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Review

Recent progress in structural studies of carotenoids in animals and plants

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ABSTRACT

About 750 naturally occurring carotenoids had been reported as of 2004. Furthermore, annually, more than 20 new structures of carotenoids are reported. Improvements of analytical instruments such as NMR, MS, HPLC, etc., have made it possible to perform the structural elucidation of very minor carotenoids in nature. Interesting new structural carotenoids can still be identified in aquatic animals and higher plants. The present paper provides a review of new structural carotenoids isolated from aquatic animals and higher plants by our group over the last five years.

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]. Improvements of analytical instruments such as NMR[1], MS, HPLC, etc., have made it possible to perform the structural elucidation of very minor carotenoids in nature [2,3]. Annually, more than 20 new structures of carotenoids are being reported.

Our research group has developed structural analysis of naturally occurring carotenoids [3] using NMR, MS, MS/MS [4], LC/MS [5], etc., over the last decade. Herein, I describe the progress in structural studies of naturally occurring carotenoids of our group.

Carotenoids in aquatic animals

Marine animals, especially bivalves (oysters, clams, scallops, etc.) contain various carotenoids, which show structural diversity [6–9]. Bivalves accumulate carotenoids from their dietary algae and modify them through metabolic reactions. Many of the carotenoids present in bivalves are metabolites of fucoxanthin, peridinin, and diatoxanthin [6–9].

In 1992, our group isolated two unique carotenoids, named crassestreaxanthin A (**1**) and B (**2**), from the Japanese oyster *Crassostrea gigas* [10]. These two carotenoids have unique structural end groups, which have not been previously reported. These structures were determined to be 3′,6′-epoxy-3-hydroxy-6′-methyl-7,8-didehydro-1′,2′,5′,6′,7′,8′-hexahydro-16′-nor-β, ψ-carotene-1′,8′-dione (**1**) and 3,3′-dihydroxy-7,8-didehydro-1′,2′,7′,8′-tetrahydro-6′-methyl-16′-nor-β, ψ-carotene-1′,8′-dione (**2**), respectively, by the detailed analysis of MS and NMR data [10] as shown in Scheme 1. Based on the structural similarity, crassostreaxanthin A and B were assumed to be metabolites of halocynthiaxanthin. The (3*R*, 3′*S*) configurations of crassestreaxanthin B were confirmed based on total synthesis by Tode et al. [11]. Further studies of carotenoids in marine animals revealed that crassostreaxanthin A and B and their 3-acetates were widely distributed in marine bivalves. Moreover, two new carotenoids, **3** and **4**, possessing a structural moiety of crassostreaxanthin A, were isolated from the oyster as minor components [12,13].

Mytiloxanthin, which has a unique enol hydroxy group at C-8′ in the polyene chain and a 3′-hydroxy-6′-oxo-κ-end group, is a characteristic carotenoid in marine mussels and oysters [9]. Furthermore, three mytiloxanthin analogs containing a 3,6-epoxy-end group (**5**), an allenic end group (**6**), and a 3,4-dihydroxy-β-end group (**7**) were isolated from the oyster [12,13]. Compound **6** was assumed to be a metabolic intermediate from fucoxanthin to mytiloxanthin. A 3,6-epoxy derivative of diadinoxanthin, named cycloidadinoxanthin (**8**), was also isolated from the oyster [12]. In addition to these C_{40}-skeletal carotenoids described above, two new C_{37}-skeletal carotenoids, **9** and **10**, were isolated from the oyster [12,13]. Compound **9**, having a 6-hydroxy-3-oxo-ε-end group, was assumed to be a metabolite of peridinin (Scheme 1).

Some edible clams have a bright orange or red color due to the presence of carotenoids. Fucoxanthin 3-ester (**11**) and fucoxanthinol 3-ester (**12**) were found to be major carotenoids in *Mactra chinensis* [14], *Ruditapes philippinarum*, and *Meretrix petechialis*.

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[1] *Abbreviations used*: NMR, nuclear magnetic resonance; MS, mass spectrometry; HPLC, high performance liquid chromatography; MS/MS, mass spectrometry/mass spectrometry; LC/MS, Liquid chromatography mass spectrometry; CD circular dichroism spectrometry, LC/NMR Liquid chromatography nuclear magnetic resonance.

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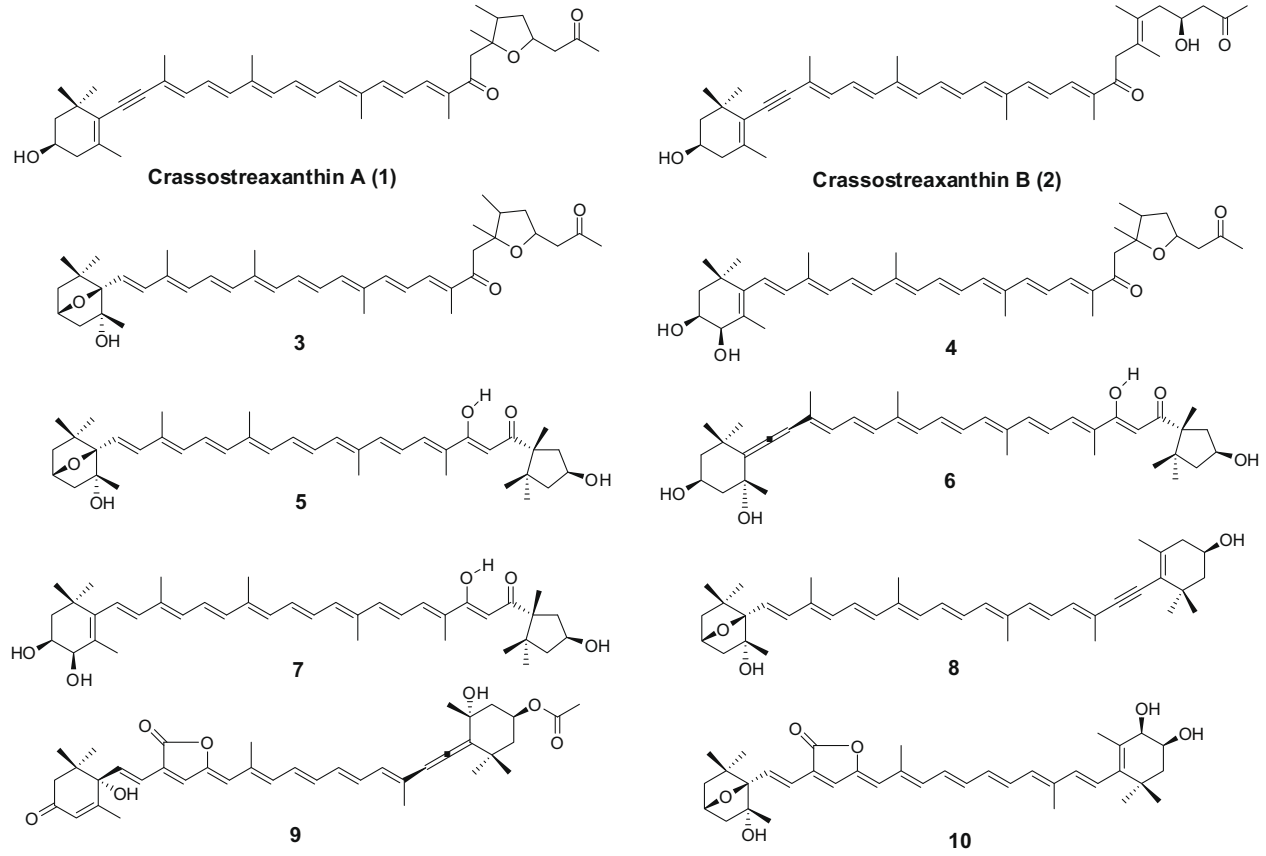
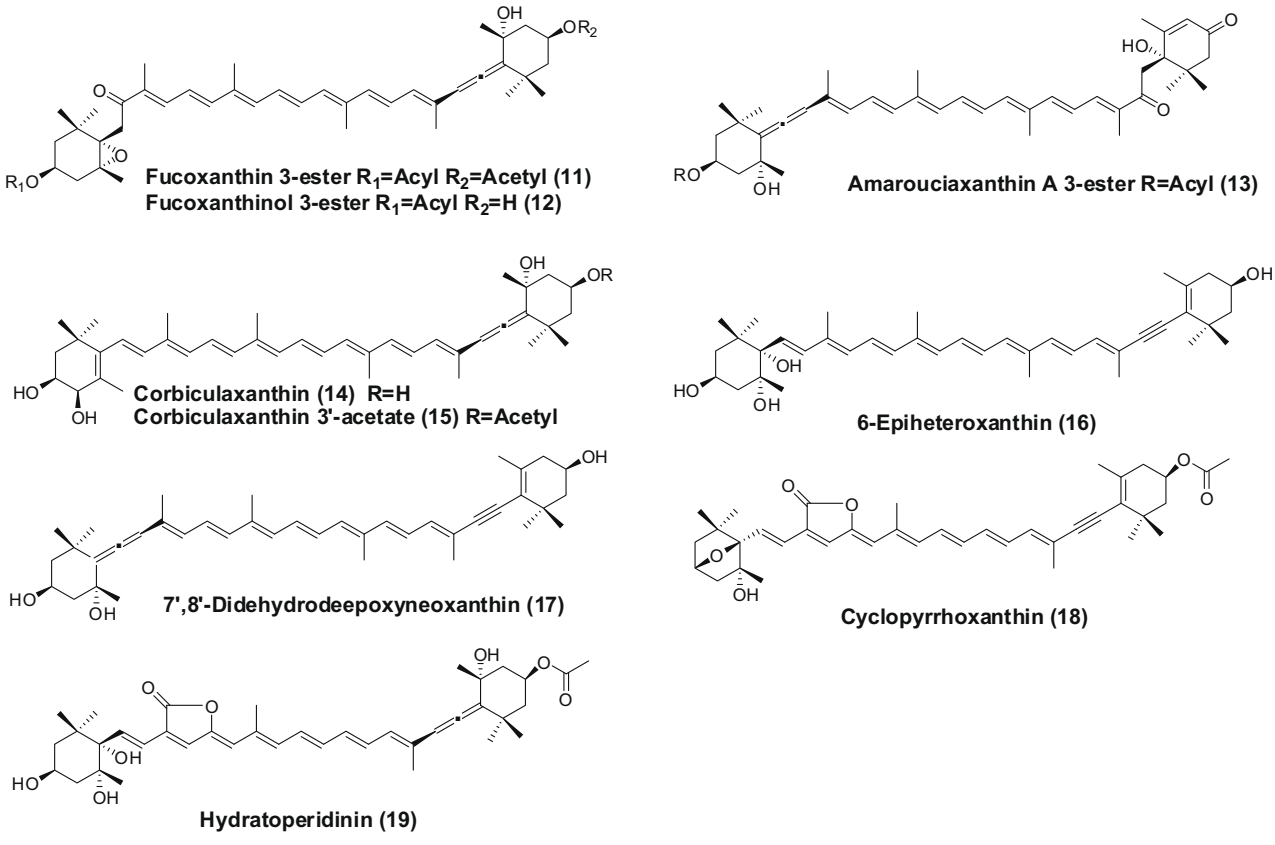


Scheme 1.



Scheme 2.

Amarouciaxanthin A 3-ester (**13**) was also identified as a major carotenoid in *Paphia amabills*. These structures were characterized using ^{1}H NMR and MS spectrometry. Fatty acids esterified with these carotenoids were assigned as C24:6, C22:5, C22:6, C20:5, C20:0, C20:1, C18:0, C18:1, C16:0, C16:1, and C14:0 based on FAB-MS data.

There are many reports on carotenoids in marine shellfish [6–9]. However, there are few reports on the carotenoids of shellfish inhabiting brackish or fresh water [6–9]. In Japan, *Corbicula japonica* inhabits brackish water and *C. sandai* and *C. leana* inhabit fresh water. Therefore, we studied the carotenoids of these three species of *Corbicula* clams.

Forty-three carotenoids, including six new ones, were isolated from the three species of *Corbicula* clams [15,16]. Among them, peridinin derivatives were found only in *C. japonica*, which inhabits brackish water. On the other hand, lutein and loroxanthin were found only in *C. sandai* and *C. leana*, which inhabit fresh water. Fucoxanthin and diatoxanthin derivatives were found in all three species [15]. From the carotenoid patterns in *Corbicula* clams, it can be concluded that the brackish water clam mainly feeds on dinoflagellates and diatoms and freshwater clams mainly feed on green algae and diatoms.

Four new carotenoids, corbiculaxanthin (**14**), corbiculaxanthin 3′-acetate (**15**), 6-epiheteroxanthin (**16**), and 7′,8′-didehydrodeepoxyneoxanthin (**17**), were found in all the above three species of clam but not in other clam species [15,16]. Therefore, they are assumed to be carotenoids peculiar to the genus *Corbicula*. 7′,8′-Didehydrodeepoxyneoxanthin (**17**) has an interesting structure with both allenic and acetylenic moieties. Two new metabolites of peridinin, cyclopyrrhoxanthin (**18**), and hydratoperidinin (**19**), were isolated from *C. japonica*, which inhabits brackish water [15,16] (Scheme 2). Carotenoids found in these animals provide a key to the food chain as well as metabolic pathways [9].

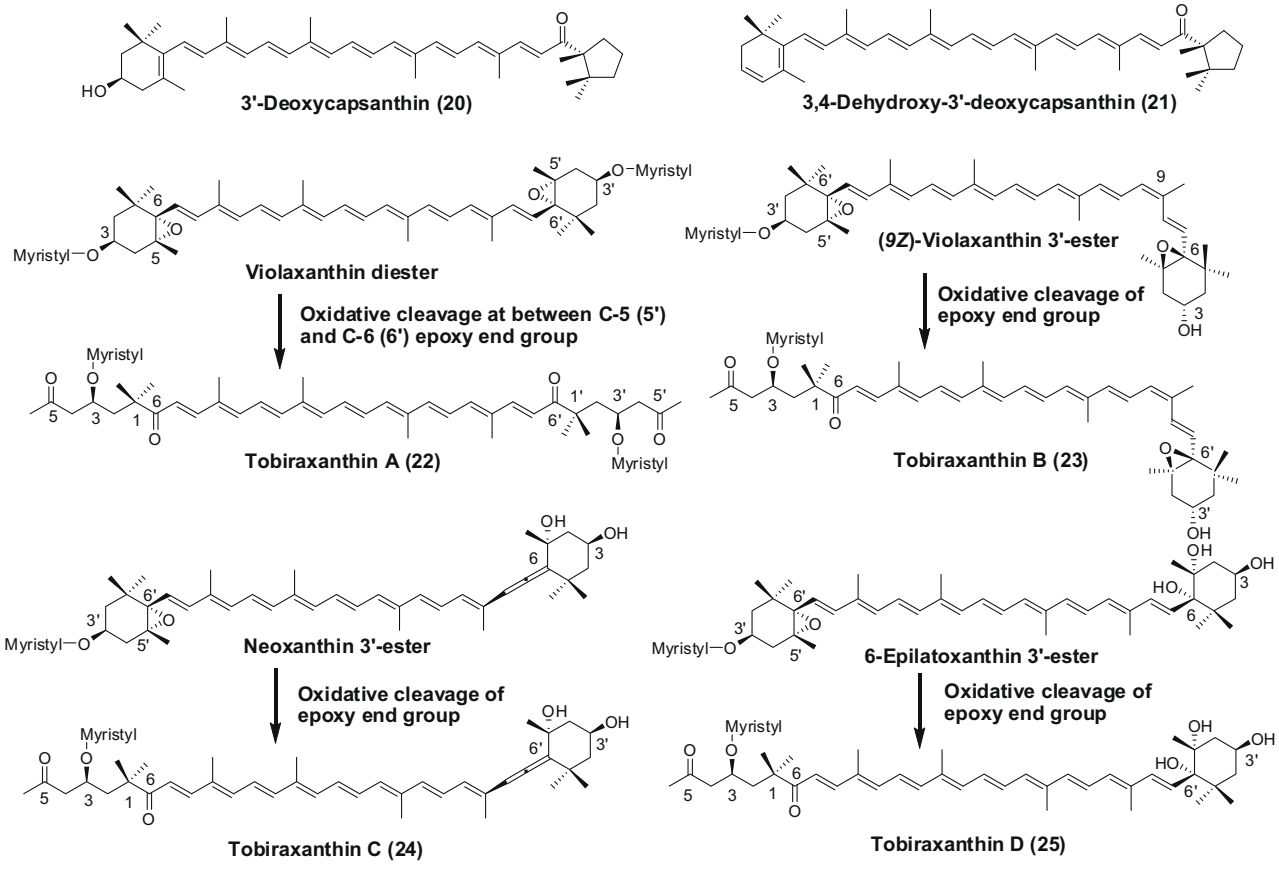
Carotenoids in higher plants

Carotenoids present in photosynthetic organs have been extensively investigated. These biosynthetic roots were revealed by enzymatic and gene level experiments [17]. On the other hand, carotenoids present in non-photosynthetic organs such as fruits, seeds, and flowers, showed structural diversity. Some of the carotenoids found in non-photosynthetic organs were formed by secondary metabolic reactions, such as oxidation, the cleavage of polyene chains, (*Z*/*E*) (*cis*–*trans*) isomerization, etc. [17]. Therefore, new structural carotenoids can still be found in fruits, seeds, and flowers in higher plants.

Carotenoids with 6-oxo-κ-end group in paprika

Paprika, *Capsicum annuum*, is a good source of carotenoids. Major carotenoids in paprika are capsanthin and capsorubin, containing a 3-hydroxy-6-oxo-κ-end group. These carotenoids show a strong singlet oxygen quenching activity [18] and also the inhibition of lipid peroxidation induced by free radicals [19]. They also possess excellent anti-cancer activities [20].

Several unique structural carotenoids have been reported in paprika by the University of Pécs group in Hungary [21]. Our group also isolated a series of apocarotenoids derived from capsanthin from paprika [22]. Furthermore, two new carotenoids, named 3′-deoxycapsanthin (**20**) and 3,4-dehydroxy-3′-deoxycapsanthin (**21**), were isolated from paprika as very minor components [23]. These carotenoids have a 6-oxo-κ-end group. Carotenoids with a

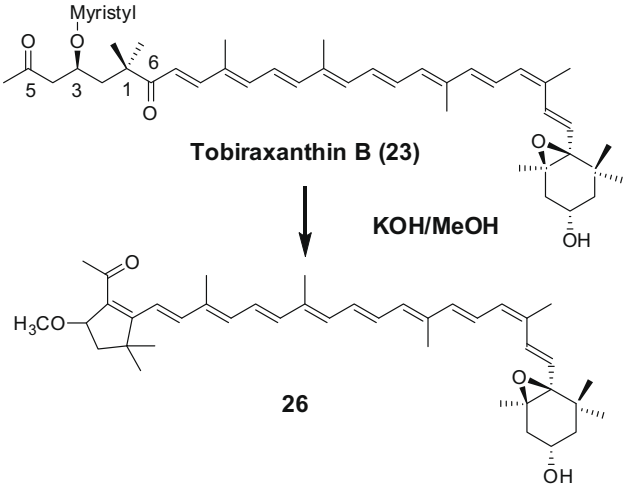


Scheme 3.

6-oxo-κ-end group have not been reported previously in nature. These carotenoids were assumed to be formed from β-cryptoxanthin 5′,6′-epoxide by pinacolic rearrangement through capsanthin capsorubin synthase [23].

Seco-carotenoids in the seeds of Pittosporum tobira

Pittosporum tobira is a small, slender, evergreen tree growing in southern Japan. In summer, the seeds have a pale yellow color. In autumn, the seeds are exposed to sunlight and change color from yellow to red.



Scheme 4.

The major carotenoids in the yellow seeds are violaxanthin, (9*Z*)-violaxanthin, antheraxanthin, and neoxanthin esters. On the other hand, a series of acylated seco-carotenoids having a 3-acyloxy-5,6-diseco-5,6-diketo-β-end group, named tobiraxanthin A (**22**), B (**23**), C (**24**), and D (**25**), were isolated from red seeds as major carotenoids [24]. 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′) bond(s) in the violaxanthin diester, (9*Z*)-violaxanthin 3′-ester, neoxanthin 3′-ester, and 6-epilatoxanthin 3′-ester, respectively as shown in Scheme 3.

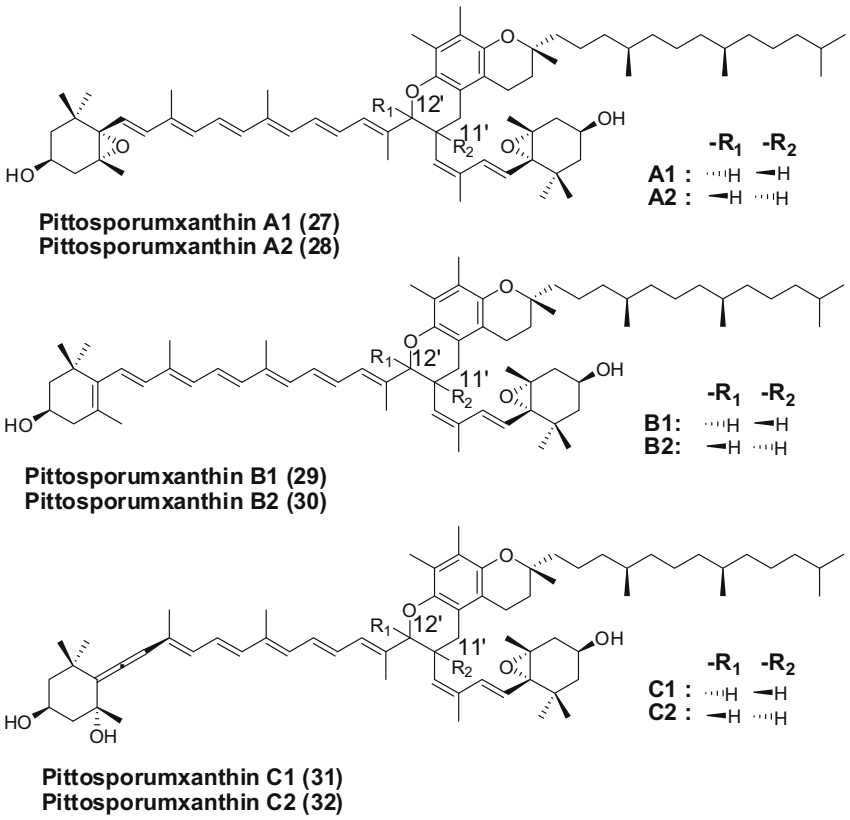
These seco-carotenoids (**22**–**25**) showed on approximately 15–30 nm longer wavelength shift than their corresponding precursor epoxy carotenoids because of the introduction of conjugated carbonyl group(s) at C6 (C6′) by the oxidative cleavage of C5–C6 (C5′–C6′) bond(s). Therefore, it was revealed that the color change from yellow to red in the seeds is due to the formation of these seco-carotenoids from epoxy carotenoids. Furthermore, it was assumed that these seco-carotenoids protected the seeds from oxidation induced by sunlight and singlet oxygen because it was reported that the introduction of the conjugated double bond system in carotenoids enhanced the singlet oxygen quenching activity [25,26].

Moreover, seco-carotenoids with a 3-acyloxy-5-hydroxy-5,6-diseco-6-keto-β-end group were also isolated from the red seeds [27].

These seco-carotenoids showed lability in alkaline medium. Tobiraxanthin B was converted to a carotenoid with a 3-methoxy-5-keto-5,6-seco-4,6-cyclo-β-end group (**26**) on treatment with KOH/MeOH [24] as shown in Scheme 4.

Carotenoid and α-tocopherol complexes in the seeds of P. tobira

A series of carotenoid and α-tocopherol complexes named pittosporumxanthins were isolated from the red-colored seeds of *P.*



Scheme 5.

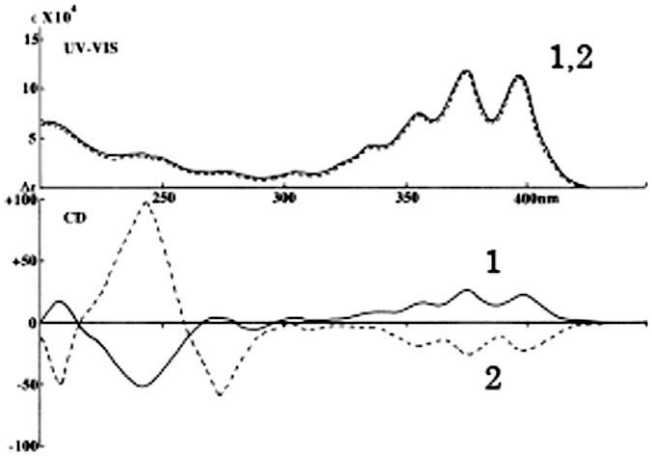


Fig. 1. UV–vis (top) and CD (bottom) spectra of **1**: pittosporumxanthin A1 and **2**: pittosporumxanthin A2 in ether.

tobira [28,29]. Pittosporumxanthin A1 (**27**) and A2 (**28**) 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 (**29**) and B2 (**30**) and (9′*Z*)-neoxanthin, and α-tocopherol complexes, named pittosporumxanthin C1 (**31**) and C2 (**32**), were isolated. These structures were elucidated by the detailed analysis of NMR and MS, including MS/MS experiment and CD spectral data as shown in Scheme 5. The absolute configurations of the carotenoid end groups in the pittosporumxanthins were determined by a modified Mosher's method using ^{1}H NMR spectroscopy. Through the addition of α-tocopherol at the C-11′ and C-12′ positions in the polyene chain of carotenoids, two diastereomeric pairs were formed. Mirror image CD spectra were observed between diastereomeric pairs of pittosporumxanthins A1 and A2, between B1 and B2, and between C1 and C2. Fig. 1 shows the UV–vis and CD spectrum of pittosporumxanthins A1 and A2. These CD spectra were assumed to have originated from excitation coupling of $\pi \rightarrow \pi^*$ absorption of hexaene (or heptaene) around 370 nm and diene around 240 nm. Therefore, from the excitation chirality rule of the CD spectrum [30], the (11′*R*, 12′*S*) chirality for pittosporumxanthins A1, B1, and C1 and (11′*S*, 12′*R*) chirality for pittosporumxanthins A2, B2, and C2 were determined.

Pittosporumxanthins are reaction products of epoxy carotenoids and α-tocopherol. Therefore, it was assumed that epoxy carotenoids such as violaxanthin, antheraxanthin, and neoxanthin in seeds of *P. tobira* might quench α-tocopherol radical through the formation of adduct products in their polyene chain.

Conclusion

Interesting new structural carotenoids can still be found in plants and animals. The improvement of analytical tools such as NMR, MS, CD, HPLC, LC/MS, LC/NMR etc., has made it possible to perform the structural elucidation of very minor carotenoids. Structures of new carotenoids provide information on the function, biosynthesis in plants, and a key to the food chain as well as metabolic pathways in animals.

Acknowledgments

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