Chemical Constitution of Renieratene

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Renieratene is the main consistuent of carotenoids of a sponge "*Reniera japonica*"[1,2]. The most conspicuous characteristic of the pigment is that it has the most unsaturated composition of all the carotenoids hitherto reported. The light absorption maxima of the pigment, however, are located at nearly the same position as those of γ-carotene, which has only twelve double bonds in the molecule.

It has been reported in the previous paper[2] that renieratene has the molecular formula $C_{40}H_{48}$ or $C_{40}H_{50}$ (the former is more probable). Further confirmation of its molecular weight has been given by means of isothermal distillation. On catalytic hydrogenation, renieratene absorbs 15 moles of hydrogen. It is therefore considered to be a cyclic compound with one or two rings.

The quasi-equilibrium mixture of *cis-trans* isomerization of the pigment with iodine, shows the absorption maxima at a shorter wavelength region than those of the original solution, and a marked *cis*-peak also appears (Fig. 1). It can therefore, be concluded that renieratene is not a *cis*-isomer of a carotenoid with the absorption maxima of longer wavelength but has an all-*trans* form as to the configuration of conjugated double bonds. High melting point and low solubility of the pigment also support the conclusion.

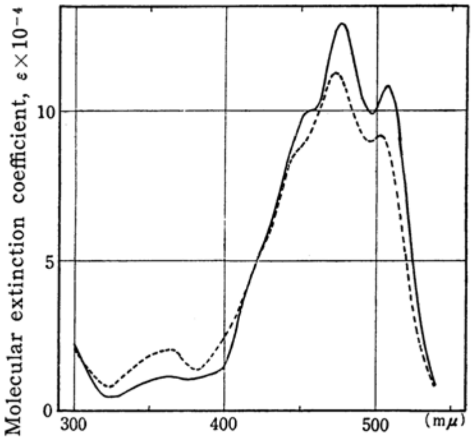

Fig. 1. Absorption curves of renieratene
—— fresh solution (benzene)
---- isomerized solution with iodine.

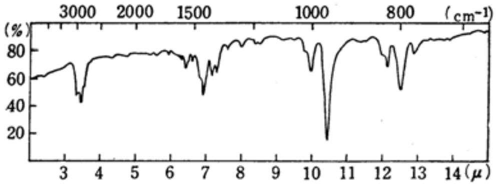

Fig. 2. Infra-red spectrum of renieratene.

The infrared spectrum (Fig. II) indicates the absence of vinyl group, which is further supported by the fact that renieratene is a rather stable carotenoid and has little tendency to polymerize. When a C_{40}—carotenoid with two 2,2,6-trimethylcyclohexyl-skeletons has no vinyl group, the maximum number of double bonds which are allowed to exist is fourteen, as in the case of anhydroeschscholtzxantin[3], I. If the carotenoid had throughout an open chain structure it might of course be possible to contain fifteen double bonds in the molecule. However even if it were so, all these double bonds would be in conjugation as in the case of dehydrolycopene[4], II; therefore the absorption maxima should be situated at much longer wavelength regions. These considerations lead to a presumption that a highly unsaturated group with a slight bathochromic effect must exist in the molecule of renieratene. There are two presumable cases which satisfy this requirement, i. e. the presence of triple bonds or the presence of benzene rings in the molecule of renieratene, although the former is less likely because of the reasons described below.

In the infra-red spectrum (Fig. II), no bands are observed which are characteristic of triple or allenic bond. On the other hand, a sharp absorption at 800 cm^{-1}, which seems to be characteristic of renieratene

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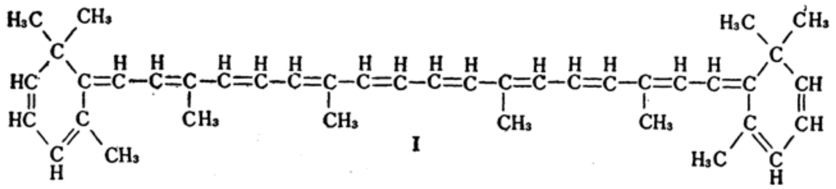
* Pigments of Marine Animals, IV.; III of this series; This Bulletin, **30**, 111 (1957).

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

[2] M. Yamaguchi, This Bulletin, **30**, 111 (1957).

[3] P. Karrer and E. Leumann, ibid., **34**, 445 (1951).

[4] P. Karrer and J. Rutschmann, *Helv. chim. Acta* **28**, 793 (1945).



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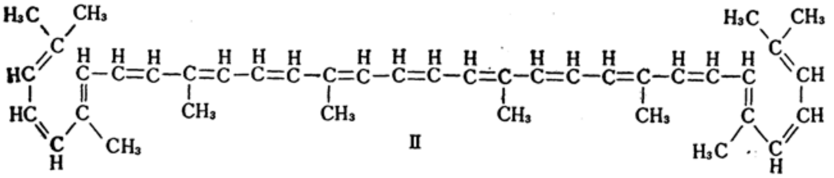

II

because of the general lack of this absorption in common carotenoids, is considered to give an indication of the presence of the benzene ring with two or three adjacent free hydrogen atoms. However this interpretation is not decisive, for the absorption in 3030 cm^{-1}, 1500—1600 cm^{-1} and other regions which are useful for the identification of aromatic structures, are not distinctive, because of the simultaneous presence of the absorption due to the polyene structure in the same regions.

Renieratene gives only a very small amount of acetone (0.03—0.05 mole) on ozonolysis. It has been reported that one isopropylidene group gives 0.8—0.9 mole of acetone[5], one isopropyl group 0.3 mole[5] and an α- or a β-ionone group 0.15 mole[6]. Therefore it seems that renieratene has neither isopropylidene group nor ionone ring. This result seems to be in favor of the presumption of the presence of the benzene ring.

Renieratene has no provitamin A activity in the rat when applied in daily doses of 60 γ. This is in agreement with the results of the ozonolysis.

By the mild oxidation of reniertene in benzene-acetic acid soultion with chromic acid corresponding to two atoms of oxygen per mole of the pigment, followed by chromatographic separation, two pigments are obtained in small yield. One of them forms purple crystals of m. p. 199°, and the other forms brown crystals of m. p. 127°. Both the pigments have the formula of $C_{30}H_{36}O$ and are considered to be polyene aldehydes. Therefore these two pigments seem to be in an isomeric relation. The term *renieral* is tentatively proposed for the pigment of m. p. 199°, and *isorenieral* for the pigment of m. p. 127°. On catalytic hydrogenation, both the pigments absorb 12 moles of hydrogen. This

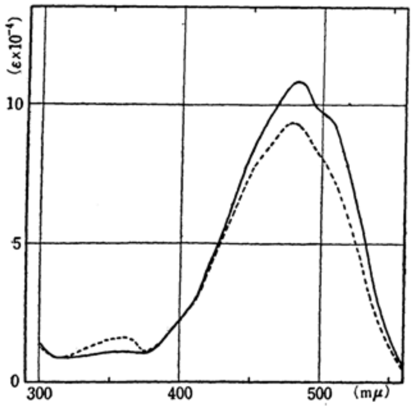

Fig. 3. Absorption curves of renieral.
—— fresh solution (benzene).
---- isomerized solution with iodine

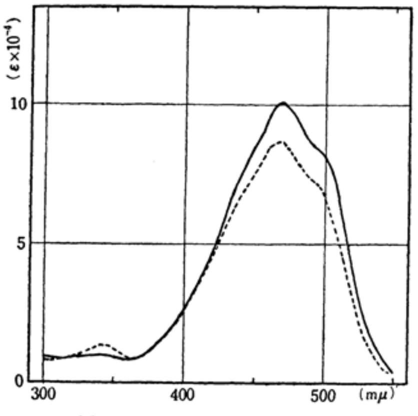

Fig. 4. Absorption curves of isorenieral.
—— fresh solution (benzene)
---- isomerized solution with iodine.

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5) R. Kuhn and H. Roth, *Ber.*, **65**, 1285 (1932).

6) L. Zechmeister and W. A. Schroeder, *Arch. Biochem.*, **1**, 231 (1942).

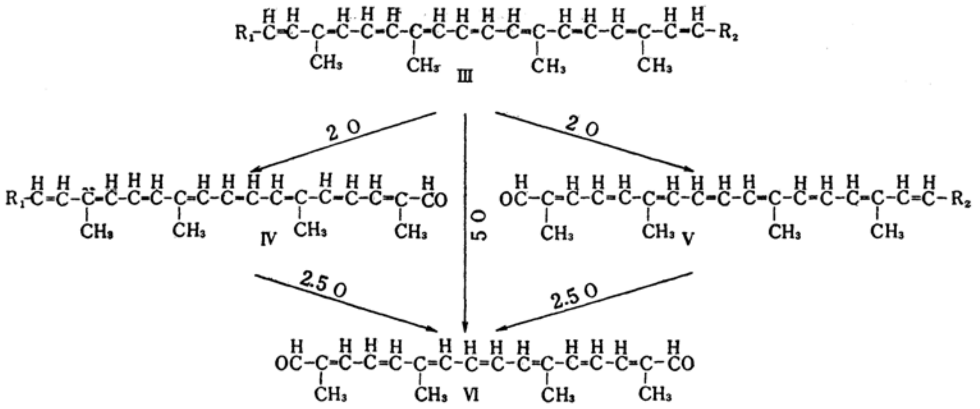

Fig. 6. Mild oxidation of renieratene.

fact indicates that they have 11 carbon-carbon double bonds in their molecules. Renieral exhibits the absorption maxima at 540 and 501 mμ in (carbon disulfide). Isorenieral exhibits absorption maxima at 526 and 489 mμ (in carbon disulfide) and gives phenylhydazone of m. p. 207°. Absorption curves in benzene with and without iodine are given by Fig. 3 and Fig. 4.

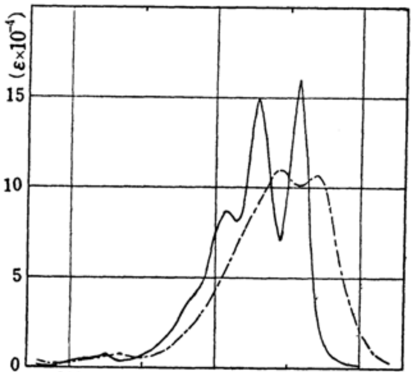

Fig. 5. Absorption curves of the pigment of m. p. 190°.
—— in petroleum ether,
-·-·- in ethanol

When renieratene is oxidized with chromic acid corresponding to five atoms of oxygen, the principal product is the pigment of m. p. 190°, which forms orange red plates with purple reflex. The molecular formula of the pigment is $C_{20}H_{24}O_2$, and the absorption maxima are at 457, 430 and 407 mμ (in petroleum ether). The absorption curves are given in Fig. 5. As the pigment gives a bisphenylhydrazone of m. p. 239—242°, it seems to be a dialdehyde. These facts and the other properties of the pigment indicate that it is identical with crocetin dialdehyde, VI, (violet red plates, m. p. 190—191°, $C_{20}H_{24}O_2$, absorption maxima in petroleum ether, 458, 430 and 408 mμ; bisphenylhydrazone, m. p. 243°), which has been synthesized by Isler and coworkers⁽⁷⁾. It is for the first time that crocetin dialdehyde is obtained by the degradation of a carotenoid. Both renieral and isorenieral give crocetin dialdehyde by the oxydation with chromic acid corresponding to 2.5 atoms of oxygen. However, renieral does not give isorenieral on oxidation and neither does the reverse occur. These relationships are illustrated in Fig, 6, and the facts described above lead to the conclusion that renieratene, renieral and isorenieral are expressed by the partial formulae III, IV and V in Fig. 6, respectively. Both R_1– and R_2 groups seem to correspond to C_9H_{11}– which probably contain one ring and three double bonds.

From the positions of the absorption maxima of renieral and isorenieral, it is indicated that at least nine double bonds in these pigments should be in conjugation⁽⁸⁾. Therefore at least one double bond in R_1– and one double bond in R_2– group should be in conjugation with principal chromophore of renieratene. Numerous instances⁽⁹⁾ of the mild oxidation of carotenoids have shown that the oxidative fission of double bond generally occurs at the outer end of the conjugated double bond system. However, in the case of renieratene, none of the pigments which have the absorption maxima at a longer

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7) O. Isler, H. Gutmann, H. Lindlar, M. Montavon, R. Rüegg, G. Ryser and P. Zeller, *Helv. chim. Acta*, **39**, 463 (1956).

8) Calculated according to K. Hirayama's method, *J. Chem. Soc. Japan, Pure Chem. Sec.*, **75**, 674 (1954).

9) For example, R. Kuhn and C. Grundmann, *Ber.*, **65**, 900 (1932); R. Kuhn and H. Brockmann, ibid., **65**, 894 (1934); *Ann.*, **516**, 123 (1935).

wavelength region are obtained i. e. those which have a longer conjugated double bond system, than the above-mentioned aldehydes have. Therefore, in renieratene, the oxidation of double bond does not occur at the outer end of the conjugated system but at the inner position of the system. This indicates that the partial double bond systems contained in R_1- or R_2- group may be highly sablet towards oxidation, which also suggests the presence of benzene rings.

However, it seems to be contradictory to the view of the presence of benzene ring, that renieratene gives 7 moles of acetic acid on determination of side chain methyl groups. If renieratene had benzene rings in the molecule, non-volatile benzene polycarboxylic acid would be formed by such a serious oxidation as is employed in the determination; because the benzene ring in renieratene might be replaced by several methyl groups, as would be expected from the formula. In such a case, the amount of volatile acid should be no more than 4 moles when estimated as acetic acid. The account of this result can not be given in the present stage of the study.

Experimental

Renieratene prepared in the way described in the preceding papers[1,2] was employed. It formed fine purple red needles of m. p. 185° and was chromatographically homogeneous. Absorption maxima, 532, 496, 463 mμ (in carbon disulfide).

Anal. Found: C, 90.95; H, 9.26. Calcd. for $C_{40}H_{48}$; C, 90.85; H, 9.15%.

Infrared spectrum.—It was taken in a potassium bromide pellet, with a Perkin-Elmer 21 double beamins trument equipped with a potassium bromide prism (Fig. 2).

Molecular weight.—Signer's method[10] (method of isothermal distillation) was slightly modified. The solutions (128.1 mm^3 and 194.1 mm^3) of renieratene (5.913 mg. and 6.270 mg.) in benzene exhibited the same vapor pressures as those of the solutions (133.6 mm^3 and 193.8 mm^3) of benzil (2.531 mg. and 2.501 mg.) in benzene respectively. Found; 526.8, 536.7. Calcd. for $C_{40}H_{48}$, 528.8. In a parallel experiment on lycopene, 539.2 was obtained (Calcd. 536.9).

Catalytic hydrogenation (Kuhn and Möller's method[11]).—Pure sorbic acid was employed for the test substance. Catalyst, platinum oxide. Solvent, decalin-acetic acid (1:1). Found: 15.23, 15.17, 14.98 moles hydrogen. In parallel experiments, lycopene absorbed 13.03 moles hydrogen and β- carotene 10.94 moles hydrogen.

Isopropylidene group (Kuhn and Roth's method[5]).—On ozonolysis, renieratene (15.116 mg. and 8.912 mg.) gave acetone (0.077 mg. and 0.031 mg.) after subtraction of the blank value. These corresponded to 0.05 and 0.03 mole of acetone per mole of renieratene. In parallel experiments, lycopene and β-carotene gave 1.5 and 0.35 mole of acetone, respectively.

Side-chain methyl group (Kuhn and Roth's method[12]).—Renieratene (4.603 mg. and 4.196 mg.) gave volatile acid corresponding to 6.07 cc. and 5.73 cc. of 0.01 N sodium hydroxide solution. These corresponded to 6.97 and 7.22 moles of acetic acid. A parallel determination on lycopene gave 5.61 moles of acetic acid.

Vitamin A activity.—Renieratene was examined in experiments on four groups of rats in daily doses of 7, 15, 30 and 60 γ respectively. The rats of these four groups lost 26 g., 28 g., 27 g. and 26 g. (average on the rats of each group) in bodyweight, and finally died. The rats of the group for the positive control, which 7 γ of β-carotene were applied in daily doses, gained 33 g. on the average in body-weight in 30 days.

Cis-trans isomerization[13].—The solution (25 cc.) of renieratene (1.175 mg.) in benzene was prepared in a dark room, and 10 cc. of the solution was exposed to scattered light after the addition of iodine (ca. 6 γ) in benzene (0.8 cc.). The remainder was kept in the dark after the addition of benzene (1.2 cc.). After 30 minutes, 1 cc. of each solution was diluted to 25 cc. with benzene and the absorption curves were determined. They are given in Fig. 1. A remarkable *cis*-peak was ovserved at 362 mμ in the isomerized solution.

Chromic acid oxidation.—*a*) *Renieral and isorenieral.* Renieratene (133 mg.) was dissolved in a mixture of benzene (130 cc.) and acetic acid (130 cc.). To 10 cc. of this solution (containing ca. 5 mg. of the pigment), was added the mixture of the aqueous chromic acid (0.1 N, 0.2 cc.) and acetic acid (2 cc.) in one portion with vigorous stirring. The color changed into deep red. After 4—5 seconds the mixture was poured into water (20 cc.) covered with petroleum benzine (10 cc.), and washed with water several times. The procedure was repeated with other 10 cc. portions. All oxidized portions were combined together and dried with anhydrous sodium sulfate. The concentrated solution (30 cc.) was chromatographed on alumina (Merck) (column, 2.5 cm × 20 cm; developer, benzene) and gave the following zones in the sequence of decreasing adsorbability. 1) A thin yellow zone. It gave a small quantity of oily brown residue having absorption maxima at 454 and 425 mμ in petroleum benzine. 2) A thin yellow zone. It gave a small quantity of oily yellowish brown residue with absorption maxima at 455 and 425 mμ in petroleum benzine. 3) A thin orange zone. On treatment with petroleum benzine the residue afforded a minute quantity of red crystals having absorption maxima

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10) R. Signer, ibid., **478**, 246 (1930).

11) R. Kuhn and E. E. Möller, *Angew. Chem.*, 145 (1934),

12) R. Kuhn and H. Roth, *Ber.*, **66**, 1274 (1933).

13) L. Zechmeister and A. Polgár, *J. Am. Chem. Soc.*, **65**, 1522 (1943).

at 456 and 429 mμ in petroleum benzine. It was crocetin dialdehyde. 4) A deep purple zone. After rechromatography on alumina, the residue of the eluate gave 8.5 mg. of renieral when recrystallized once from benzene-methanol. 5) A purplish red zone. After rechromatography on alumina and recrystallization of the residue of the eluate from petroleum benzine, 17.5 mg. of isorenieral was obtained. 6) A red zone. A small quantity of renieratene was recovered. Another experiment with 60 mg. of renieratene afforded 3.3 mg. of renieral and 7 mg. of isorenieral.

Renieral (further purified by recrystallization twice from benzene-methanol and twice from benzene-petroleum benzine) forms purplish brown needles, m. p. 198—199° (total 8.1 mg). It is soluble in benzene and difficultly soluble in petroleum benzine and methanol. Absorption maxima; 540, 501 mμ (in carbon disulfide); 499, 468 mμ (in hexane). Absorption curves (in benzene) with and without iodine (ca. 1.5% of the pigment) are given in Fig. III. On catalytic hydrogenation, 12.2 moles of hydrogen were absorbed.

Isorenieral (further purified by recrystallization thrice from petroleum benzine) forms brown crystals, m. p. 125—127° (12.5 mg.). It is soluble in benzene, petroleum benzine and hot ethanol, but is less soluble in methanol. Absorption maxima: 526, 489 mμ (in carbon disulfide); 486, 455 mμ (in hexane). Absorption curves (in benzene) with and without iodine are given in Fig. 4.

Anal. Found: C, 87.19; H, 8.88. Calcd. for $C_{30}H_{36}O$: C, 87.33; H, 8.80%.

On catalytic hydrogenation, 12.1 moles of hydrogen were absorbed. Phenylhydrazone, prepared by mixing the pigment (2 mg.) with phenylhydrazine in acetic acid and recrystallized once from benzene-methanol, formed purplish red needles, m. p. 207° (in an evacuated tube). It melted at 185° in an open capillary tube.

Anal. Found; N, 5.56. Calcd. for $C_{36}H_{42}N_2$; N, 5.57%.

b) *Crocetin dialdehyde.* Renieratene (120 mg.) was dissolved in a mixture of benzene (120 cc.) and acetic acid (120 cc.), and the solution was divided into 24 portions. Each portion (10 cc.) was oxidized with the mixture of the chromic acid solution (0.1 N, 0.5 cc.) and acetic acid (2 cc.), under vigorous stirring for one minute. The color of the solution changed into deep red and then gradually into orange yellow. The products were treated as in the case of a), and chromatographed on alumina. The main zone was orange red (it corresponded to the zone 3 of the chromatogram described in a)), while the zones of renieral and isorenieral were fairly faint. The residue of the eluate was recrystallized thrice from benzene-methanol and gave crocetin dialdehyde in purple red plates m. p. 190° (7.7 mg.). All the mother liquors of crystallizations of reneiral and isorenieral were combined together and oxidized as above with sufficient chromic acid to change the deep red color of the solution into orange yellow; 4.7 mg. of crocetin dialdehyde was further obtained. Absorption curve is given in Fig. 5.

Anal. Found: C, 81.46; H, 8.12%. Calcd. for $C_{20}H_{24}O_2$; C, 81.04; H, 8.16%.

Bisphenylhydrazone. Purple crystals, m. p. 256° (in an evacuated tube). In an open capillary tube, it melted at 239—242°.

Anal. Found; N, 11.79% Calcd. for $C_{32}H_{36}N_4$ 11.76%.

c) *Oxidation of renieral.* Renieral (0.8 mg.) was oxidized as above with chromic acid solution (0.1 N, 0.03 cc.), and the product was chromatographed. The principal zone gave a minute quantity of crocetin dialdehyde (m. p. 189°, mixed m. p. 189—190°).

d) *Oxidation of isorenieral.* Isorenieral (1.4 mg.) was oxidized with chromic acid solution (0.04 cc.). Crocetin dialdehyde was obtained as the main product (m. p. 189°, mixed m. p. 189—190°).

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