Two Natural 7-*Cis* Aromatic Carotenoids from Sea Sponge *Tethya amamensis*^{*1}

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Two novel *cis* aromatic carotenoids, isorenieracistene and renieracistene, have been isolated from a sea sponge *Tethya amamensis*. The structures 7-*cis*- ϕ , ϕ -carotene for isorenieracistene and 7-*cis*- ϕ , χ -carotene for renieracistene were proposed on the basis of their spectral data and iodine-catalyzed photoisomerization.

In the previous papers,^{1,2)} five major pigments, ϵ -carotene, α -carotene, β -carotene, tethyatene and renieratene have been isolated from a sea sponge *Tethya amamensis* and identified or structurally elucidated. Two other minor pigments have remained structurally unsolved. Recently, considerable amounts of the pigments have been isolated and evidences have been obtained to elucidate that they have structures of 7-*cis* aromatic carotenoids, I (pigment I) and II (pigment II), respectively.

On the other hand, the majority of natural carotenoids possess the all *trans* configuration and in regard to the natural occurrence of *cis*-isomer of carotenoids, 7-*cis* carotenoids were until recently not known.³⁾ Though many kinds of aromatic carotenoids have been isolated from sea sponge, 7-*cis* aromatic carotenoids have been neither isolated nor known synthetically. We now wish to report the first isolation of 7-*cis* aromatic carotenoids from a sea sponge *Tethya amamensis*.

Materials and Methods

Specimens of sea sponge were collected in Kinko Bay, Kagoshima and the carotenoids were extracted with acetone. The crude carotenoids were separated on a MgO column,⁴⁾ using petroleum ether as developing solvent and six fractions were obtained. Repeated chromatographic separation of the second fraction on a MgO column was necessary to obtain homogeneous band. The isolated carotenoid (pigment I) was purified on a sucrose column and crystallized from petroleum ether.

Pigment II was isolated from the third fraction by the same way as that of pigment I.

The spectral data were obtained with following instruments: Visible absorption spectrum; Shimadzu UV-200S, IR; Shimadzu IR-27R, MS; JEOL-JMS-300, ¹H-NMR; JEOL-model FX90Q (pigment I) and JEOL-100 (pigment II).

Iodine-catalyzed photoisomerization was carried out to know the characteristics of pigment I and pigment II. The carotenoid (ca. 1 mg) in benzene (50 ml) was isomerized by exposure to scattered light for 3 hours after addition of iodine (ca. 0.12% of the weight of the carotenoid).⁵⁾

Results and Discussion

Pigment I

This carotenoid has the following characteristics: red needles, mp.: 151-153°C, HRMS: 528.3761 (C₄₀H₄₈), λ_{max} (petroleum ether): 423, 447, 474 nm, MS: M⁺ (528), M-79 (449), M-92 (436), M-106 (422), M-133 (395), M-158 (370), M-199 (329), 133

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Fig. 1. Visible absorption spectra of pigment I (1) and isorenieratene (2).

(base peak), $^1\text{H-NMR}$: δ 1.43 (s, 3H), 1.93 (s, 3H), 1.99 (s, 3H), 2.09 (s, 3H), 2.16 (s, 6H), 2.25 (s, 3H), 2.28 (s, 9H), 6.06–6.68 (m, 14H), 6.97 (s, 4H), IR (KBr): 3040, 3025, 2975, 2940, 2920, 2850, 1460, 1440, 1395, 1366, 1005, 970, 964, 891, 830, 809, 754, 720, 579, 522 cm^{-1} .

Mass spectral analysis indicated that this carotenoid has the same molecular formula as that of isoreieratene (*trans*- ϕ , ϕ -carotene). When pigment I was treated with iodine-catalyzed photoisomerization and the components on TLC were examined, the original pigment disappeared and isoreieratene and three kinds of *cis*-isomers (stereoisomeric equilibrium mixture) were formed as shown in Fig. 2. Isorenieratene, after iodine-catalyzed photoisomerization, yielded an equilibrium mixture of the same composition and pigment I did not occur in any detectable amount in the mixture. Tentative assignment of neo A to 9-*cis*, neo B to 13-*cis* and neo C to di-*cis* is based on shifts in λ_{max} and relative *cis* peak intensities⁶⁾ as shown in Table 1 and pigment I has unique characteristics of absorption spectrum. From

Fig. 2. Thin layer chromatograms of isorenieratene, pigment I and their stereoisomeric mixtures on Kieselgel 60 (Merck). Developing solvent: Petroleum ether

I: Isorenieratene II: Pigment I
III: Stereoisomeric mixture of isorenieratene
IV: Stereoisomeric mixture of pigment I

the results of the isomerization, pigment I should be sterically hindered *cis*-isomer of isorenieratene. The splitting of 964 cm^{-1} absorption in the IR spectrum also supports the conclusion (Fig. 3). From general rule of *cis* carotenoids and the small blue shift of λ_{max} (4 nm in petroleum ether) in visible absorption spectrum observed in going from isorenieratene to this carotenoid, it can be assumed that pigment I has only one *cis* double bond.⁷⁾

Four kinds of methyl protons in $^1\text{H-NMR}$ spectrum at δ 2.28, 2.25, 2.09 and 1.99 indicated that the half moiety of this carotenoid is ϕ -carotene terminal group and the corresponding polyene chain as same as isorenieratene. It is noteworthy that one methyl group (δ 1.43) in polyene chain and two (δ 2.16) out of six aromatic methyl groups are significantly shielded when compared with that of isorenieratene [δ 1.98 (s, 6H), 2.07 (s, 6H), 2.22 (2, 6H), 2.27 (s, 12H), 6.10–6.90 (m, 14H), 6.93 (s, 4H)]. If pigment I is 7-*cis*-isomer of isorenieratene, these shifts can be reasonably

Table 1. Visible absorption characteristics of isorenieratene stereoisomeric set and pigment I

Stereoisomer	Absorption λ_{max} *1				Spectra D_B/D_{II} *2 (%)	III/II *2 (%)	Tentative assignment
Trans	426	451	478		—	37	all- <i>trans</i>
Neo A	339	423	446	473	18	31	9- <i>cis</i>
Neo B	339	421	444	469	25	5	13- <i>cis</i>
Neo C	339	419	442	467	21	10	di- <i>cis</i>
Pigment I		423	447	474	—	60	

*1 in petroleum ether. *2 refer to 6).

Fig. 3. IR spectrum of pigment I.

Fig. 4. ¹H-NMR spectrum of pigment I.

Table 2. Visible absorption characteristics of renieratene stereoisomeric set and pigment II.

Stereoisomer	$\lambda_{\max}^{*1}$			Absorption spectra D_B/D_{II}^{*2} (%)	III/II ^{*2} (%)	Tentative assignment
Trans	436	459	488	—	29	all- <i>trans</i>
Neo A	350	434	454	19	23	9 or 9'-mono-
Neo B	350	433	454	31	4	<i>cis</i> -13 or 13'-
Neo C	350	425	449	24	8	mono- <i>cis</i>
Pigment II	433	456	486	—	44	di- <i>cis</i>

*1 in petroleum ether *2 refer to 6)

explained in terms of anisotropic effect by assuming that the location of *cis* double bond in polyene chain is in the position neighbouring one ϕ -carotene terminal group. The two *ortho*-standing methyl groups may be considered to become more shielded owing to proximity of these methyl to C-19 methyl group in 7-*cis* configuration. And the observed great shift at δ 1.43 could be assumed that C-19 methyl group lies at the vicinity of perpendicular position to the plane of aromatic ring. Such these significant shifts could not be expected on the 9-, 11- or 13-*cis*-isomer when ¹H-NMR of *cis* compounds of vitamin A analogues were considered.⁶⁾

Therefore, the structure of pigment I should be 7-*cis*- ϕ , ϕ -carotene as shown in I and the trivial name, isorenieracistene is proposed for it.

Pigment II

This carotenoid has the following characteristics: mp.: 161–164°C, $\lambda_{\max}$ (petroleum ether): 432, 456,

486 nm, MS: M⁺ (528), M-29 (436), M-106 (422), M-133 (395), M-158 (370), 133 (base peak,) ¹H-NMR: δ 1.42 (s, 3H), 1.91 (s, 3H), 1.96 (s, 3H), 2.04 (s, 3H), 2.15 (s, 6H), 2.19 (s, 3H), 2.23 (s, 3H), 2.27 (s, 6H), 6.04–7.02 (m, 18H).

The mass spectrum and visible absorption spectrum are similar to those of renieratene (Fig. 5). However, this carotenoid has also clearly different R_f value on TLC from that of renieratene as shown in Fig. 6. The results of photoisomerization suggested that this carotenoid is one kind of mono-*cis*-isomer of renieratene.

In ¹H-NMR spectrum, four kinds of methyl protons at δ 2.27, 2.19, 2.04 and 1.96 indicated that the half moiety of this carotenoid is χ -carotene terminal group and the corresponding polyene chain. When compared with the spectrum of renieratene [δ 1.96 (s, 6H), 2.04 (s, 6H), 2.19, 2.22, 2.24, 2.27 (m, 18H), 6.00–7.03 (18H)], the significant upshifts of the *ortho*-standing methyl groups and one methyl group in polyene chain can be

Fig. 5. Visible absorption spectra of pigment II (1) and renieratene (2).

Fig. 6. Thin layer chromatograms of renieratene, pigment II and their stereoisomeric mixtures on Kieselgel 60 (Merck). Developing solvent: Petroleum ether.

I: Renieratene II: Pigment II
III: Stereoisomeric mixture of renieratene
IV: Stereoisomeric mixture of pigment II.

Fig. 7. ^1H -NMR spectrum of pigment II.

observed as same as pigment I and the same explanation can apply to this carotenoid.

From the results of ^1H -NMR, MS data and isomerization, this carotenoid should be 7-*cis*- ϕ , χ -carotene as shown in II. The name, renieracistene is proposed for this carotenoid.

The *cis* forms of carotenoids can be divided into sterically hindered and unhindered types, and unhindered types such as 9- or 13-*cis* have weak hindrance between hydrogen atoms. While in the hindered type, hindrance is caused mainly by the conflict of a hydrogen atom and a "in-chain" methyl group. The 7-*cis* configuration in polycopene (7,9,7',9'-tetra-*cis*- ϕ , ψ -carotene) which is only one example of 7-*cis* form in natural origin, classes the hindered *cis* type as mentioned above.

However, in the 7-*cis* configuration of isorenieracistene and renieracistene, a spatial conflict should arise between *ortho*-standing methyl groups and "in-chain" methyl group. Such a unique sterically hindrance is a new type of hindered *cis* form and the two kinds of these carotenoids isolated from *Tethya amamensis* should be novel type of carotenoids.

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