Biochemical Studies on Carotenoids

(I) Carotenoids in Green Leaves of Spinach and Carrot

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The purification and identification of carotenoids in spinach leaves, carrot leaves and roots were carried out by column chromatography on silica gel. Especially, the three major carotenoids in spinach leaves which were more polar than chlorophyll were identified as lutein, auroxanthin and crocetin against the lutein, violaxanthin and neoxanthin which have been known as carotenoids in green leaves. Furthermore, the latter two were foundto contain galactose as structural components. From these results, it woud be decided that the major carotenoids in green leaves were β-carotene, lutein, auroxanthin and crocetin. This conclusion will be important to discussion for the physiological function and the roles of carotenoids, and therefore further investigation will be necessary.

In comparison with carrot leaves and its roots, non polar hydrocarbon carotenoid (β-carotene etc.) was much more exsistent in root than the polar, in which especially the most polar carotenoid crocetin was negligible small, while, in leaves the polar carotenoids occupied more than 60 %. From the fact that this tendency in leaves was also recognized in spinach leaves, and therefore the polar carotenoid was much more existent in green leaves which have photosynthetic function than root, it was suggested that the polar carotenoids might be physiologically active.

Introduction

Chlorophyll, one of the representative pigments in green leaves, is related closely to the photosynthesis, and its features in green leaves and the physiological roles has been considerably resolved. On the other hand, carotenoid pigments, which widely distributed in green leaves in the same manner with chlorophyll and might be conceivable to play important roles, has been scarecely elucidated on its existence from in nature. In plants carotenoids have been found as fatty acid ester[1)2)], glycosides[3)-6)] and carotenoid chromoproteins[7)] as well as free form, while it has been known[8)] that all green leaves contained carotenes, lutein, violaxanthin and neoxanthin as the general major carotenoids in a free form. Because in green leaves carotenoids were found to be located specifically in the grana of the chloroplast under the electromicroscopic observation, and the chlorophylls were also located in the same sites, it has been considered that the carotenoids might not be existent in a simple free form, but rather complexed form in some interaction with other lipids, saccharides and proteins.

Up to date, various physiological roles have been suggested for carotenoids in photosynthesis, such that are energy transfer to chlorophyll[9)-11)], electron transfer[12)], oxygen evolution[13)], and protection of chlorophyll against photoxidation[14)].

Carotenoids have been classified to carotenes and xanthophylls according to the structure and the polality. In algae it has been found that more polar carotenoids containing saccharides were existent[3)-6)]. The carotenoid carboxylic acid and its ester have been also reported as the carotenoids containing saccharide by ZECHMEISTER et

al (bixin, crocetin and crocin)[15)-17)]. Crocin, bis-gentiobiosyl crocetin, was the major pigment of safflan and this carotenoid was found to be present in this plant as the glycoside picrocrocin[18)].

It has been obvious that xanthophylls in green leaves were lutein, zeaxanthin, cryptoxanthin, violaxanthin and neoxanthin[19)], and it has been regarded that these xanthophylls were not existent as the derivatives with other component, though some of neoxanthin was not yet clarified for the structure.

Hitherto, the extractive and preparative method of these carotenoids were mainly in accordance to AOAC methods, which was direct extraction from green leaves. By this method, it was conceivable with probability that only carotenoid components were seperated from the complexed carotenoids in vivo. Especially, in preparation of carotenoid chromoprotein, it was ascertained by the fact that as increasing acetone ratio to water, more carotenoid was extracted increasingly and the ratio of carotenoid to protein decreased[7)].

This report describes the extraction of carotenoids by Folch method, subsequent to purification by the relatively mild mehtod, to clarify the existent form of carotenoids in green leaves and their physiological roles. From the results, the different carotenoid pattern from the known one in green leaves was obtained and it was also revealed that the carotenoids containing galactose were existent.

Experimental

1. Materials

Spinach and carrot were used for materials, which were cultured at the experimental farm in Okayama University. Spinach leaves were washed with water, removed water by allowing to stand at dark place for one night, and its roots were cut off. Carrot leaves were treated as the same of spinach. Carrot root were washed and wiped by filter paper.

2. Methods

(1) Visible and UV absorption spectrum estimation

Shimadzu Maltipurpose MPS—50 L autorecording spectrophotometric apparatus was used for this purpose. Solvents used mainly were petroleum ether, or diethyl ether.

(2) IR absorption spectrum estimation

The estimation was carried out by general KBr tablet method with Hitachi EPI-G 3 spectrophotometric apparatus.

(3) Quantitative analyses of carotenoids

Sample carotenoid solution in petroleum ether was messed up to appropriate volume and an aliquot was put into cell followed by spectrophotometric estimation with 450 nm wave length.

The standard curve, which was previously drawn in relation to β-carotene concentration and its optical density at 450 nm, was used for this analyses of sample carotenoids. Therefore, the carotenoid contents might be expressed as β-carotene.

(4) Qualitative analyses of carotenoids

(HCl—Ether Test)

Carotenoids was dissolved in 2 ml ether to adjust to O.D. 1.0 and then 1 ml of conc. HCl was added. If both 5, 6 and 5, 8 epoxides were present, the color from light green to dark blue was formed.

(HCl—Ether—MtOH Test)

19 ml of carotenoid solution dissolved in ether (10 ml) and methanol (9 ml) was prepared. To aliquot of this solution, and 1 ml of water, and to another 1 ml of

conc. HCl. The solution added with HCl will often become greener at once if an epoxide is present. After 1 hr., acid treated diepoxide solution was generally greenish blue, monoepoxide yellowish green.

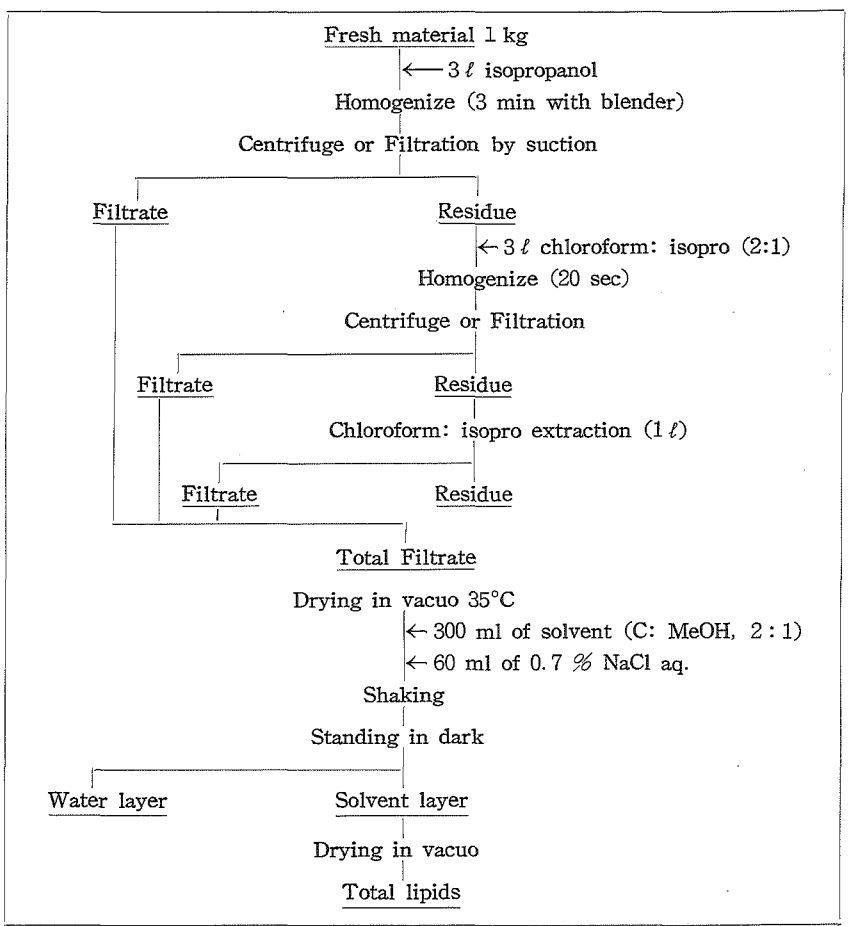
(Test for 5, 6 Epoxides)

Pipet out equal aliquots of the carotenoid solution (10 ml of O.D. 1.0 solution). Evapolate in vacuo and dissolve the residues in 5 ml of ether and transfer to test tubes of the same diameter. To one, add 5 ml of methanol and to another, 5 ml of a 1 % solution of citric acid in methanol. Spectrophotometric curves were run after 1 hr over the range 340—750 nm. If a shift to short wave side is observed, 5, 6 epoxides is present.

(Test for allylic hydroxyl group[21])

A carotenoid was dissolved in 9 ml methanol and 1 ml of concentrated HCl. It was allowed to stand for 10 min. An excess of 20 % KOH in methanol was added. It was then diluted with distilled water, extracted with ether and evaporated under reduced pressure. A bathochromic shift in spectral maxima denotes dehydration of allylic hydroxyls.

Table 1. Preparation of total lipids



(Detection of ketonic carotenoids[21])

The carotenoid solution in 95 % ethanol was kept over night in the refrigerater after several crystals of $NaBH_4$ were added. It was extracted with ether, washed several times with distilled water and then evaporated under reduced pressure. Any hypsochromic shift in absorption maxima is indicative of the presence of ketone groups.

(Detection of glycolipids and phospholipids)

Detection of glycolipids on TLC was carried out by using Bial reagent and diphenylamine reagent as usual. Detection of phosphlipid was carried out by using Dittmer reagent ant Hans-Isherwood reagent.

Results and Discussion

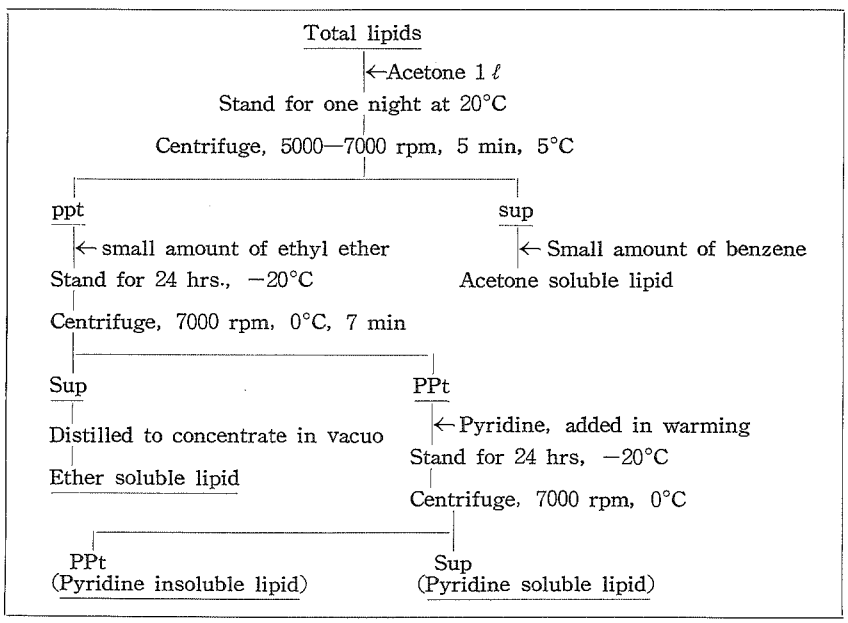
1. Extraction and preparation of total lipids

Total lipid of spinach and carrot leaves was prepared by the Folch method[22] as indicated in Table 1. In the treatment of carrot root the residue at the last extraction was homogenated in morter with sea sand untill the residue became colorless.

2. Solvent fractionation of total liplds with acetone, ether and pyridine

The total lipids were solvent fractionated with acetone, ether and pyridine by the method shown in Table 2.

Table 2. Solvent fractionation of total lipids



The lipid components in each fraction were detected by TLC on Kiesel Gel G. From the result, it was found that most part of carotenoids were contained in acetone soluble lipid. Fig. 1 showed the detection of glycolipids and phospholipids in these fractions. The spots corresponding to glycolipid were in agreement with the carotenoid yellow spots, while no phospholipid was detected at the part of these yellow spots. Therefore, it was conceivable that the carotenoids which were developed as spot S—II and S—III in Fig. 1 might be the one containing saccharide as structural components. The same detection was carried out on the carrot leaves and its roots (Fig. 1).

From these results, it was found that some carotenoids positive to glycolipid test were existent in the more polar fraction than chlorophyll on TLC, but no carotenoid positive to phospholipid test.

As indicated above, carotenoids in acetone soluble fraction were consisted of four groups, these being non polar carotenes, and other three groups (I, II and III) which were more polar than chlorophyll. The latter three groups were designated S—I, S—II and S—III for spinach leaves and C—I, C—II and C—III for carrot to discriminate material sources. Moreover, the II and III groups were found to have a saccharide as the structural component.

3. Fractionation of each carotenoid with column chromatography

A preliminary fractionation was carried out by partition column chromatography on silica gel (Wakogel C—200) saturated with petroleum ether, because the acetone soluble lipid could be seperated at least four components at the TLC on Kieselgel G. The petroleum ether or benzene solution of the acetone soluble lipid was placed on the column and washed twice with small amounts of petroleum

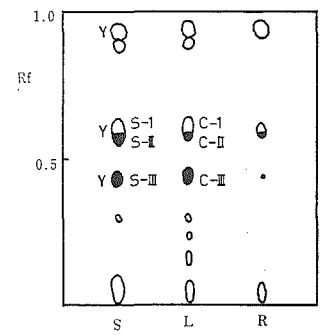


Fig. 1. TLC of acetone soluble fraction of spinach leaves, carrot leaves and root, followed by detection of glycolypid.

S : spinach leaves; L : carrot leaves; R : carrot root
Solvent : Be : EtAc : MtOH (6 : 3 : 1)
Plate : kiesel gel G, 0.25 mm thick
"Y" indicate yellow spots, and black spots is the positive to Bials reagent (see experimental).

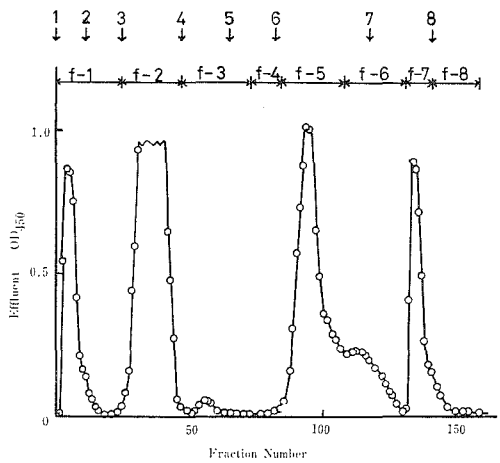


Fig. 2. Chromatography of spinach acetone soluble lipids Wakogel C—200 column.

Arrows over the chromatogram indicate the exchanged point of solvents.

Column, 25 × 20 cm. 1 fraction, 10 g. Elution was carried out stepwisely. Solvents and fractions, see Table 3. Total carotenoids in the sample, 150 mg. Recovery of carotenoid, more than 95 %.

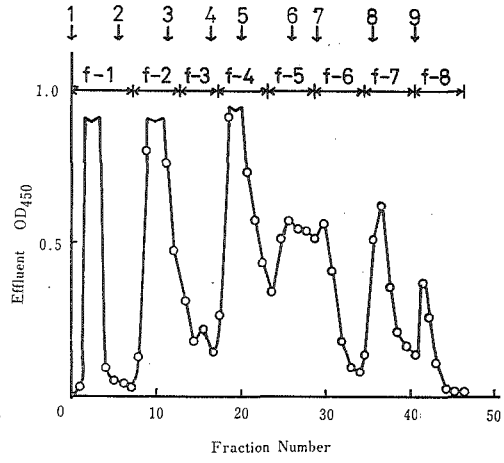


Fig. 4. Chromatography of carrot leaves acetone soluble lipids on Wakogel C—200 column.

Arrows over the chromatogram indicate the exchanged points of solvents. Column, 1.0 × 15 cm. 1 fraction, 10 g. Elution was carried out stepwisely. Solvents and fractions, see Table 4. Recovery, more than 95 %.

Table 3. Relation between solvents and fractions at chromatography of spinach acetone soluble lipid

No.	Solvent	Eluting volume	Fraction No.	Component of fraction*
1	Benzene	200 ml	f—1	Carotenes
2	Be:E (8:2)	200 ml	f—1	Carotenes
3	Be:E (7:3)	200 ml	f—2	Chlorophyll mainly
4	Be:E (6:4)	300 ml	f—3	Tailing component of f—2
5	Be:E (5:5)	200 ml	f—4	〃
6	Be:EtAc (5:5)	400 ml	f—5	Carotenoid S—I
			f—6	〃 S—II
7	Be:MeOH (8:2)	200 ml	f—6	〃 S—II
			f—7	〃 S—III
8	MeOH	450 ml	f—8	Lipid other than carotenoid

* Concluded by the results on TLC

Table 4. Relation between solvents and fractions in chromatography of carrot leaves acetone soluble lipid

No.	Solvent	Eluting volume	Fraction No.	Component of fraction*
1	Benzene	80 ml	f—1	Carotenes
2	Be:E (8:2)	80 ml	f—1	〃
			f—2	Chlorophyll mainly
3	Be:E (7:3)	40 ml	f—3	Xanthophylls
4	Be:Ace (8:2)	40 ml	f—3	〃
			f—4	Carotenoid L—I, II
5	Be:Ace (7:3)	70 ml	f—4	〃
6	〃 (6:4)	50 ml	f—5	Carotenoid L—III
7	〃 (4:6)	80 ml	f—6	Lipid other than carotenoid
8	Be:MeOH (8:2)	70 ml	f—7	〃
9	MeOH	100 ml	f—8	〃

*Concluded by the results on TLC

Table 5. Relation between solvents and fractions in chromatography of carrot root acetone soluble lipid

No.	Solvent	Eluting volume	Fraction No.	Component of fraction*
1	Benzene	100 ml	f—1	Carotenes
2	Be:E (8:2)	65 ml	f—1	〃
3	Be:E (7:3)	70 ml	f—2	Chlorophyll
4	Be:Ace (8:2)	50 ml	f—3	—
5	Be:Ace (7:3)	100 ml	f—3	—
6	Be:Ace (6:4)	50 ml	f—4	Carotenoid R—I, II
7	Be:Ace (4:6)	50 ml	f—4	〃
8	MeOH	150 ml	f—5	〃 R—III
			f—6	Lipids other than carotenoid

*Concluded by the results on TLC

ether (or mixture with benzene), followed by the first elution with benzene. The typical chromatogram were given in Fig. 2, 4 and 5 and the relation between solvents and eluting fractions were also given in Table 3, 4 and 5, and furthermore typical TLC of each fraction in Fig. 3. It was found that the more polar carotenoids were fairly purified with preliminary silica gel column chromatography.

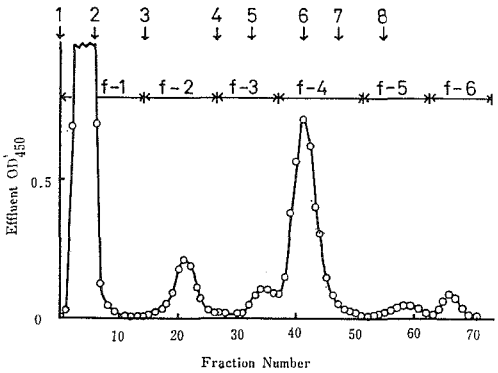


Fig. 5. Chromatography of carrot root acetone soluble lipids on Wakogel C—200 column.

Arrows over the chromatogram indicate the exchanged points of solvents. Column, 1.0 × 15 cm. 1 fraction, 7 g. Elution, stepwisely. Solvents and fractions, see Table 5.

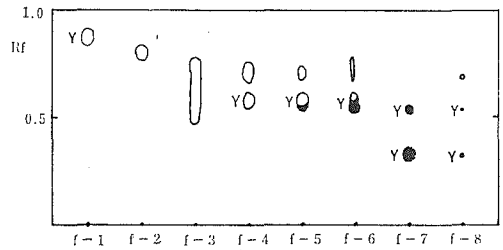


Fig. 3. TLC of each eluting fraction of spinach acetone soluble lipids on Kiesel gel G using Be : EtAc : MeOH (6 : 3 : 1).

Black spots, positive to Bial's reagents. "Y" indicate carotenoids spots.

4. Quantitative ratio of carotenoids in spinach and carrot

After saponification of each fraction in Table 3, 4 and 5 at 20°C for 2 days, carotenoid in each fraction was extracted with ether and its optical density estimated at 450 nm followed by converting its value of individual carotenoids to that of β-carotene.

The relative amounts of individual carotenoid are given in Table 6.

Table 6. Carotenoids contents in each fraction of column chromatography of spinach and carrot lipids (%)

Fraction	Spinach leaves	Carrot leaves	Carrot root	Carrot*
f—1	46.5	37.5	74.2	55.9
f—2	13.1	3.5	4.7	4.1
f—3		8.1	2.0	5.0
f—4	19.4 (S—I)	48.8 (L—I, II)	17.8 (R—I, II)	33.3 (I, II)
f—5		6.7 (L—III)	1.0 (R—III)	3.9 (III)
f—6	7.2 (S—I, II)	1.2	0.3	
f—7	11.9 (S—III)			
f—8	1.9			
Total	100	100	100	

*Carotenoid contents in total carrot

From these results it was found that in spinach leaves f—1 was the most abundant in carotenoid followed by f—4 (S—I), f—7 (S—III) and f—6 (S—I, II). However, in carrot leaves f—1 was less than f—4 (L—I, II), while in carrot root f—1 came to

74 % and f—5 (R—Ⅲ) was very small. The average value (the fourth column in Table 6) of each carotenoid in total carrot was similar to that in spinach leaves, and especially a similar pattern might be indicated between the non porlar carotenoids (f—1) and teh polar (I, Ⅱ and Ⅲ).

It was deduced from these results that the more polar carotenoids (I, Ⅱ and Ⅲ) existent abundantly in green leaves would be physiologically active in photosynthesis, while non polar carotenoids (f—1) which was major component in root carotenoids would have the different roles, such as preserved compounds, precursor of other carotenoids or photoprotective stabilizer.

5. Isolation and identification of spinach leaves carotenoids

(1) Rechromatography

Carotenoids S—I, Ⅱ and Ⅲ in spinach leaves which were prepared by column chromatography mentioned above were placed into rechromatography to isolate. The whole sample were dissolved in benzene. Rechromatography was carried out on Wakogel C—200 column with the following chromatographic conditions.

(Rechromatography of S—I)

Column; 2.5×25 cm. Eluting solvents; 1) Be : Ether (8 : 2) 200 ml, 2) Be : Ether (7 : 3) 300 ml, 3) Be : Ether (6 : 4) 500 ml, 4) Be : Ether (5 : 5) 400 ml, 5) Be : MeOH (8 : 2) 200 ml, 6) MeOH 200 ml. With batch system the colored bands were fractionated to 8 fractions. The fractions eluted by No. 3 and 4 solvent gave a single spot, negative to Bial reagent, slightly soluble in ether (20°C) and very soluble in ethyl acetate. When these fractions were dissolved in ether in the water bath and standed for at −20°C, the dark reddish crystal could be obtained. The visible spectrum of crystalized S—I was shown in Fig. 7.

(Rechromatography of S—Ⅱ)

It was found that S—Ⅱ shown in Fig. 3 and Table 3 was the mixture with S—I and gave spot positive to Bial reagent just under the S—I spot.

Column, 20×20 cm. Eluting solvents; 1) Be : Ether (8 : 2) 2000 ml, 2) Be : Ether

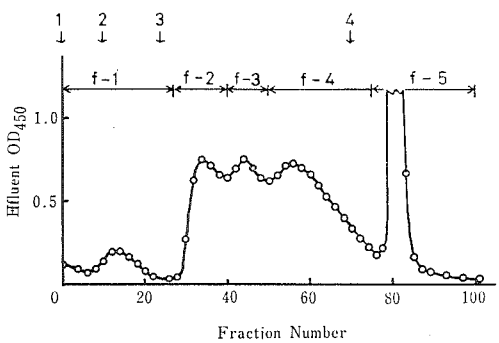


Fig. 6. Rechromatography of spinach carotenoid S—Ⅲ on Wakogel C—200 column.

Column, 2.0×30 cm. 1 fraction, 10 g. Eluting solvents, (1) Be : Ether (8 : 2) 200 ml, (2) Be : Ether (7 : 3) 500 ml, (3) Be : EtAc (5 : 5) 1000 ml, (4) Be : EtAc : MeOH (5 : 5 : 1) 200 ml.

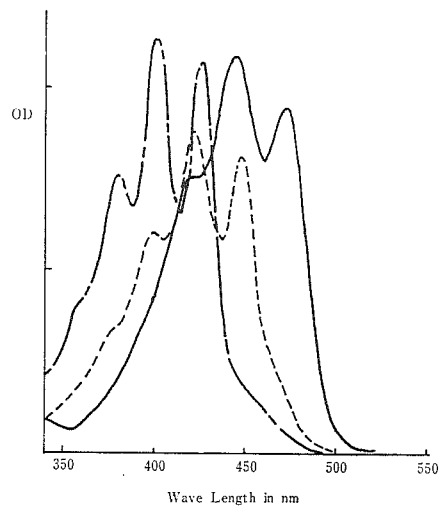


Fig. 7. Visible absorption spectrum of spinach carotenoids, S—I, S—Ⅱ and S—Ⅲd issolved in ether.

——S—I; — - S—Ⅱ; - - - S—Ⅲ.

(7 : 3) 400 ml, 3) Be : Ether (6 : 4) 200 ml, 4) Be : EtAc (5 : 5) 200 ml, 5) Be : MeOH (8 : 2) 100 ml, 6) MeOH 200 ml. Rechromatography was repeated twice for reasons of difficulty to fractionate S—II from S—I.

Finally, by batch system 5 fractions were obtained. It was found that the fraction eluted by No. 3 or 4 solvent was isolated as S —II carotenoid and this carotenoid discolored to blue by exposure to air after the development of TLC.

(Rechromatography of S—III)

S—III was found to have the strongest polarity in green leaves carotenoids, and positive to Bial reagent. By rechromatography shown in Fig. 6, five fractions were obtained. Fractions eluted by No. 3 solvent might be indicated as isolated materials, and these visible spectra were shown in Fig. 7.

(2) Identification of carotenoid components in spinach carotenoids S—I, S—II and S—III

It was found from the results described above that spinach carotenoids might be generally constituted from four groups, in which S—II and S—III contained saccharides. From the difference of the visible spectrum in Fig. 7, it was obvious that these two carotenoids had different carotenoid components. The visible absorption maxima in each solvent were shown in Table 7 to compare with the reference. For detection of carotenoid epoxides, HCl—Ether test, HCl—Ether—MeOH thst and citric acid test were carried out (Table 8). In addition to these detection, test for allylic hydroxyl groups and for ketonic groups were carried out, and no shift at λ_{max} in S—I, S—II and S—III was observed, which no allylic hydroxyl and ketonic groups in these carotenoids.

Table 7. Visible absorption maxima of carotenoids (nm)

Carotenoid	PE	n—Hexane	CS_2	Benzene	EtOH	Chloroform
S—I	425, 445, 473	425, 445, 473	445, 475, 506	436, 459, 488	428, 447, 475	436, 455, 483
S—II	380, 401, 426	380, 402, 427	405, 427, 454	389, 411, 437	381, 403, 427	388, 410, 436
S—III	398, 420, 447	400, 421, 446	425, 447, 476	408, 431, 459	400, 423, 449	407, 430, 458
Lutein	420, 447, 477	—	445, 475, 508	—	420, 446, 476	428, 456, 458
Violaxanthin	443, 472	—	440, 470, 501	428, 453, 483	420, 441, 471	424, 451, 482
Auroxanthin	382, 402, 427	—	423, 454	—	381, 402, 427	385, 413, 438
Neoxanthin	415, 437, 466	—	463, 493	447, 477	417, 438, 467	421, 447, 477
Crocetin	400, 420, 445	—	426, 453, 482	—	—	434, 463

Table 8. Coloring test for epoxied carotenoids of S—I, II and III

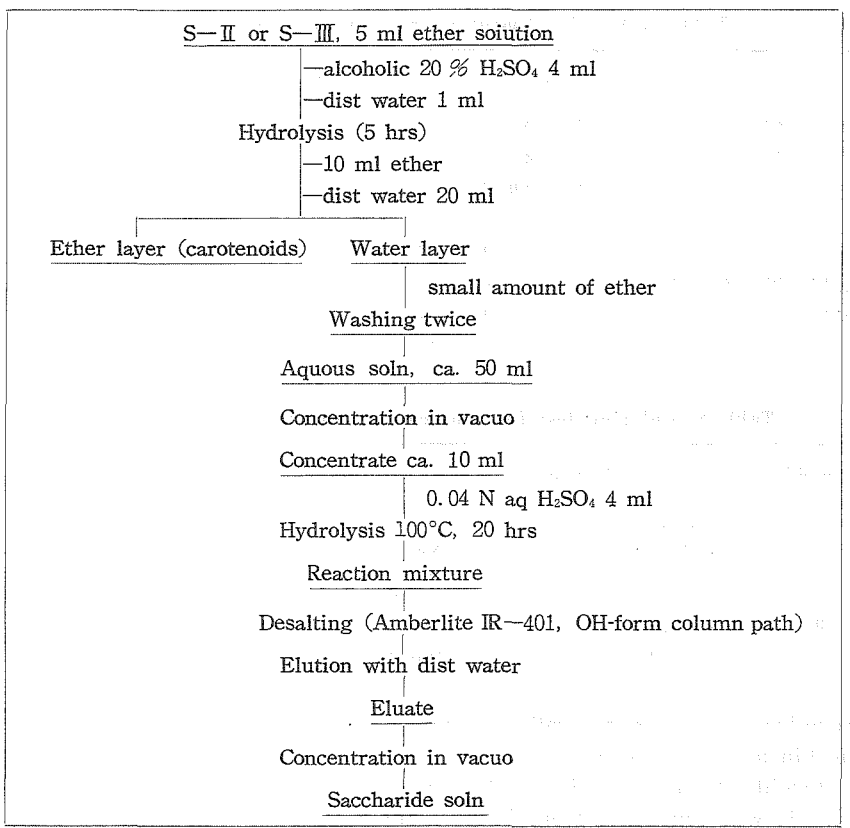
Carotenoid	HCl—Ether test	HCl—Ether—MeOH test	Citric test
S—I	− (Pale)	—	—
S—II	+ (Blue)	Yellow green, 10 min Blue, 60 min Purple, 24 hrs	—
S—III	+ (Pale Blue) (Violet)	Green, 1 hr Purple, 24 hrs	—

In green leaves occured generally four major carotenoids that were β-carotene, lutein, violaxanthin and neoxanthin[8]. The latter three carotenoids might be correspond to the carotenoids (S—I, II and III) in this report which was more polar than chlorophyll on TLC. It was confirmed from Table 7 and 8 that the carotenoid S—I was lutein.

Up to date the reports on violaxanthin and neoxanthin[23)-26)] have presented only the visible absorption maxima as the most confidencely data. The comparison of visible absorption maxima between these reference carotenoids (violaxanthin and neoxanthin) and spinach carotenoids (S—II and S—III) was shown in Table 7. From these data, spinach carotenoid S—II did not correspond to violaxanthin reported by GOODWIN[23)] but auroxanthin closely. This might be supported by the fact that carotenoid S—II was positive to general epoxide test but negative to 5, 6 epoxide test and S—II might, therefore, have 5, 8 epoxide groups. Furthermore, S—III might correspond to crocetin rather than neoxanthin from the data in which the visible absorption maxima (Table 7) and the fine structures of the visible spectrum were both in good agreement with crocetin. However, from the fact that in infra red spectrum of S—III the weak absorption at 1735 cm^{-1} and 1160—1190^{-1} (absorption of $c=o$) was observed, and the greater part of the carotenoid to ether layer after alcoholic KOH saponification of S—III, it might be necessary to correct the aspect that crocetin so called was the carotenoid dicarboxylic acid with C_{20}. This conclusion on S—III was also supported by the fact that S—III was positive to epoxide test although crocetin had no epoxide group.

From the results comparing with the experimental data and the reference, it might be indicated that S—I was lutein, S—II auroxanthin and S—III crocetin. However, as it appears to have a little relation between the polar carotenoid structures and the behaviors in chromatography and color test, it was conceivable that further investigations were necesary.

Table 9. Preparation of saccharides iu carotenoids S—II and S—III



Furthermore, in these results it deduced that the major xanthophylls in green leaves of higher plants might be lutein, auroxanthin and crocetin instead of lutein, violaxanthin and neoxanthin described in reference[19].

(3) Identification of saccharide component

The identification of the saccharides which were contained in S—II and S—III carotenoide was carried out. The preparative method of the saccharide was shown in Table 9. In this preparation, it was the reasoning of twice acid hydrolyses that in the first hydrolysis the color reaction and behavior on paper chromatography of the products were different from those of general saccharides, and so the existence of alkoxy delivatives might be suggested.

(Color reaction of saccharides)

Initially, the color reactions of carotenoid S—II and S—III were carried out for detection of saccharides. In these results, it was found that both S—II and S—III might contain aldohexose or aldopentose. Subsequently the color reaction of saccharide solution prepared in Table 9 was carried out (Table 10). It was assumed that the

Table 10. Coloring test of saccharides S—II and S—III

Test	S—II	S—III	Glu	Gal	Glu. acid	Rham	Xyl
Anthron	+	+	+	+	+	+	+
α-naphthol	+	+	+	+	+	+	+
Cysteine-H_2SO_4	Yellow	Yellow	Yellow	Yellow	Red	Yellow	Yellow
o-amino diphenyl acetic acid	Dark green	Dark green	Green	Dark green	Red Green	Red brown	Red orange
Orcin-HCl	−	−	−	−	Green	−	Blue

saccharide might correspond to galactose or glucose from these results.

(Paper chromatography and gas chromatography)

Owing to decide whether the saccharide in S—II and S—III carotenoids might be galactose or glucose, the analysis by paper chromatography was carried out. In Fig. 8 it wes confirmed that these saccharides might be galactose.

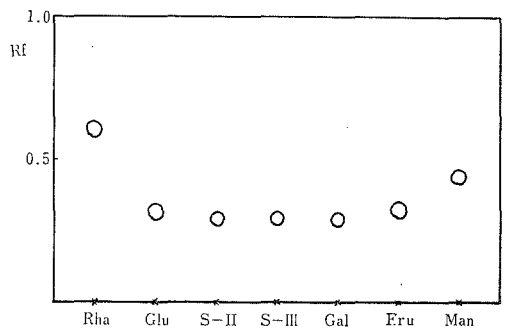


Fig. 8. Paper chromatogram of saccharides on Toyo No. 51 A.
Solvent; iso-Buthanol : Pyridine : Water : AcOH (12 : 6 : 4 : 1). Detection; Aniline—Phthalate reagent spray followed by 110°C, 10 min heating.

Furthermore, the identification by gas chromatography was carried out. This method is the qualitative analysis to detect the pattern on the chromatogram of trimethyl silylated (TMS) saccharides. TMS—saccharides were prepared according to the method of SWEELEY et al[27]. One to ten mg of sugar were weighed and dissolved in 0.5 ml of pyridine. To these solution 0.1 ml of hexamethylene disilazane and 0.05 ml of trimethyl chlorosilane were added. After shaking vigorously, these mixture were stand for 10 min, followed by gas chromatographic analyses. Sample saccharides (S—II and S—III saccharides) were evaporated to dryness under reduced pressure at 70°C, and 0.1 ml of pyridine were added, followed by of trimethyl silylation. The results was shown in Fig. 9

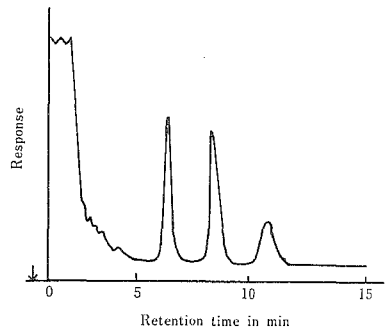


Fig. 9. Gas-chromatography of TMS S—II and S—III saccharides.

Analytical conditions : Apparatus; Shimadzu 3 AF Type. Column; Stainless steel 3 mm × 3 m, dual column. Packing; EGS 15%. Support; Neopak 1 A 60—80 mesh. Carrier gas; N_2, inlet pressure of 1.7 kg/cm². Detector; FID. Temperature of column bath detector bath and injection port; 160°C.

Table 11. Retention time TMS saccharides

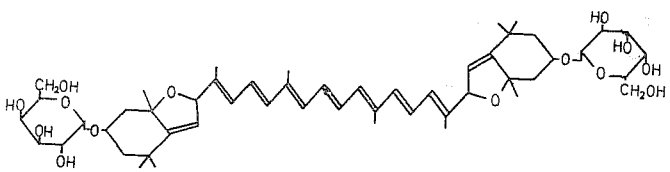
Saccharide	Retention time in min
Glu	8.6, 14.6
Gal	7.7, 8.3, 11.1
Man	5.7, 10.4
Fru	8.6, 11.2
Rham	2.4, 3.3
S—II sacch	7.6, 8.2, 11.1
S—III sacch	7.7, 8.2, 11.1

and Table 11.

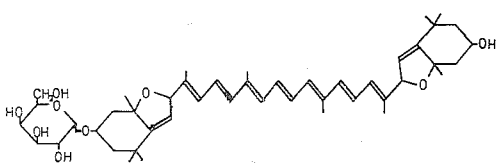
The retention time of these peaaks of S—II and S—III on chromatogram correspond to that of galactose. Therefore, both saccharides of S—II and S—III were identified with galactose.

(4) The structure of carotenoid galactose compound

It was confirmed that carotenoid component of S—II might be auroxanthin and that of S—III crocetin, while the saccharide component was both galactose. Therefore, the following 4 structural formula might be considered (Fig. 10 and 11).

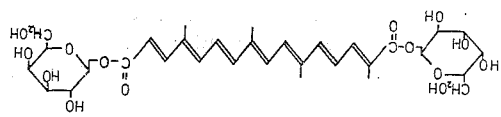


3, 3′—digalactosyl auroxanthin

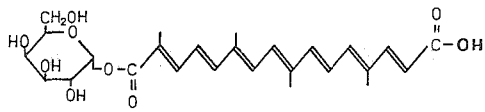


3 (Or 3′)—galactosyl auroxanthin

Fig. 10. Spinach S—II garotenoid—galactose compound.



Digalactosyl crocetin



Monogalactosyl crocetin

Fig. 11. Spinach S—Ⅲ carotenoid—galactose compound.

As the molar ratio between carotenoid and galactose was not found, the accurate structure of S—Ⅱ and S—Ⅲ were not identified at present. As found in these structure, the polality of the carotenoids were higher than that of general carotenoid. However, it seemed that the behaviors on the chromatography does not correspond to the higher polality. Therefore, it was conceivable that the alcoholic hydroxyl of galactose were alkoxylated (methylated or ethylated), and this has been remained as future problem.

Reference

1) Kuhn R. et al. (1931) : Ber., 64, 326
2) Karrer P. et al. (1929) : Helv. Chim. Acta., 12, 790
3) Hertzberg S. and Liaaen—Jensen S. (1967) : Acta Chem. Scand., 21, 15
4) ibid (1969) : Phytochem., 8, 1259
5) ibid (1969) : Phytochem., 8, 1281
6) Aasen A. J., Francis G. W. and Liaaen—Jensen S. (1969) : Acta Chem. Scand., 23, 2605
7) Nishimura M. and Takamatsu K. (1957) : Nature (London), 180, 699
8) Goodwin T. W. (1965) : Chem. and Biochem. of Plant Pigments, p. 127, Acad. Press, London and New York
9) Warburg O. et al. (1954) : Angew. Chem., 66, 493
10) Cholnoky L. et al. (1956) : Nature, 178, 410
11) Hattorri S. and Shimokoriyama M. (1967) : Natural Pigments, p. 19, Asakura, Tokyo
12) Yamashita K., Konishi K., Itoh M. and Shibata K. (1969) : Biochim. Biophys. Acta, 172, 511
13) Dorough G. D. and Calvin M. (1951) : J. Amer. Soc., 73, 2362
14) Griffith M., Sistrom W. R., Cohen-Bazire G. and Stanier R. Y. (1955) : Nature (London), 176, 1211
15) Zechmeister L. et al. (1944) : J. Amer. Chem. Soc., 66, 322
16) Inhoffen I. et al. (1953) : Liebigs Ann. Chem., 58, 7
17) Zechmeister L. (1962) : Cis-Trans Isomeric Carotenoids Vitamin A and Arylpolyenes; wien Springer-Verlag
18) Kuhn R. and Winterstein A. (1934) : Ber., 67, 344 Pigman W. and Horton B. (1970) : The Carbohydrates, p. 229, Academic Prese, London
19) Livingston B. E. M. et al. (1954) : Agri. Food Chem., 2, 563
20) Curl A. L. (1961) : Agri. Food Chem., 9, 403
21) ibid (1962) : Agri. Food Chem., 10, 504
22) Rosalita R. De. La Mar, et al. (1969) : J. Food Sci., 34, 287
23) Goodwin T. W. (1955) : In "Modern Methods of Plant Analysis" Vol. Ⅲ, p. 272, Springer, Heiderberg
24) Wierzchowski Z. et al. (1962) : Roczn. Nauk. Roln., 81, B. 87

25) Eugster C.H. and Karrer P. (1957) : Helv Chim. Acta, 40, 69
26) Krinsky N.I. and Goldsmith T.H. (1960) : Arch Biochem. Biophys., 91, 271
27) Weeley C.C.S., Betley R., Makita M. and Wells W.W. (1963) : J. Amer. Chem. Soc., 85, 2497

カロチノイドの生化学的研究

(第1報) ホウレン草と人参緑菜のカロチノイドについて

高木茂明・白石正英

ホウレン草緑葉，人参葉及び人参根から Folch 法によってカロチノイドを調製し，精製を行なった．なかでもホウレン草中のカロチノイドについてはクロロフイルよりも極性の大きい3種の（S—I，S—II，S—III）カロチノイドを単離し，カロチノイド部分の同定を行なった結果，S—I は Lutein，S—II は Auroxaanthin，S—III は Crocetin であった．S—II とS—III は糖陽性であり，その構成糖を分析の結果ガラクトースであることが確認された．

従来，緑葉中の主要カロチノイドとして β-Carotene，Lutein，Violaxanthin，Neoxanthin が挙げられているが，本報告の結果からは β-Carotene，Lutein，Auroxanthin，Crocetin が明らかとなっており，この点は緑葉の機能とカロチノイドの生理活性などを論じる場合重要な事項となるので，さらに検討を進めて行きたい．

人参葉と根のカロチノイドを比較すると，炭化水素系の非極性カロチノイド量が根に圧倒的に多く，極性の大きいそれは約20%に過ぎない．とくにS—III（Crocetin）に相当するものの含量比が小さい．いっぽう葉では前者が少なく，極性カロチノイドは約60%に達する．この葉におけるカロチノイド含有比率の傾向はホウレン草葉についても認められ，極性カロイドが根よりも光合成機能を有する緑葉に多い事実は，これらカロチノイドの生理活性の示唆するものである。

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〃	下から1行	and	add
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