*On Carotenoids of a Sponge “Reniera japonica”**

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The orange-colored sponge “*Reniera japonica*” which is widely distributed in the tidal zone in our shores contains a number of polyene pigments consisting of various carotenoid hydrocarbons with different types of chromophores as well as of several oxygenated carotenoids.

The six carotenoid hydrocarbons isolated in crystalline states from this sponge have already been reported in the preceding paper[1]. In the present paper are reported the results obtained by further investigations which have clarified to a considerable extent the total composition of the coloring matters of this sponge by the isolation of nine different polyene pigments in crystalline states in addition to the above six hydrocarbons.

The materials were collected near the Amakusa Marine Laboratory attached to the Faculty of Science, Kyushu University. The sponge was extracted with acetone and petroleum benzine, and after saponification the crude pigment which consisted mainly of carotenoid hydrocarbons was precipitated from the petroleum benzine solution by the addition of ethanol. The crude pigment and the mother liquor were separately subjected to chromatography. Almost all zones in the chromatograms afforded crystallized pigments. All pigments obtained are listed in Table I in the sequence of increasing adsorbability. Several of these pigments appear to be new pigments hitherto unknown.

It should be emphasized that only one species of sponge contains such numerous polyene pigments. Of course it may be possible that a few minor pigments do not belong to this sponge but, in reality, to a small quantity of algae with which the sponge is inevitably contaminated. Of these pigments listed above, renieratene predominates in quantity, the pigments which melt at 199° and β-carotene are the next, and the quantities of other pigments are much smaller.

TABLE I

PIGMENTS OBTAINED FROM “*Reniera japonica*”

Spectral absorption maxima in CS_2; in mμ	Melting point	Yield from 5.7 kg. of sponge; in mg.
507, 478, 447 (α-carotene)	185°	2
520, 485, 452 (β-carotene)	183°	20
520, 484, 452	199°	45
524, 488, 453	194°	—[a]
532, 496, 463 (renieratene)	185°	93
536, 499, 467	205°	1.1
544, 504, 475 (renierapurpurin)	230°	4.4
531, 494, 463	123°	—[a]
558, 521, (492) (renieraxanthin)	161°	2.6
548, 512, (471)		—[a]
495, a single maximum		—[a]
507, 475, 445		—[a]
518, 483, 452[b]	153–4°	0.4
530, 493, 461[c]	173–4°	1.0
558, 517, (485)		—[a]

a) As the yields of these pigments were very small, they were not weighed.
b) Not identical with cryptoxanthin.
c) Not identical with rubixanthin, nor probably with gazaniaxanthin.

The main pigment “renieratene” seems to have a molecular formula $C_{40}H_{48}$ or $C_{40}H_{50}$ on the basis of the analytical data, and it shows the highest carbon content of the carotenoid pigments hitherto reported. The study on the structure of this unique carotenoid hydrocarbon is now in progress.

The pigment of m. p. 199° is of interest, since it shows the same spectral properties as those of β-carotene. It closely resembles leprotin[2] ($C_{40}H_{54}$; abs. max. in CS_2, 517, 479, 447 mμ; m. p. 198–200°) especially in melting point and in spectral properties. However, as the elementary analysis showed 90.91% carbon and 9.38% hydrogen, which corresponds to the formula $C_{40}H_{48}$ or $C_{40}H_{50}$, it may not be said safely that

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* “Pigments of Marine Animals”, III. II of this series; *J. Chem. Soc. Japan.* (*Pure Chem. Sect.*), **75**, 605 (1954)

[1] T. Tsumaki, M Yamaguchi and T. Tsumaki, *J. Chem. Soc Japan.* (*Pure Chem. Sect.*) **75**, 297 (1954).

[2] C. Grundmann und Y. Takeda, *Naturwissenschaften*, **25**, 27 (1937); Y. Takeda und T. Ohta, *Z. Physiol. Chem.* **258**, 6 (1939).

the two pigments are identical with each other.

The pigment of m. p. 230° also appears to be a new carotenoid having a molecular formula $C_{40}H_{50}$. This pigment is termed renierapurpurin by the author.

These three hydrocarbons have a common feature of high carbon content, and therefore these pigments appear to be closely related to one another in their constitutions.

Although the pigment of m. p. 161° forms only a small proprotion of the total amount of all the pigments obtained, it is of importance, since it is the greatest in quantity among the oxygenated pigments isolated. This pigment resembles rhodovibrin[3] which has been isolated by Karrer and his co-workers from rhodovibrio-bacteria, but the identity is improbable. The name renieraxanthin is tentatively proposed for this pigment by the author. The elementary analysis shows that this pigment has the molecular formula $C_{40}H_{56}O_2$. Both the oxygen atom in the molecule do not appear to exist in the form of hydroxyl groups or a carboxyl group, since the pigment exhibits an almost epiphasic nature in the partition test. The possibility of the presence of epoxide groups may also be excluded on the basis of the negative result in the color test with concentrated hydrochloric acid. The solution of this pigment in petroleum benzine is orange, while the solution in ethanol is wine-red, and, though the former exhibits an absorption spectrum with three sharp maxima, the latter shows a single banded absorption spectrum. These facts indicate that renieraxanthin contains at least one carbonyl group in the molecule, since these phenomena are generally accepted as the typical properties of carotenoids containing systems of conjugated double bonds terminated by carbonyl groups. This conclusion is also in accord with the long-wavelength locations of the absorption maxima of the pigment.

Other pigments were not analysed because of the small quantities of materials.

The coloring matter of the sponge has hitherto often been studied by different investigators on various species[4]. However, more precise investigations are relatively few. Lederer[5] observed by means of chromatography that the red sponges, "*Suberites domuncula*" and "*Ficulina ficus*" contained the following pigments: epiphasic pigments with absorption maxima similar to those of α-, β-, and γ-carotene, respectively; the corresponding oxygenated carotenoids; and a somewhat hypophasic pigment with absorption maxima similar to those of tolurin. Of these pigments, β-carotene was isolated in the crystalline state. Drumm and his coworkers[6] studied the red sponge "*Hymeniacidon sanguineum*" and isolated from its α-, β-, γ-carotene and echinenone in crystalline states. Karrer and his coworkers[7], on the other hand, described the presence of the crustacean pigment, astacene, in the red sponge of the Mediterranean Sea, "*Axinella crysta galli*". However, no elementary analysis of the pigments were reported in these miscel laneous reports.

Being compared with these results, the sponge "*Reniera japonica*" has proved to have the following characteristic features with regard to the constituents of the coloring matter.

1. The main pigment exhibiting the same spectral properties as those of γ-carotene is not γ-carotene but is a new carotenoid hydrocarbon ($C_{40}H_{48}$ or $C_{40}H_{50}$), for which the name renieratene has been proposed in the preceding paper.
2. Two hydrocarbons which show the same absorption maxima as those of β-carotene are present in considerable quantities. One of them is β-carotene and the other is leprotin-like carotenoid hydrocarbon ($C_{40}H_{48}$ or $C_{40}H_{50}$).
3. A pigment which shows the spectral properties similar to those of torulin is isolated, but it has the nature of a carotenoid ketone ($C_{40}H_{56}O_2$). It is termed renieraxanthin.
4. In addition to the pigments above described, several hitherto unknown pigments are present in small quantities, one of which has proved to be a hydrocarbon ($C_{40}H_{50}$) and is termed renierapurpurin.

Experimental

Solutions of the pigments were evaporated under reduced pressure in the stream of carbon dioxide. In chromatography benzene containing a little methanol was employed for elution. In the partition tests, petroleum benzine b. p. 70–80° and 90% methanol were employed unless otherwise mentioned. In the color tests with conc.

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[3] P. Karrer und U. Solmssen, *Helv. Chim. Acta*, **19**, 1019 (1936).

[4] P. Karrer und E. Jucker, "Carotinoide", Basel (1948), P. 95.

[5] E. Lederer, *Bull. soc. chim. biol.*, **20**, 567 (1938).

[6] P. J. Drumm, W. F. O'Connor and L. P. Renouf. *Biochem. J.*, **39**, 208 (1945).

[7] P. Karrer und U. Solmssen, *Helv. Chim. Acta*, **18**, 915 (1935).

sulfuric acid or with antimony trichloride, the pigments were dissolved in chloroform. Melting points were determined in evacuated tubes.

Extraction and the Separation of the Crude Pigment.—The sponge, 5.7 kg. (wett weight), was extracted with acetone and petroleum benzine by the addition of water. After the saponification with ethanolic potassium hydroxide, the solution was concentrated and the oily residue was treated with acetone to remove most of the colorless lipids which were less soluble in this solvent. The concentrate, 35 g., was dissolved in 60 cc. of petroleum ether, and by the addition of 50 cc. of ethanol, the crude pigment was precipitated. The crude pigment, which weighed 120 mg. after the recrystallization from benzene ethanol, was separated by chromatography on alumina into six pigments[8]. Sixtyseven mg. of renieratene, 29 mg. of the pigment of m. p. 199°, 4 mg. of renierapurpurin, 1 mg. of the pigment of m. p. 205°, and small quantities of the pigment of m. p. 194° and β-carotene were thus obtained.

Anal. (Renieratene), Found: C, 90.98, 90.62; H, 9.22, 9.34%. Molecular weight (cyclopentadecanone), 510, 579, 530.

Anal. (The pigment of m. p. 199°), Found: C, 90.91: H, 9.38%.

Anal. (Renierapurpurin), Found: C, 90.12; H, 9.36.

Calcd. for $C_{40}H_{48}$: C, 90.85; H, 9.15. Calcd. for $C_{40}H_{50}$: C, 90.51; H, 9.49%; molecular weight, 531.2.

Separation of Pigments of the Mother Liquor.—The mother liquor of the crude pigment and the filtrate of recrystallization were combined together, washed alcohol-free with water, dried with anhydrous sodium sulfate, and concentrated. The residue was treated with acetone and the colorless lipids were removed repeatedly as completely as possible. The concentrate was finally dissolved in petroleum ether and chromatographed on alumina (Merck, standardized according to Brockmann) (6×20 cm.). Petroleum benzine-benzene mixture with increasing percentage of the latter (finally 50% benzene) was employed for the developer. All zones observed are listed in Table II, the lower six of these, from 9 to 14, being separated by means of liquid chromatography.

Zone 1.—No crystalline pigment was obtained. It seems to be decomposition product.

Zone 2.—The pigment was only incompletely eluted. The eluted fraction contained a large quantity of colorless lipid which melted at 113° after recrystallization from methanol. Rechromatography on calcium carbonate gave seven red zones, but none of these zones afforded any crystallized pigment. The concentrates of the eluates showed blue colorations with conc. sulfuric acid or with antimony trichloride.

Zone 3.—This consisted of several zones, which were eluted together. The concentrate of the eluate was dissolved in hot ethanol. On cooling, colorless lipids crystallized out, which were removed by filtration. The concentrate was dissolved in a small quantity of petroleum ether and left to stand in an ice chamber. The red brown precipitates were redissolved in a small quantity of benzene, diluted with petroleum benzine and adsorbed on calcium carbonate (3×20 cm.). Petroleum benzine containing 10% of benzene was employed as the developer. The chromatogram observed is given in Table III in the sequence of decreasing adsorbability.

TABLE II

No. of the Zones	Width of the Zones (in mm.)	Color of the Zones
1	5	Dark brown
2	40	Deep red
	15	Colorless
3	12	Brown (heterogeneous)
	9	Colorless
4	3	Purplish brown
	5	Colorless
5	3	Reddish brown
	9	Colorless
6	30	Purple
7	26	Faint purple
	14	Colorless
8	18	Pale yellow
9	Liquid chromatography	Pale pink
10		Pale red
11		Orange red
12		Yellowish orange
13		Yellow
14		Bright yellow

TABLE III

	Width of the Zones (in mm.)	Color of the Zones
a	2	Purplish red
	6	Colorless
b	5	Pale yellow
c	18	Orange
d	20	Yellow
	7	Colorless
e	12	Yellow
f	15	Pale yellow

Zone a.—On recrystallizing the residue of the eluate from benzene-methanol, a minute quantity of purplish glistening fine needles was obtained. Absorption maxima, in carbon disulfide, 558, 517 485 mμ. In the partition test, the pigment colored both the layers.

Zone c.—Recrystallization from benzene-methanol gave purplish brown plates, m. p. 173–4°, 1 mg., absorption maxima; in carbon disulfide, 530, 493, 461 mμ; in hexane, 493, 462 mμ. In the partition test, the pigment colored both the layers.

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[8] Operations were carried out in the way described in the previous paper[1].

Zone d.—Recrystallization from petroleum benzine-methanol gave fine long orange needles, m. p. 153-4°, 0.4 mg., absorption maxima; in carbon disulfide, 518, 483, 452 mμ; in hexane, 482, 454 mμ. In the partition test both the layers also become colored. The solubility of the pigment was larger than that of the pigment of the zone c.

Zone e.—A minute quantity of brown plates of absorption maxima, 507, 475, 445 mμ in carbon disulfide was obtained.

Zone 4.—No crystalline pigment was obtained. The carbon disulfide solution gave a single maximum at 480 mμ.

Zone 5.—A minute quantity of almost black needles with a single maximum at 495 mμ in carbon disulfide was obtained.

Zones 6 *and* 7.—They were eluted together and the reddish black residue of the eluate was reecrystallized with a little petroleum benzine containing ethanol, and then with benzene-methanol. The crystals obtained were again adsorbed on alumina (2×20 cm.) from a petroleum benzine-benzene solution. The upper narrow purple zone afforded a minute quantity of red needles with absorption maxima 548, 512, (471) mμ in carbon disulfide. Partition test, almost epiphasic. The lower purple zone contained renieraxanthin. The residue of the eluate was recrystallized twice from a small quantity of benzene by the addition of methanol, the crystals being washed with a little cold petroleum ether and then with boiling methanol after each crystallization. Renieraxanthin, 2.6 mg., m. p. 161°, had a glistening purplish brown appearance and under microscope the crystals showed purplish red hexagonal plates. Absorption maxima; in carbon disulfide, 558, 521. (492) mμ; in hexane, 521, 487, 456 mμ; in benzene, 536, 511 mμ; in ethanol, a broad band with a single maximum at 500 mμ. The absorption curves in hexane and in ethanol are given in Fig. 1. In the partition test using 95% methanol, the pigment was almost epiphasic, but the methanol phase also somewhat colored. The pigment was very easily soluble in chloroform, easily soluble in benzene, considerably less soluble in petroleum ether, and very slightly soluble in ethanol and in methanol. In the color tests, conc. sulfuric acid gave a deep blue coloration and antimony trichloride a purple coloration which gradually changed into blue. On treating an ethereal solution with conc. hydrochloric acid no blue color was observed.

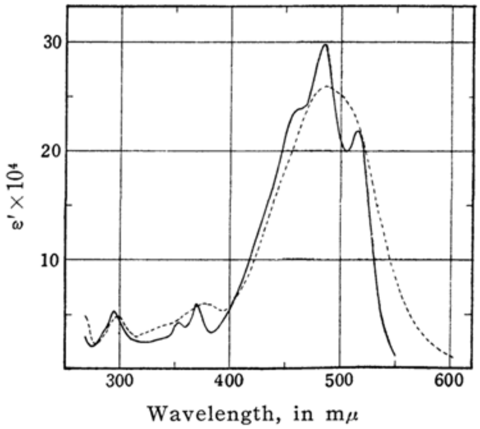

Fig. 1. Absorption spectra of renieraxanthin.
—— in hexane solution
- - - in ethanol solution
ε′ is given by $\varepsilon'=2.3/cd\times\log(I_0/I)$

Anal. Found: C, 84.64; H, 10.08. Calcd. for $C_{40}H_{56}O_2$: C, 84.45; H, 9.92%.

Zone 8.—Rechromatography on alumina and recrystallization from benzene-methanol gave a minute quantity of fine glistening copper red needles which sintered at 123°, changed simultaneously somewhat pale in color but did not melt even above 200°. This sample did not seem to be in an entirely pure state. Absorption maxima: in carbon disulfide, 531, 494, 463 mμ; in hexane, 494, 463, (435) mμ. Partition test, entirely epiphasic.

Zones 9, 10, 11 *and* 12.—Each fraction obtained by liquid chromatography was repeatedly chromatographed on alumina when necessary. Recrystallization from benzen-ethanol or from chloroform-methanol gave the following pigments; 0.4 mg. of renierapurpurin, a minute quantity of the pigment of m. p. 205°, 26 mg. of renieratene and a small quantity of the pigment of m. p. 194°, and 16 mg. of the pigment of m. p. 199°, corresponding to the fractions 9, 10, 11 and 12, respectively. These pigments were exactly identical with those obtained by the chromatography of the crude pigment already described.

Zones 13 *and* 14.—Both solutions were operated together. Recrystallization from benzene-methanol gave carotene mixture, m. p. 170°, 29 mg. Chromatography on calcium hydroxide (4×30 cm 2 columns) gave α-carotene, m. p. 185°, 2 mg, absorption maxima, in carbon disulfide, 507, 478, 447 mμ; and β-carotene, m. p. 183°, 20 mg, absorption maxima in carbon disulfide, 520, 485, 452 mμ. The colorimetric determination[9] showed that the ratio of α-carotene to β-carotene was 14 : 86.

Anal. α-Carotene, Found: C, 89.53; H, 10.52. β-Carotene, Found: C, 89.35; H, 10.65. Calcd. for $C_{40}H_{56}$: C, 89.49; H, 10.51%.

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