The Carotenoids Pigments of Diatoms in Plankton Ice in Antarctica

Keiji HARASHIMA*, Mitsuko ISHIDA* and Hiroshi MEGURO**

プランクトンアイス中の ケイソウのカロチノイド色素

原島圭二*・石田満子*・目黒　熙**

要　　旨

南極 Lützow-Holm 湾沖で採集したプランクトンアイス中の植物性プランクトン（主として硅藻 *Fragilariopsis cylindrica* の carotenoids 色素を抽出し，アルミナのカラムにより9つの分画を得た．アルミナに対する吸着度及び吸収スペクトルにより最も多量に含まれるのは fla-voxanthin であり，他に lutein 様 carotenoids も確認した．従来硅藻に通常含まれていると考えられている carotenes の分画がなく，親水性の水酸基を2個以上持つと考えられる分画が8分画を占めた．プランクトンアイス中の硅藻が通常の硅藻とその carotenoids の組成が異なる点は，その特殊な光合成環境から考えて興味深いものがある．

Plankton ice is a phenomenon of bloomings of phytoplanktons in sea ice of Antarctic ocean. The ecological and glaciological meanings were reported by MEGURO, H[1]. According to FUKUSHIMA, H, the flora consists of diatoms, and *Fragilariopsis cylindrica* was the dominant species of 24 examples among 25 samples. It is significant that plankton ice may be considered as a natural selective culture of special diatoms under a special condition. As a place of photosynthesis the sun light is supplied through a filter of snow 30 to 40 cm thick which screens ultraviolet, infrared and near infrared region of visible light.

In order to get some information of photosynthetic pigments of the diatoms the carotenoids pigments were studied.

The sample was collected in pack ice on Jan. 25, 1961 off Lützow-Holm Bay, Antarctica (Lat. 68°11′5″ S, Long. 38°42′ E) during the fifth Japanese Antarctic Research Expedition. It was stored in refrigerator at −20°C in dark until September 1961 for about eight months.

Nine carotenoids fractions were separated by column chromatography and the absorption spectra were measured. Some comparisons with the carotenoids of familiar

* Department of Agricultural Chemistry, Faculty of Agriculture, the University of Tokyo. 東京大学農学部農芸化学教室.
** Faculty of Agriculture, Tohoku University. 東北大学農学部食糧化学教室.

diatoms and standard pigments were also carried out. The results are summarized as follows.

1) The main component (H_1+H_2) that consists of about 40% of the total carotenoids was identical with flavoxanthin absorption spectrum, chromatographic behaviour (Fig. 1) and colour reaction with hydro-chlorlicacid. H_1 and H_2 are considered as the same compound.

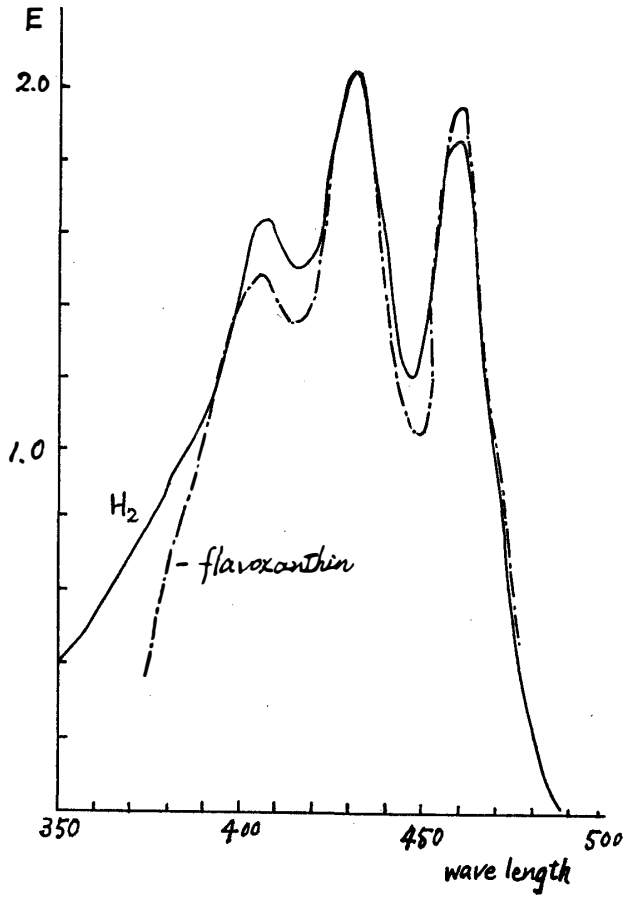
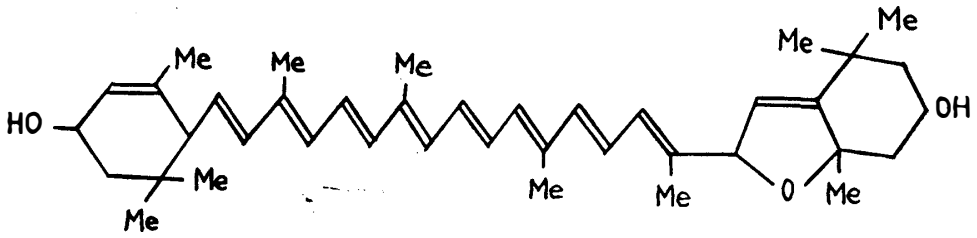


Fig. 1. Absorption spectra of flavoxanthin and fraction H_2 in benzene.
** R. Kuhn and H. Brockman: Z. Physiol, Chem., **213**, 192 (1932)*



2) Carotenes were not found. As an epiphatic carotenoid fraction A which had absorption maximum of 380 $m\mu$ was detected, but it was too small to be identified (Fig. 2).

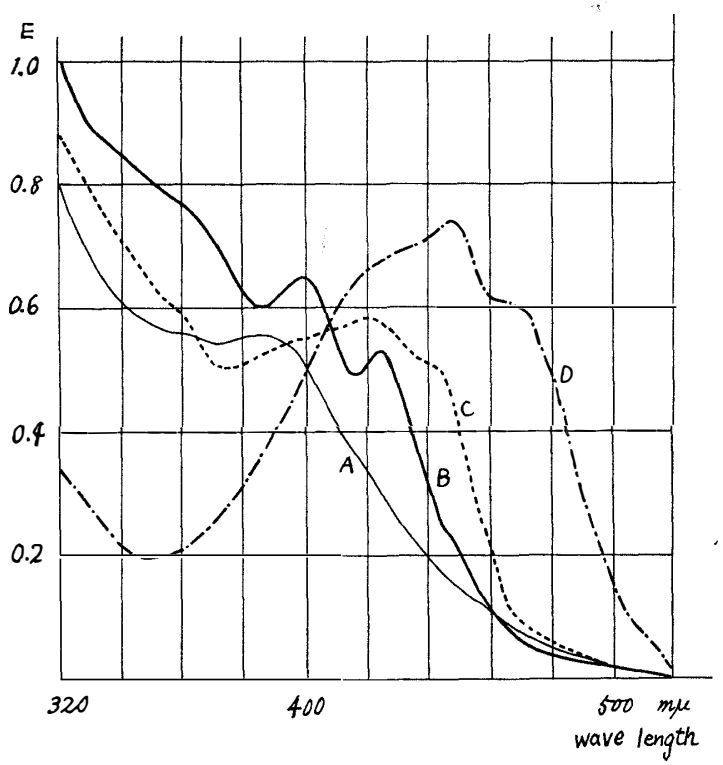
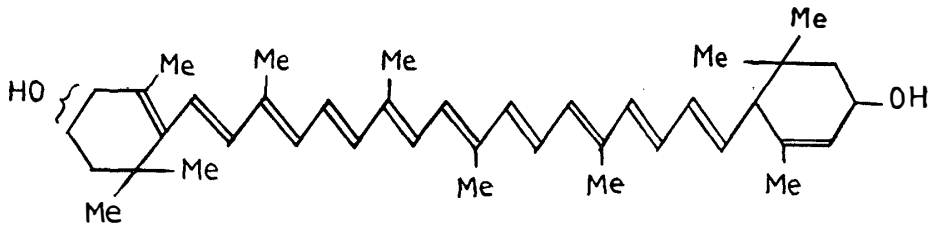


Fig. 2 Absorption spectra of fraction A, B, C, D in petroleum benzin.

3) Fraction F that consists of about 10% of the total carotenoids was identical with lutein from the absorption spectra and the chromatographic behaviour (Fig. 3).

The same fraction was found also in diatoms (*Skeltonema*) that was sampled at Aburatsubo in Tokyo Bay and cultured for the comparison.



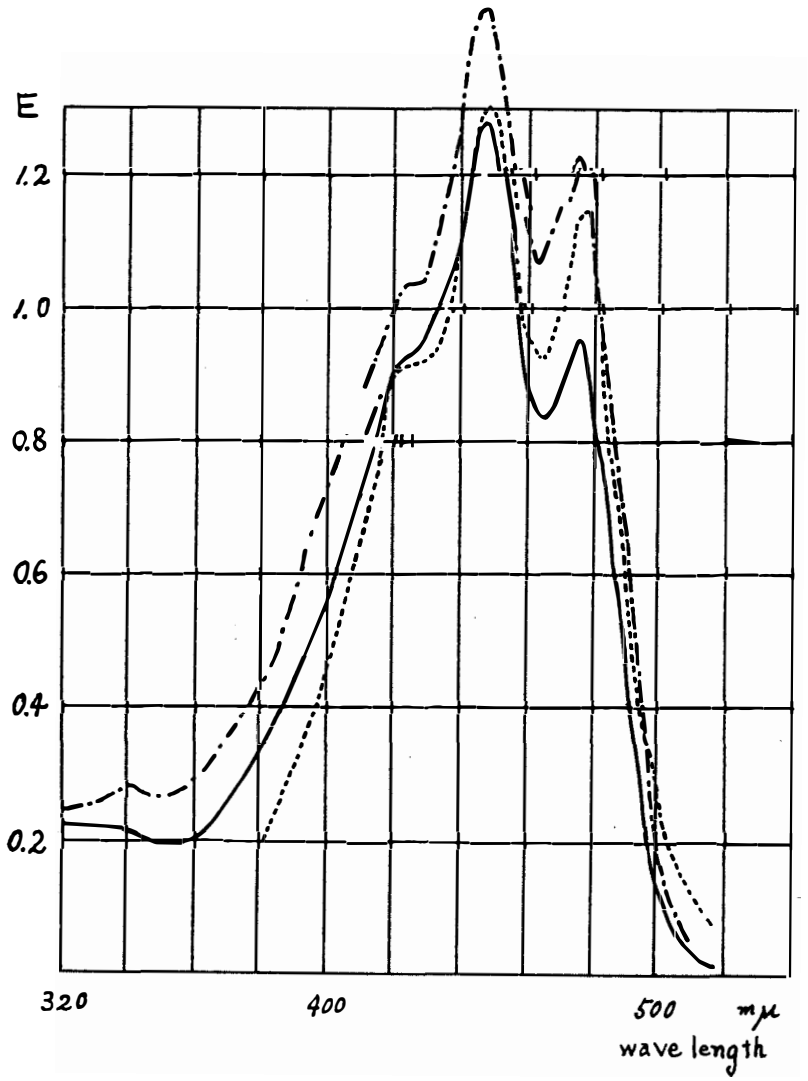


Fig. 3 Absorption spectra of
*..... lutein in ethanol**
——— fracton F in petroleum benzin
-·-·-·-·- lutein-like fraction obtained from cultured diatoms in ethanol
** F. P. Zscheile et al.: Plant Physiol., 17, 331 (1941).*

4) Fraction I consists 20% of the total carotenoids. It was also found in carotenoids of a diatoms mixture (*Chaetoceros, Coscinodiscus, Bacteriastrum, Rhizolema*) which was sampled in Tokyo Bay. It was not identified yet (Fig. 4).

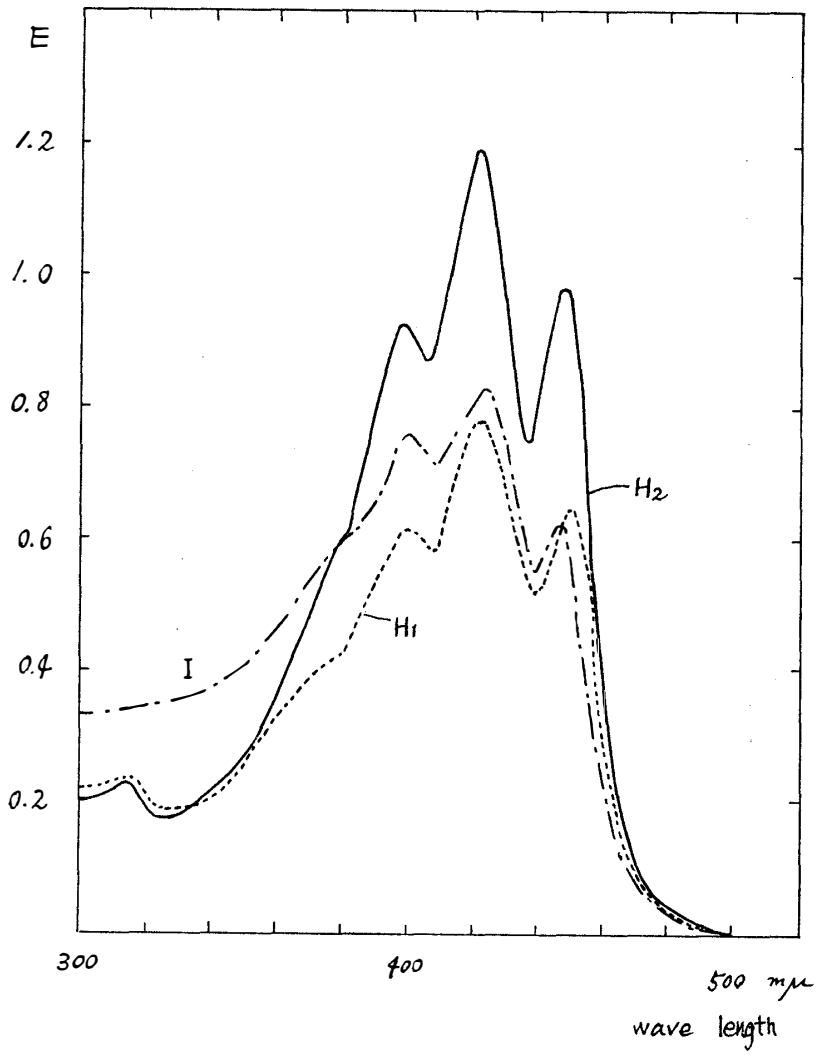


Fig. 4. Absorption specta of fraction H_1, H_2 I in ethanol.

5) Fraction E and G respectively consist of 10% of the total carotenoids. From their chromatographic behaviour they are considered to have more than two hydroxyl groups. The absorption spectra were shown in Fig. 5 but not yet identified.

6) Fraction B, C and D are trace components in total carotenoids and from their chromatographic behaviour and partition test they are all considered to have more than two hydroxyl groups. Absorption spectra were shown in Fig. 2.

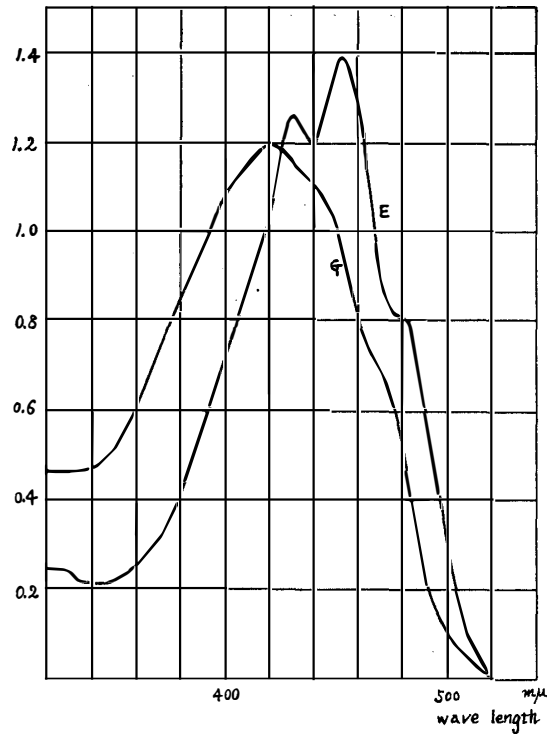


Fig. 5. Absorption spectra of fraction E, G in petroleumbenzin.

Discussion

It must be noted that carotenes were not found. All the fractions, except Fraction A, were hysopasic and considered to have more than two hydroxyl groups. β-carotene[2] has been considered to be present in most diatoms. Fucoxanthin was also not found, although it has been reported to be present in many diatoms. It means that carotenoids composition of the diatoms in plankton ice was different from that of the common diatoms. It is a future problem if the differences are due to the species (*Fragilariopis cylindrica*) or due to physiological conditions.

We must also consider some biological or chemical oxidations during the storage. The expedition could not allow us to carry out this experiment just after the sampling.

Experimental

1) Plankton ice 9.3 kg was melted at room temperature. Diatoms were collected on filter paper by filtration under reduced pressure. This filter paper was blendered with 80% acetone and extracted repeatedly with the same solvent. The extract was extracted with petroleum benzin until all the yellow pigments were transferred to petroleum benzin layer. It was condensed to 100 ml under a reduced pressure an 1-N ethanolic-potassium hydroxide solution was added and allowed to stand over a night. It was

Table 1. Carotenoids fractions of diatoms in plankton ice on column chromatography.

Fractions	Effluent acetone% in petroleum benzin	Volume of effluent	Partition test between 90% MeOH and petroleum benzin	Percentage in total carotenoids
A	0.2%	10 ml	epiphatic	trace
B	4-7%	20 ml	hysophasic	〃
C	10-15%	20 ml	〃	〃
D	15-20%	10 ml	〃	〃
E	15-20%	10 ml	〃	10 %
F	15% (with 1-2ml MeOH)	10 ml	〃	10 %
G	50 %	—	〃	10%
H_1		—	〃	40%
H_2		—	〃	
I		—	〃	10%

washed with dil-alkali solution and water, dried and condensed for chromatographic separation.

2) The sample was absorbed on a weakly activated alumina column (diametre 8 mm, length 80 mm). The column was washed with petroleum benzin and eluted with acetone-petroleum benzin mixtures. As shown in Table 1, six fractions were eluted with increased concentration of acetone. More strongly absorbed pigments were taken out directly from cutting off the colour bands of the column followed by extraction with acetone-benzine solution (1:1). Each fraction was named according to the order of elution A, B, C, D, E, F, G, H_1, H_2 and I.

Absorption spectra were measured by Carry-spectrophotometer (Figs. 2-5).

3) In order to examine the number of hydroxyl radical partition test between petroleum ether and 90% methanol was carried out. The results are shown in Table 1, by the term of hysophasic or epiphatic.

4) When the main component H was evaporated, the colour became green and when its etherial solution was treated with hydrochloric acid, the colour changed to blue. Both the colour reactions were the same as those of flavoxanthin.

References

1) Meguro, H.: Antarctic Record, No. *14*, 72 (1962).

2) Goodwin, T. W.: The Comparative Biochemistry of the Carotinoid (1952), Chapman & Hall, London.

(Received Dec. 27, 1963)