our Research Group During the Three Decade

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Abstract – Since 1978, our research group determined more than one hundred natural carotenoids. In the present paper, structural studies of natural carotenoid during the three decade by our group were reviewed.

1. Introduction

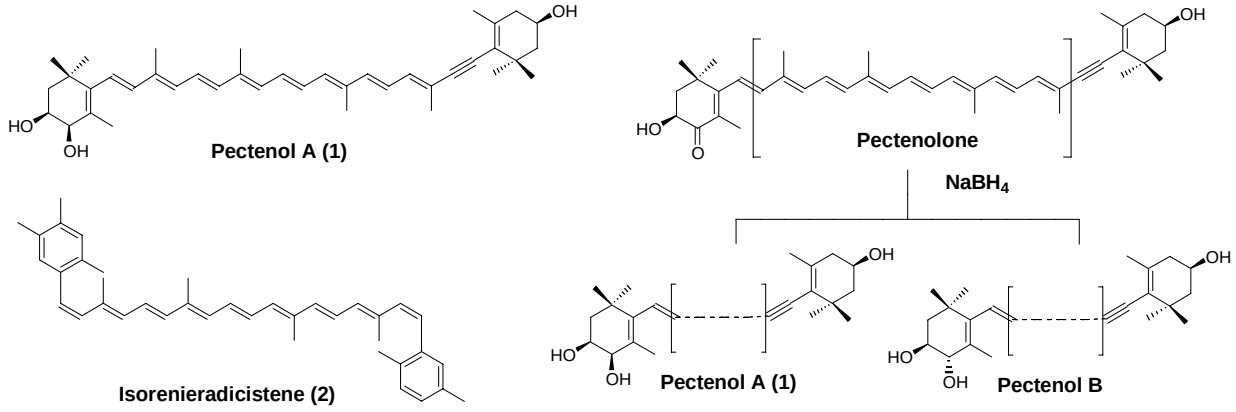
More than 750 naturally occurring carotenoids have been reported as of 2004 [1]. Since 1978, I have contributed structural studies of various natural carotenoids. In the previous paper [2], I described some experimental techniques for structural elucidation and analysis of natural carotenoids. In the present paper, I will review the structural studies of natural carotenoids done in Kyoto Pharmaceutical University and Research Institute for Production Development during the three decade.

2. Structural study of carotenoid using 80 MHz NMR and chemical derivatizations (1978-1985)

In 1970s, column chromatography and preparative thin layer chromatography were mainly used for separation tools of natural carotenoid. Purification of carotenoids was accomplished by re-crystallization. UV-Vis, IR, ^{1}H NMR using 60-100 MHz continuous wave instrument, and electro inonization mass (EI MS) were available for instruments of structural elucidation. Chemical derivatizations such as acetylation, trimethyl sililation, methylation, epoxide-furanoxide rearrangement reduction of carbonyl group etc. were also essential procedures for characterization. Therefore, more than 10 mg crystal state carotenoid was required for structural elucidation.

In the graduate thesis of Kyoto Pharmaceutical University (1978), carotenoids of a red dragonfly, *Sympetrum frequens* were investigated. About 0.2 mg of unidentified carotene (β,γ-carotene) was isolated from one thousand dragonflies, along with β-carotene and β-zeacarotene. Because of limitation amount, NMR spectrum of this compound could not be measured. Therefore, this compound was identified by UV-Vis and chromatographic behavior compared with semi-synthetic β,γ-carotene, which was derived from β-carotene [3].

In the thesis at master course in Kyoto Pharmaceutical University (1980), carotenoids of a sea mussels, *Mytilus coruscus* were investigated. A new carotenoid named, pectenol A (**1**) (3 mg) was isolated from 10 kg of shellfish. This structure was characterized from UV-Vis, IR, EI-MS, ^{1}H NMR (using 80 MHz electro magnetic Fourier-transform instrument) spectral data and some chemical derivatizations such as acetylation, trimethyl sililation, and methylation [4]. In 80 MHz ^{1}H NMR, only methyl signals in **1** could be assigned by comparison with alloxanthin and diatoxanthin. Signals of methin and metylen protons could not be assigned because of overlapping. Therefore, stereochemistry of 3,4-dihydroxy group in **1** was determined by comparison with semi-synthetic diastereomeric triols



Scheme 1

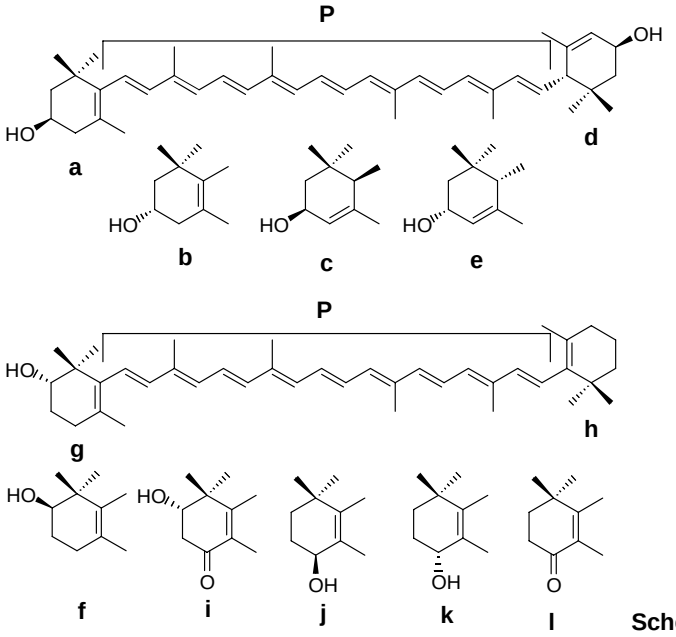
obtained from pectenolone by $NaBH_4$ reduction as shown in Scheme 1 [5].

A novel sterically hindered (7*Z*,7'*Z*)-aryl-carotenoid, named isorenieradicisten (**2**), was isolated from the sea sponge, *Suberites sericeus* and its structure was characterized by 80 MHz ^{1}H NMR, IR, EI-MS spectral data and I_2-catalysis isomerization comparing with isorenieratene [6].

3. Separation and characterization of stereo (optical)-isomers of natural carotenoids using chiral phase HPLC (1985-1989)

Carotenoids in animals are sometimes presented as mixture of optical isomers. European research group used diastereomric derivatization method for the separation of optical isomers of carotenoids [7]. On the other hand, our group achieved the separation of optical isomers of carotenoids using chiral phase columns, Sumichiral OA-2000 or Chiral cell OD [8].

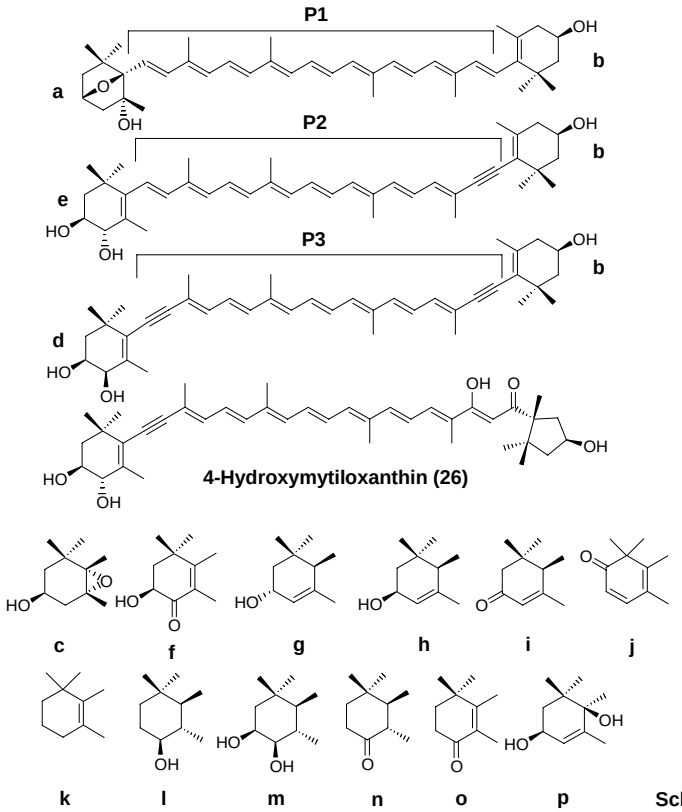
Three new stereoisomers of luteins, (3*R*,3'*S*,6'*S*)-lutein [lutein D (**3**), first report as a natural product], (3*R*,3'*R*,6'*S*)-lutein [lutein F (**4**)], and (3*S*,3'*R*,6'*S*)-lutein [lutein G (**5**)], from marine fish [9], (3*S*,6*R*,3'*R*,6'*S*)-tunaxanthin [tunaxanthin D



Lutein D (3) : a-P-d
Lutein F (4) : a-P-e
Lutein G (5) : b-P-e
Tunaxanthin D (6) : c-P-e
***meso*-Zeaxanthin (7) : a-P-b**
(3*S*,3'*S*)-Zeaxanthin (8) : b-P-b

(2*S*)-β,β-caroten-2-ol (9) : g-P-h
***meso*-β,β-carotene-2,2'-diol (10) : f-P-g**
(2*S*,2'*S*)-β,β-carotene-2,2'-diol (11) : g-P-g
(2*S*)-2-Hydroxyechinenone (12) : i-P-h
(4*S*)-β-Isocryptoxanthin (13) : j-P-h
***meso*-Isozeaxanthin (14) : j-P-k**
(4*S*,4'*S*)-Isozeaxanthin (15) : j-P-j
(4'*S*)-4'-Hydroxyechinenone (16) : l-P-j

Scheme 2



Cucurbitaxanthin A (17) : a-P1-b
Cucurbitaxanthin B (18) : a-P1-c
Pectenol B (19) : f-P2-b
4-Hydroxyalloxanthin A (20) : d-P3-b
4-Hydroxyalloxanthin B (21) : e-P3-b
4,4'-Dihydroxydiatoxanthin (22) : e-P2-e
4,4'-Dihydroxyalloxanthin (23) : e-P3-e
4-Keto-4'-hydroxydiatoxanthin (24) : f-P2-e
4-Keto-4'-hydroxyalloxanthin (25) : f-P3-e
4-Hydroxymytiloxanthin (26)
4-Hydroxyzeaxanthin (27) : d-P1-b
4-Hydroxylutein A (28) : d-P1-g
4-Hydroxylutein B (29) : d-P1-h
4-Keto-4'-hydroxyalloxanthin (30) : f-P3-d
4-Keto-4'-hydroxydiatoxanthin (31) : f-P2-d
2-Keto-3,4-didehydro-β-carotene (32) : j-P1-k
4,4'-Dihydroxypirardixanthin (33) : l-P1-l
3,4,4'-Trihydroxypirardixanthin (34) : m-P1-l
3,4,3',4'-Tetrahydroxypirardixanthin (35) : m-P1-m
4-Keto-4'-hydroxypirardixanthin (36) : n-P1-l
5',6'-dihydro-4'-hydroxyechinenone (37) : o-P1-l
Pectenolone (38) : f-P2-b
Papirioerythrinone (39) : f-P1-i
4-Ketoalloxanthin (40) : f-P3-b
Salmoxanthin (41) : c-P1-p
Deepoxysalmoxanthin (42) : b-P1-p

Scheme 3

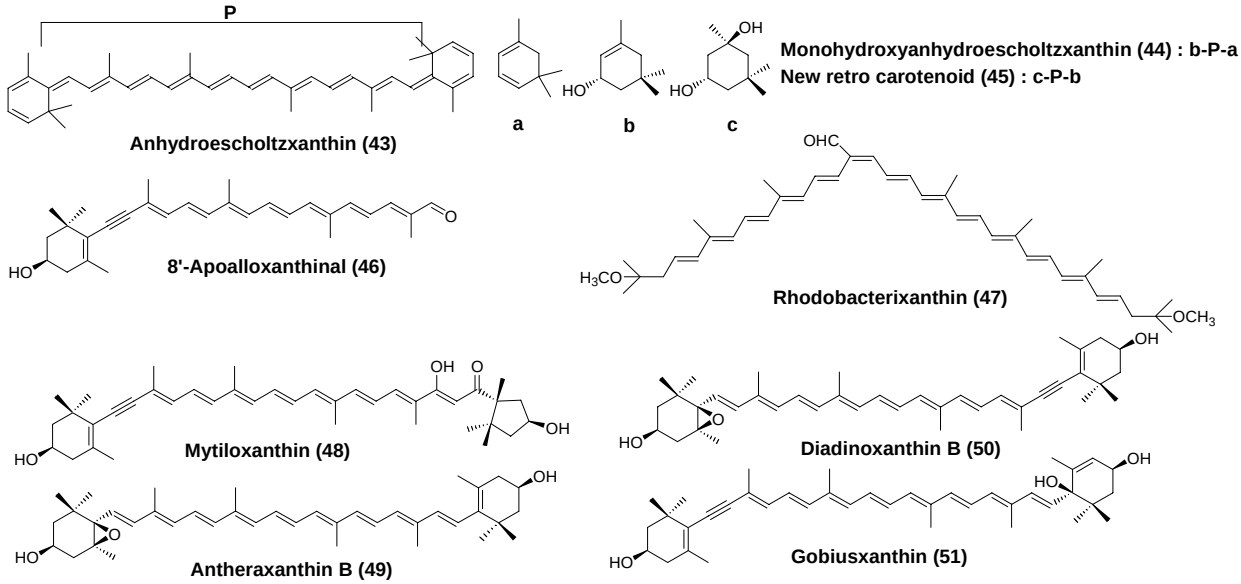
(6)] from marine fish [10], two new optical isomers of zeaxanthins, *meso*-zeaxanthin (**7**) and (3*S*,3'*S*)-zeaxanthin (**8**) [first report as natural products] from marine fish [11], four new optical isomers of 2-hydroxy carotenoids, (2*S*)-β,β-caroten-2-ol (**9**) [first report as a natural product], *meso*-β,β-carotene-2,2'-diol (**10**), (2*S*,2'*S*)-β,β-carotene-2,2'-diol (**11**), and (2*S*)-2-hydroxyechinenone (**12**) from the sea louse, *Ligia exotica* and the stick insect, *Neophirosea japonica* [12], four new optical isomers of 4-hydroxy carotenoids, (4*S*)-β-isocryptoxanthin (**13**), *meso*-isozeaxanthin (**14**), (4*S*,4'*S*)-isozeaxanthin (**15**), and (4'*S*)-4'-hydroxyechienone (**16**) from the sea urchins [13] were isolated and were characterized by CD and NMR spectroscopy. They were my Ph. D. thesis works in Kyoto Pharmaceutical University.

4. Structural study of carotenoid using 300 MHz NMR and preparative HPLC (1985-1995)

In middle of 1980s, 300 MHz NMR instrument was introduced in our university. This instrument strongly improved the sensitivity and resolution of signals than 80 MHz instrument and also enable to routinely measurement of ^{13}C NMR of carotenoids with several mg scale. Unique 3,6-epoxy carotenoids, named cucurbitaxanthin A (**17**) and cucurbitaxanthin B (**18**) were isolated from the pumpkin, *Cucurbita maxima* as crystal states. These structures were elucidated by detailed analysis of ^{1}H, ^{13}C NMR, EI MS, CD spectral data and by chemical derivatization such as reductuin with $LiAlH_4$ [14]. Our group reported the structures of **17** and **18** in *Phytochemistry* (1986) [14]. Immediately after our publication, Hungarian research group reported the isolation and structural elucidation of **17** from the red paprika, *Capsicum annuum* in *Tetrahedron Letters* [15].

In middle of 1980s, preparative HPLC was routinely used for isolation tool. By using ^{1}H NMR (300 MHz), EI MS, and CD spectroscopy, it made possible to structural determination of natural carotenoids with a few hundred μg. Our research group studied various animal carotenoids from the comparative biochemical points. Through this research, several new carotenoids were isolated. Structures of three new acetylenic carotenoids, pectenol B (**19**), 4-hydroxyalloxanthin A (**20**), and 4-hydroxyalloxanthin B (**21**) from the sea mussel, *Mytilus coruscus* [16], five new acetylenic poly oxygenated carotenoids, (3*S*,4*S*,3'*S*,4'*S*)-4,4'-dihydroxydiatoxanthin (**22**), (3*S*,4*S*,3'*S*,4'*S*)-4,4'-dihydroxyalloxanthin (**23**), (3*S*,3'*S*,4'*S*)-4-keto-4'-hydroxy- diatoxanthin (**24**), (3*S*,3'*S*,4'*S*)-4-keto-4'-hydroxyalloxanthin (**25**), and 4-hydroxymytiloxanthin (**26**) from the starfishes, *Asterina pectinifera* and *Asterias amurensis* [17], two new tri-hydroxy carotenoids, 4-hydroxyzeaxanthin (**27**) and 4-hydroxylutein A (**28**) from the chiton, *Acanthopleura japonica* [18], three tri-hydroxy carotenoids, 4-hydroxylutein B (**29**), (3*S*,3'*S*,4'*R*)-4-keto-4'-hydroxyalloxanthin (**30**), and (3*S*,3'*S*,4'*R*)-4-keto-4'-hydroxydiatoxanthin (**31**) from the gold fish, *Carassius auratus* [19], a new 2-oxo-carotenoid, 2-keto-3,4-didehydro-β-carotene (**32**) from the stick insect, *Neophirosea japonica* [20] were determined. Furthermore, five unique 5,6 and/or 5', 6'-hydro carotenoids, named pirardixanthins (**33**-**37**) from the spindle shell, *Fushinus perplexus,* were isolated and characterized by UV-Vis, ^{1}H NMR, EI MS, and chemical derivatizations such as acetylation, trimethyl sililation, reduction of carbonyl group with $NaBH_4$ [21]. Later (2001), the absolute stereochemistry of **33**-**37** were completely characterized by 500 MHz ^{1}H NMR using modified Mosher method [22].

The absolute configurations of pectenlone (**38**) [16,



Scheme 4

23], papyrioerythrinone (**39**) [24], 4-ketoalloxanthin (**40**) [16], salmoxanthin (**41**), and deepoxysalmoxanthin (**42**) [25] were determined using CD, NMR spectrometry and chemical derivatization such as reduction with $NaBH_4$. The additive rule of CD was used for estimation of the chirality of these carotenoids. (Scheme 3)

These stereochemical studies of carotenoids in animals provided the information of food chains and metabolic pathways of carotenoids in animals.

Retro-carotenoids are presented as mixtures of geometrical isomers in nature. Predominant geometrical isomer of anhydroeschscholtzanthin (**43**) in the leaves of *Buxus sempervirens* was determined to be (6*E*, 6'*E*) configuration by NOE using NOE difference experiment [26]. Furthermore, structures of two new *retro*-carotenoids, mono-anhydroeschscholtzanthin (**44**) from the leaves of *B. sempervirens* [26] and trihydroxy-retro-carotenoid (**45**) from the petals of *Eschscholtzia californica* [27] were determined by ^1H NMR, including NOE experiment, ^{13}C NMR, CD, and MS spectroscopy.

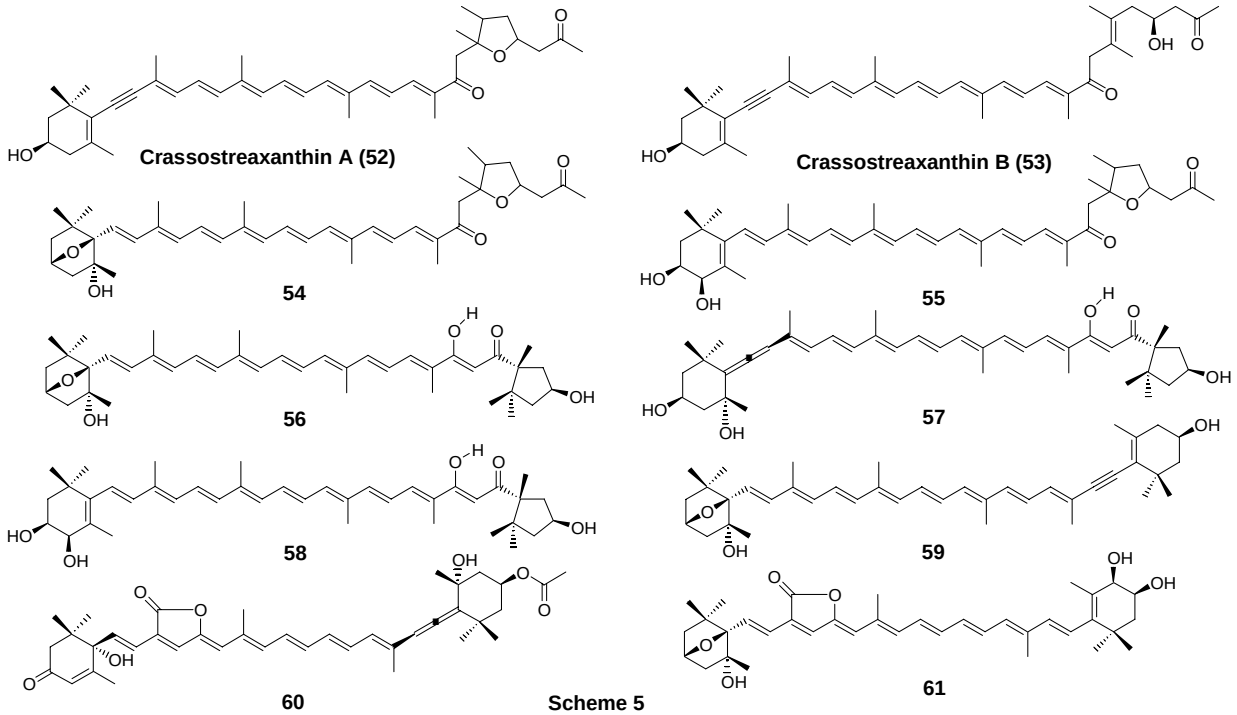
Structures of 8'-apoalloxanthinal (**46**) from the marine shellfish [28] and novel purple carotenoid, rhodobacterioxanthin (**47**) from the photosynthetic bacterium, *Rhodobactor capsulatus* [29] were determined (Scheme 4).

5. Structural study of complex structural carotenoid using high magnetic filed NMR and soft ionization MS spectrometry (1995-2008)

In 1990s, several new experimental techniques of NMR and soft ionization mass spectrometry were routinely available. In 1998, 500 MHz NMR instrument was introduced in my present workplace, Research Institute for Production Development. Nano Probe™ (Varian) enables to measuring ^{13}C NMR of several hundred μg. ^1H-^{13}C correlations were elucidated by ^1H detected spectroscopy (HSQC and HMBC). Pulse filed gradient (PFG) technique has been dramatically improved two-dimensional NMR measurement [2]. Soft ionization mass spectrometry, FAB, APCI, ESI, FD, and MALDI MS, provided the molecular ion or molecular weight related ions, such as M+H, M-H, M+Na predominantly. Therefore, these soft ionization mass spectrometry afforded molecular weight information of complex carotenoid such as carotenoid glycoside and carotenoid esters. Although soft ionization MS showed less fragment ion spectrum, MS/MS experiment provided several fragment ions which had structural informations. For example, FAB MS/MS of carotenoid, using molecular ion as a precursor ion, provides EI MS like fragmentation [2]. Furthermore, separation techniques were also improved. Reverse phase HPLC using C_8, C_{18} (ODS), and C_{30} columns provided fine separation of carotenoids. Gel filtration chromatography (GPC) could effectively remove lipid impurity from carotenoid [2]. Combination of these experimental techniques, it was possible to determine complex structural carotenoid below 1 mg.

5-1. Animal carotemoids

The absolute configurations of mytiloxanthin (**48**) [30] and a series of carotenoids with 5,6 and/or 5',6'-hydro carotenoids (**33**-**37**) [22], whose chiralities could not be determined by CD spectroscopy because



Scheme 5

lacking of chromophore neighbors of asymmetric carbon, were determined by modified Mosher method using NMR spectroscopy.

A novel 3,5-*syn* epoxy carotenoids, antheraxanthin B (**49**) and diadinoxanthin B (**50**), were isolated from the fresh water goby, *Rhinogobius brunneus*. They were first reports of 3,5-*syn* epoxy carotenoids as natural products. Furthermore, a new acetylenic triol, having a 3,6-dihydroxy-ε-end group, gobiusxanthin (**51**) was isolated. Their structures were completely characterized by 500 MHz ^{1}H NMR and CD spectral data [31] (Schem 4).

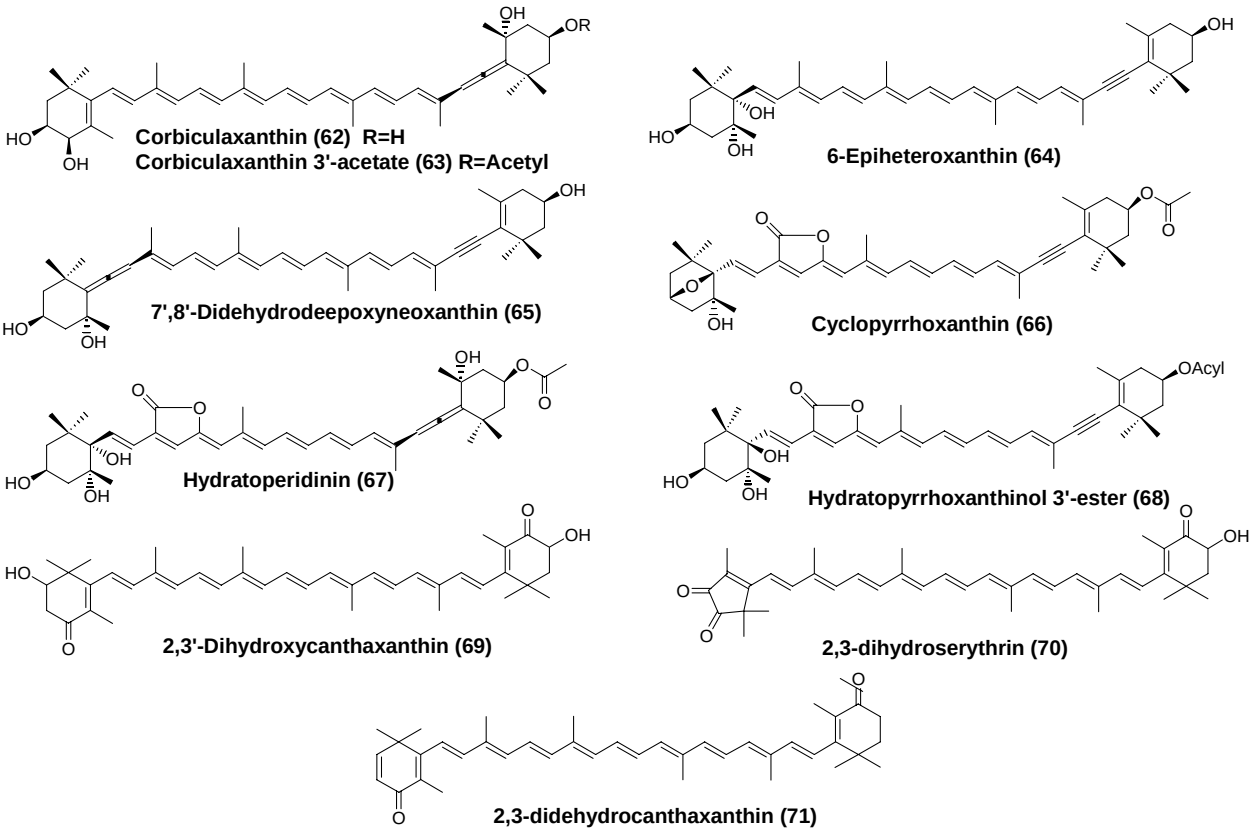
Crassostreaxanthin A (**52**) and crassostreaxanthin B (**53**), isolated from the Japanese oyster, *Crassostrea gigas*, were unique carotenoids having previous unknown end groups. These structures were elucidated by detailed analysis of NMR including two-dimensional NMR experiments [32]. Later, the (3*R*,3'*S*) configurations of crassestreaxanthin B (**53**) were confirmed based on total synthesis by Tode et al [33].

Two new carotenoids having crassestreaxanthin A end group, **54** and **55** were isolated [34, 35]. Furthermore, three mytiloxanthin analogues containing a 3,6-epoxy end group (**56**), an allenic end group (**57**), and a 3,4-dihydroxy-β end group (**58**) were isolated from the oyster [34, 35]. A 3,6-epoxy derivative of diadinoxanthin, named cycloidadinoxanthin (**59**), was also isolated [34]. In addition to these C_{40}-skeletal carotenoids described above, two new C_{37}-skeletal carotenoids, **60** and **61**, were isolated from the oyster [34, 35] (Scheme 5).

Six new carotenoids, corbiculaxanthin (**62**), corbiculaxanthin 3'-acetate (**63**), 6-epiheteroxanthin (**64**), and 7',8'-didehydrodeepoxyneoxanthin (**65**), cyclopyrrhoxanthin (**66**), and hydratedperidinin (**67**), were isolated from the brackish water clam, *Corbicula japonica* [36]. Furthermore, hydratopyrrhoxanthinol 3'-esters (**68**) was isolated from the red marine clam, *Paphia amabillis* [37]. Fatty acids consisted with compound **68** were characterized by FAB MS data. The stereochemistry of 3, 5, 6-trihydroxy-β-end group in butenolide carotenoids **67** and **68** were postulated by NOE and CD data as shown in Scheme 6. I think that synthetic approach is necessary to confirm the absolute stereochemistry of **67** and **68**.

Furthermore, the structures of some minor carotenoids in marine animals were determined. 2,3'-Dihydroxycanthaxanthin (**69**) from the hermit crab, *Paralithodes brevipes* [38], purple nor-carotenoid, 2,3-dihydroserythrin (**70**) from the crawfish, *Procambarus clarkii* [39] and 2,3-didehydrocanthaxanthin (**71**), which was first report as a natural carotenoid, from the spiny lobster, *Panulirus japonicus* [40] were characterized by ^{1}H NMR and MS spectral data. Compounds **69** and **70** were obtained as mixtures of optical isomers (Scheme 6).

5-2. Plant carotemoids



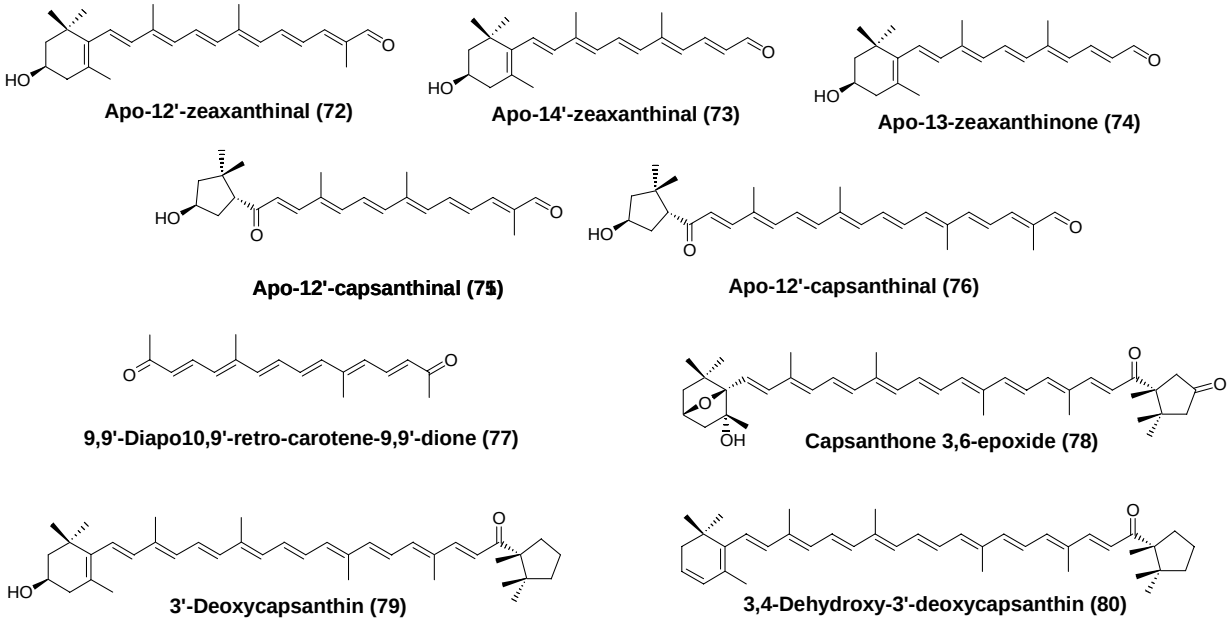
Scheme 6

Carotenoids in paprika

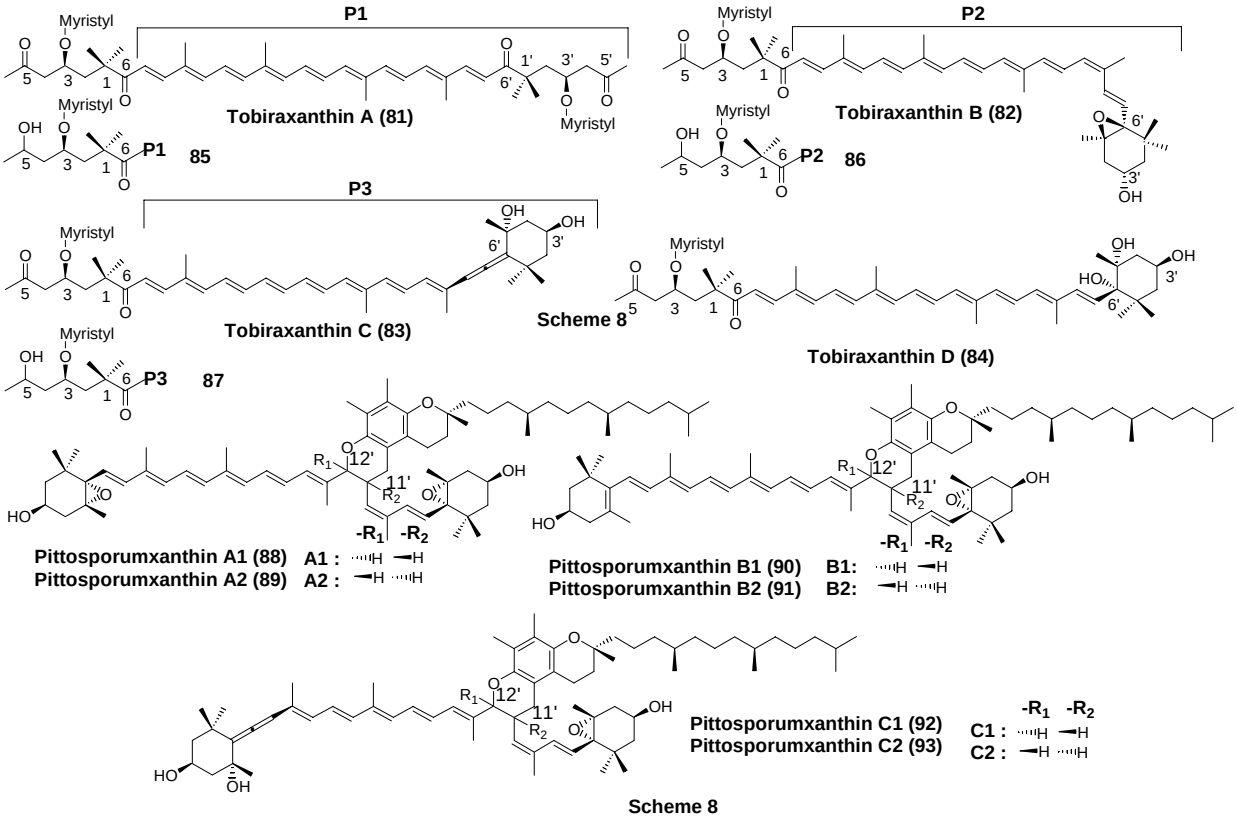
Paprika, *Capsicum annuum*, is a good source of interesting structural carotenoids. A series of new apocarotenoids (**72**-**77**), which were formed by oxidative degradation from capsanthin, were isolated from paprika [41]. A new 3,6-epoxy carotenoid, capsanthone 3,6-epoxide (**78**) were determined by NMR and FAB MS including MS/MS experiment [42]. Furthermore, two new carotenoids, named 3'-deoxycapsanthin (**79**) and 3,4-dehydroxy-3'-deoxycapsanthin (**80**), were isolated from paprika as very minor components [43]. These carotenoids have a 6-oxo-κ-end group, which had not been reported previously in nature (Scheme 7).

Seco-carotenoids in the seeds of Pittosporum tobira

The seeds of *Pittosporum tobira* contained various interesting structural carotenoids. A series of acylated seco-carotenoids having a 3-acyloxy-5,6-diseco-5,6-diketo-β-end group, named tobiraxanthin A (**81**), B (**82**), C (**83**), and D (**84**), were isolated from red seeds as major carotenoids [44].



Scheme 7



Scheme 8

These structures were elucidated by the detailed analysis of MS and NMR spectral data. Tobiraxanthin A, B, C, and D are corresponding oxidative cleavage products of C5-C6 (C5'-C6') bonds in the violaxanthin diester, (9*Z*)-violaxanthin 3'-ester, neoxanthin 3'-ester, and 6-epilatoxanthin 3'-ester, respectively. Moreover, seco-carotenoids with a 3-acyloxy-5-hydroxy-5,6-diseco-6-keto-β-end group (**85**-**87**) were also isolated from the red seeds of *P. tobira* [45]. They were corresponding reduction product of (**81**-**83**) (Scheme 8).

Carotenoid and α-tocopherol complexes in the seeds of Pittosporum tobira

A series of carotenoid and α-tocopherol complexes named pittosporumxanthins were isolated from the red-colored seeds of *P. tobira* [46, 47]. Pittosporumxanthin A1 (**88**) and A2 (**89**) were diastereomeric pairs of the cycloaddition product of (9*Z*)-violaxanthin at the C-11' and C-12' positions, with α-tocopherol showing a O-C12'-C11'-C28'' linkage. Furthermore, (9*Z*)-antherxanthin and α-tocopherol complexes, named pittosporumxanthin B1 (**90**) and B2 (**91**) and (9'*Z*)-neoxanthin, and α-tocopherol complexes, named pittosporumxanthin C1 (**92**) and C2 (**93**), were isolated. These structures were elucidated by the detailed analysis of NMR and MS, including EI MS/MS experiment and CD spectral data (Scheme 8).

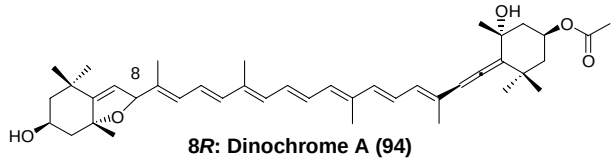
Other new carotenoids from the plants

Two epimeric pair of dinochromes, **94** and **95**, were isolated from the fresh water red tide, *Peridinium bipes* and were characterized by spectral data [48]. (3*S*,5*S*,6*R*,3'*S*,6'*R*)-5,6-dihydro-5,6-dihydroxylutein (**96**) and five novel di-*cis*-isomer of lutein 5,6-epoxides, 9*Z*,13'*Z* (**97**), 13*Z*,9'*Z* (**98**), 9'*Z*,13'*Z* (**99**), 9*Z*,13*Z* **(100**), and 9*Z*,9'*Z* (**101**) were isolated from the petals of chrysanthemum, *Dendranthema grandiflorm* and these structures were completely characterized by NMR using isomerization shift values and NOESY experiment, CD, and MS spectral data. Compounds **96** was first report as a natural product [49]. This work was done by collaboration with Dr. S. Kishimoto, National Institute of Floricultural Science. Furthermore, for new carotenoids having a 5,6-dihydroxy-β-end group named ipomoeaxanthins (**102**-**105**) were isolated from the yellow sweet potato "Benimasari", *Ipomoea batatas* [50] (Scheme 9). This work was done by collaboration with Dr. K. Ishiguro, National Agricultural Research Center for Kyusyu Okinawa Region.

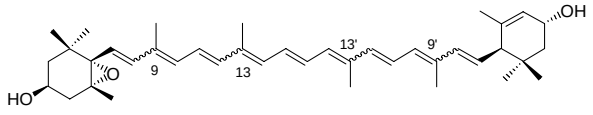
5-3. Bacterial carotenoids

Bacteria contain interesting structural carotenoids. Novel C_{50}-skeletal carotenoid, flavuxanthin (**106**) was isolated from *Corynebacterium glutamicum* [51].

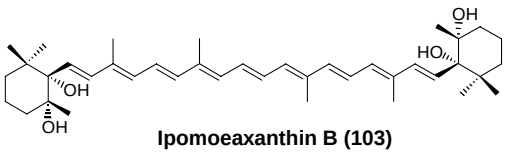
Structures of some carotenoid glycosides, dihydroxylycopene diglucoside diesters (**107)** and methoxyhydroxyylcopene glucoside (**108**) from the phototrohic purple sulfur bacteria, *Halorhodospira abdelmalekii* and *H. halochloris* [52], myxol 2'-dimethyl-fucoside (**109**) and deoxymyxol 2'-dimethyl-fucoside (**110**) from *Synechocystis* sp. PCC 6803 [53], hydroxy-diaponeurosorene glucoseide ester (**111**) from the alkaliphilic heliobacteria, *Heliorestis daurensis* and *H. baculata* [54], and adonixanthin diglucoside (**112**) from *Paracoccus schoinia* NBRC 100637^T [55] were determined by detailed analyses of NMR, soft ionization MS spectrometry such as FD and FAB MS. These sugar moiety were completely characterized by 2D NMR such as TOCSY and HSQC. Parts of them were characterized after acetylation in order to resolve overlapping oxymethin signals. The glycosylated position of carotenoid and stereochemistry of glycosyl



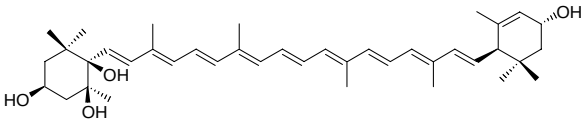
8*R*: Dinochrome A (94)
8*S*: Dinochrome B (95)



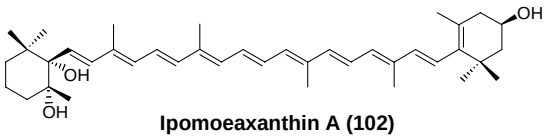
Geometrical isomers of lutein 5,6-epoxide
9*Z*,13'*Z* (97), 13*Z*,9'*Z* (98), 9'*Z*,13'*Z* (99), 9*Z*,13*Z* (100),9*Z*, 9'*Z* (101)



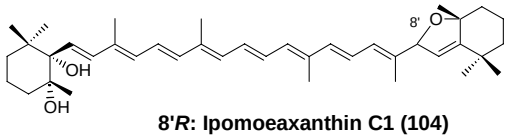
Ipomoeaxanthin B (103)



(3S,5S,6R,3'S,6'R)-5,6-dihydro-5,6-dihydroxylutein (96)



Ipomoeaxanthin A (102)



8'*R*: Ipomoeaxanthin C1 (104)
8'*S*: Ipomoeaxanthin C2 (105)

Scheme 9

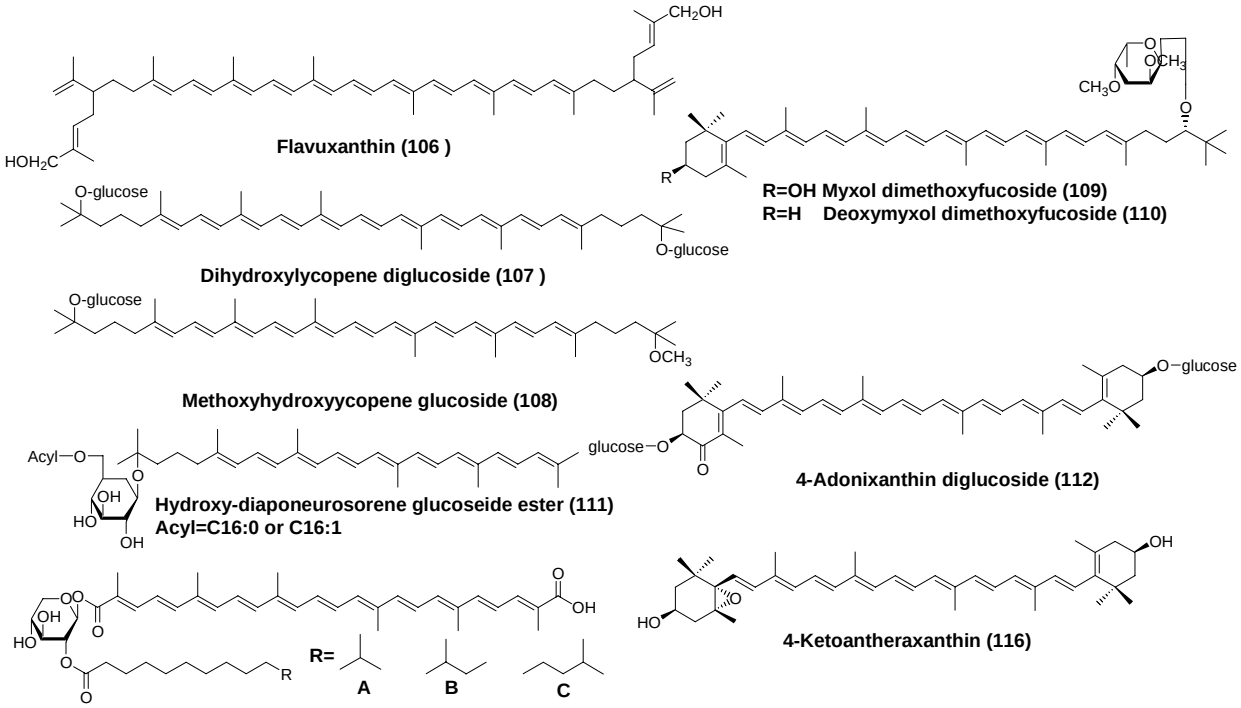
bond were determined by HMBC and NOE experiments and coupling constant of anmomeric proton. These works were done by collaboration with Dr. S. Takaichi, Nippon Medical School.

Furthermore, a series of diapolycopenedionic acid xylosyl esters (**113-115**) were isolated from the marine bacterium, *Rubritalea squallenifaciens* [56-57]. They were first report carotenoids to include xylose (Scheme 10). This work was done by collaboration with Dr. K. Shindo, Japan Women's University and Dr. N. Misawa, Marine Biotechnology Institute.

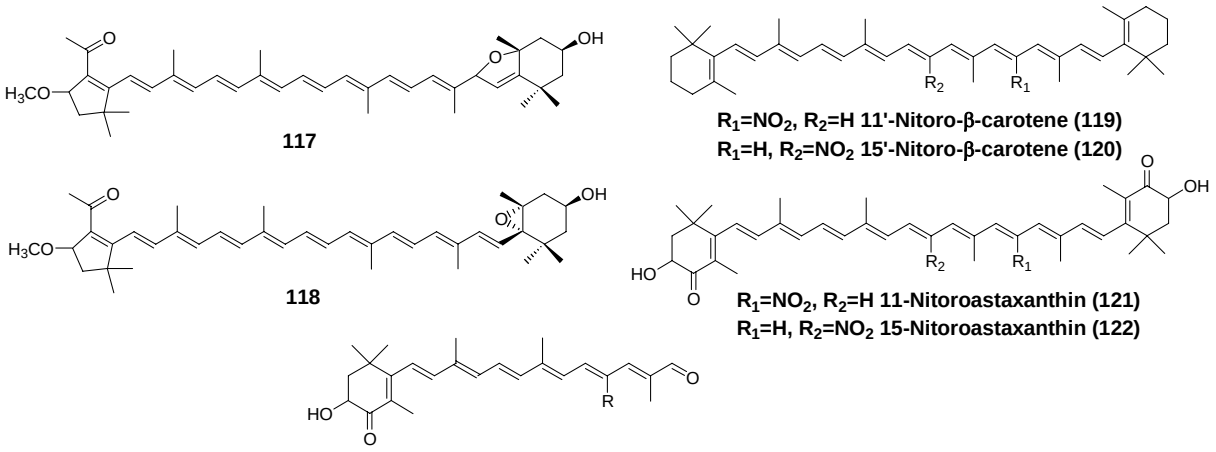
5-4. Carotenoids in transgenic plants.

A new carotenoid, 4-ketoantheraxanthin (**116**) was isolated from the leaves of tobacco plant, *Nicotiana tabacum*, introduced the genes coding CrtW (marine bacterial enzyme β-carotene ketolase) [58]. This works were done by collaboration with Dr. K. Shindo, Dr. N. Misawa, and Dr. T. Hasunuma, Research Institute of Innovative Technology for the Earth.

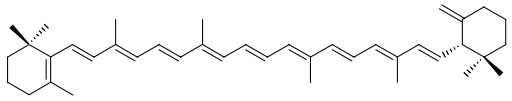
5-5. Chemical reaction products of carotenoids



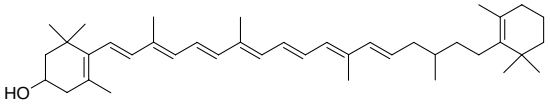
Scheme 10



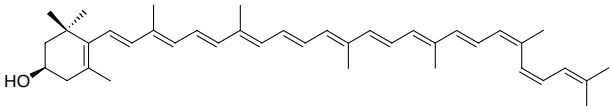
Scheme 11



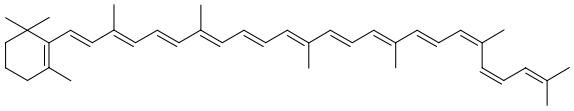
(6'S)-β,γ-Carotene



7',8',9',10'-Tetrahydro-β-cryptoxanthin



(3'Z,5'Z)-Celaxanthin



(3'Z,5'Z)-Torulene

Scheme 12

Tobiraxanthins, having a 3-acyloxy-5,6-diseco-5,6-diketo-β-end group (**81**-**84**), showed lability in alkaline medium. Structures of alkaline reaction products of tobiraxanthins, having a novel 3-methoxy-5-keto-5,6-seco-4,6-cyclo-β end group, **117** and **118** were determined [59].

Structures of nitro-β-carotenes (**119**, **120**) and nitro-astaxanthins (**121**-**123**), formed by reacted with peroxy nitrite with β-carotene and astaxanthin, were determined [60-61] (Scheme 11). This work was done by collaboration with Prof. H. Etoh, Shizuoka University.

6. Conclusion

Structures of 116 natural carotenoids and seven chemical reaction products of carotenoids were determined by our research group during the three decades. The improvement of analytical tools such as NMR, MS, CD, HPLC, etc., has made it possible to perform the structural elucidation of natural carotenoids with small amount. Structures of theses new carotenoids provide information on the function, biosynthesis in plants and bacteria, and a key to the food chain as well as metabolic pathways in animals.

Appendix

Unpublished works of natural carotenoids were described here.

The structure of β,γ-carotene including 6*S* chirality, isolated from a red dragonfly, *S. frequens*, was confirmed by ^{1}H NMR, MS, and CD spectral data [62]. Novel carotenoid having a tetrahydro-ployene-chain, 7',8',9',10'-tetrahydro-β-cryptoxanthin, was isolated from the Japanese common catfish, *Parasilurus asotus*. This structure was characterized from ^{1}H NMR, MS, and UV-Vis spectral data [63].

A series of sterically hindered *cis* carotenoids, (3'*Z*,5'*Z*)-celaxanthin and (3'*Z*,5'*Z*)-torulene were isolated from the ripe fruits of the bittersweet, *Celastrus orbicularta*s. These structures were confirmed by isomerization shift values of ^{1}H and ^{13}C NMR data and I_2 chatarized isomerization. They were first reports of carotenoids with 3'*Z*,5'*Z* geometry [64].

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[62] (6'*S*)-β,γ-carotene from dragon fly: FAB MS (*m/z*) 536 [M^+]; UV-Vis (Et_2O) 412, 442, 471 nm; ^{1}H NMR δ (in $CDCl_3$ at 300 MHz) 0.82 (3H, s, H-16' or 17'), 0.90 (3H, s, H-16' or 17'), 1.03 (6H, s, H-16, 17), ~1.46 (2H, m, H-2, 2'), ~1.63 (2H, m, H-3, 3'), 1.72 (3H, s, H-18), 1.95 (3H, s, H-19'), 1.97 (9H, s, H-19, 20, 20'), ~2.02 (2H, t, *J*=7 Hz, H-4, 4'), 2.50 (1H, d, *J*=9.5 Hz, H-6'), 4.57 (1H, br. s, H-18'), 4.73 (1H, br. s, H-18'), 5.84 (1H, dd, *J*=15.5, 9.5 Hz, H-7'), 6.12 (1H, d, *J*=11.5 Hz, H-10'), 6.13 (1H, d, *J*=15.5 Hz, H-8'), 6.14 (1H, d, *J*=16 Hz, H-8), 6.14 (1H, d, *J*=10 Hz, H-10), 6.15 (1H, d, *J*=16 Hz, H-7),

6.24 (2H, m, H-14, 14'), 6.33 (1H, d, *J*=15.5 Hz, H-12'), 6.35 (1H, d, *J*=15.5 Hz, H-12), 6.62 (1H, dd, *J*=15.5, 11.5 Hz, H-11'), 6.63 (2H, m, H-15, 15'), 6.65 (1H, dd, *J*=15.5, 11.5 Hz, H-11'); CD (in Et_2O): λ nm (Δε) 216 (+3.0), 230 (0), 238 (-2.0), 249 (0), 270 (+2.7), 225 (0), 340 (-1.0), 375 (-0.2).

[63] 7',8',9',10'-Tetrahydro-β-cryptoxanthin : FAB MS (*m/z*) 556 [M^+]; UV-Vis (Et_2O) 385, 408, 432 nm; ^{1}H NMR δ (in $CDCl_3$ at 300 MHz) 0.93 (3H, d, *J*=6.5 Hz, H-19'), 0.97 (6H, s, H-16', 17'), 1.07 (6H, s, H-16, 17), 1.40 (2H, m, H-2'), 1.48 (1H, dd, *J*=12, 12 Hz, H-2ax), 1.57 (3H, s, H-18'), 1.58 (2H, m, H-3'), 1.74 (3H, s, H-18), 1.77 (1H, ddd, *J*=12, 5, 2 Hz, H-2eq), 1.89 (3H, s, H-20'), 1.89 (2H, t, *J*=7 Hz, H-4'), 1.96 (6H, s, H-19, 20), ~2.01 (4H, m, H-7', 10'), 2.04 (1H, dd, *J*=16, 10 Hz, H-4ax), 2.39 (1H, ddd, *J*=16, 6, 2 Hz, H-4eq), 4.00 (1H, m, H-3), 5.73 (1H, dt, *J*=15, 7 Hz, H-11'), 6.10 (1H, d, *J*=16 Hz, H-7), 6.14 (1H, d, *J*=11 Hz, H-10), 6.16 (1H, d, *J*=16 Hz, H-8), 6.23 (2H, m, H-14, 14'), 6.36 (2H, d, *J*=15 Hz, H-12, 12'), 6.60 (2H, m, H-15, 15'), 6.62 (1H, d, *J*=15.5 Hz, H-11).

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