

## Bacterial Carotenoids

### XVIII.\* Aryl-carotenes from *Phaeobium*

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The carotenoids of some photosynthetic, brown bacteria have been studied. The diaryl-carotene isorenieratene (=leprotene) (I) is a major carotene. The natural occurrence of  $\beta$ -isorenieratene (II) has been demonstrated for the first time. Chlorobactene (VI), the characteristic aryl-carotene of photosynthetic green bacteria, and  $\beta$ -carotene (III) are trace carotenoids.

The carotenoids of photosynthetic bacteria, available in pure culture, have been fairly extensively studied (see for instance Refs. 1, 2).

Assisted by the Norwegian Institute of Water Research, Professor Dr. N. Pfennig, Institut für Mikrobiologie der Universität, Göttingen, was introduced to some Norwegian marine and fresh water localities. From these habitats he isolated, in pure culture, some strains or species of photosynthetic, brown bacteria, designated by him as *Phaeobium*. The relationship between *Phaeobium* and the Phaeobacteria mentioned by Utermöhl<sup>3</sup> is unknown.

The isolation and characterization of these organisms, as well as an analysis of their chlorophyll, will be published by N. Pfennig. The present paper reports on the carotenoids of four isolates of *Phaeobium*.

#### RESULTS AND DISCUSSION

In Table 1 the carotenoid composition of the four investigated isolates of *Phaeobium* is shown.

The diaryl-carotene isorenieratene (I), recently identified with leprotene<sup>4,5</sup> was a major carotene in all isolates studied. Crystalline isorenieratene was isolated in high yield, and was identified with authentic isorenieratene from identity of absorption spectra in the visible and infrared regions (see Fig. 1),  $R_F$ -values and by mixed melting point determination.

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Table 1. Carotenoid composition of four isolates of *Phaeobium*.

CAROTENOID	Carotenoid in % of total carotenoid			
	<i>Phaeobium</i> isolated			
	Polden	Blankvann	Langvik 1B	Langvik 2A
Chlorobactene (IV)	0.02	5	6	6
$\beta$ -Carotene (III)	1	1	7	4
$\beta$ -Isorenieratene (II)	33	14	38	47
Isorenieratene (I)	66	80	49	43
Carotenoid in % of dry, extracted residue	0.39	0.22	0.58	0.20
Total carotenoid in mg in sample prior to chromatography	2.44	0.72	2.20	1.09

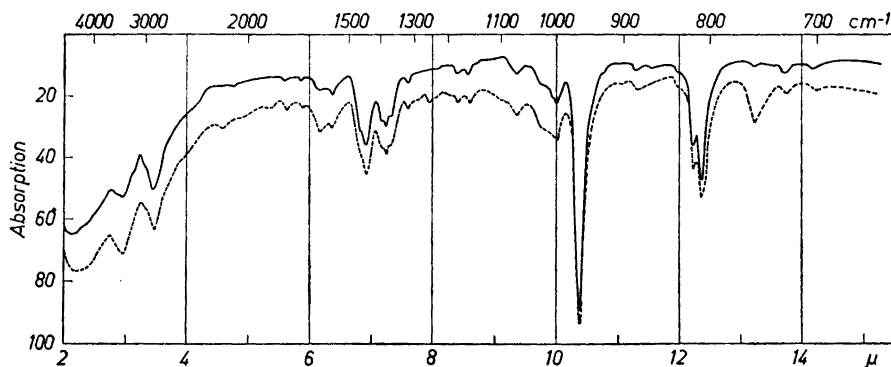


Fig. 1. Infrared spectra of ——— natural and - - - synthetic isorenieratene in KBr.

The natural occurrence of the aryl-alicyclic carotene  $\beta$ -isorenieratene (II), already made synthetically by Cooper, Davis and Weedon,<sup>6</sup> was demonstrated for the first time.  $\beta$ -Isorenieratene (II) was not obtained in the crystalline state. The identification was based on a comparative study of the iodine-catalyzed stereoisomerization mixture of the natural and synthetic pigment, following the method previously employed.<sup>7,8</sup> The result is summarized in Table 2.

$\beta$ -Carotene (III) was present as a trace carotenoid and was identified by its absorption spectrum in visible light and by co-chromatography tests with authentic  $\beta$ -carotene on aluminium oxide-containing paper.<sup>8</sup>

Finally chlorobactene (IV),<sup>2,9</sup> the characteristic carotenoid of photo-synthetic green bacteria,<sup>2</sup> was present as a trace carotenoid. Identity with authentic chlorobactene was proved by comparison of the absorption spectra

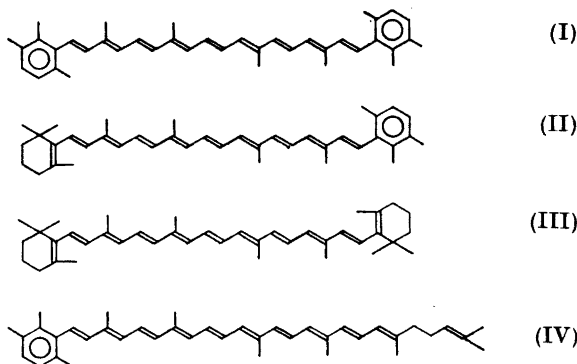
Table 2. Composition of the iodine-catalyzed equilibrium mixtures of natural and synthetic  $\beta$ -isorenieratene.

$\beta$ -Isorenieratene	Member of the stereoisomeric set	Schleicher & Schüll No. 288 paper; 3 % ether-pet.ether		In acetone		% of total
		Colour of zone	$R_F$ -value	Abs. max. in $m\mu$	% $D_B/D_{II}$	
Natural	Neo C	Yellow	0.77	} 332 (425) 448 472	26	ca. 18
	Neo B	Yellow	0.67			
	Neo A	Orange-yellow	0.60			
	<i>Trans</i>	Orange	0.50	(430) 453 480	12	ca. 10 } ca. 12 } 60
Synthetic	Neo C	Yellow	0.77	} 332 (425) 448 472	24	ca. 16
	Neo B	Yellow	0.67			
	Neo A	Orange-yellow	0.60			
	<i>Trans</i>	Orange	0.50	(430) 453 480	10	ca. 10 } ca. 12 } 62

Table 3. Chromatographic properties of isorenieratene,  $\beta$ -isorenieratene and  $\beta$ -carotene.

Carotenoid	Stereo-isomer	Approximate required eluant **	$R_F$ -values		
			Schleicher & Schüll No. 288 ( $Al_2O_3$ -paper)		
			Pet.ether	3 % Ether *	1 % Acetone *
$\beta$ -Carotene	<i>Cis</i>	5 % ether *	} 0.88	0.33	0.86
	<i>Trans</i>			0.29	
$\beta$ -Isorenieratene	Neo C	10 % ether *	} 0.85	} 0.20	0.77
	Neo B				0.67
	Neo A				0.60
	<i>Trans</i>	0.70	0.10	0.50	0.83
Isorenieratene	Neo B	20 % ether *	} 0.70	} 0.60	0.76
	Neo A				0.51
	<i>Trans</i>				0.66

\* In petroleum ether. \*\* From Woelm neutral alumina activity grade 2.



in visible light and co-chromatography tests with the main stereoisomers on circular kieselguhr-containing paper.<sup>10</sup>

The carotenes (I), (II), and (III) exhibit identical absorption spectra in visible light, and, if partly *cis*-isomerized, cannot be quantitatively separated on a deactivated alumina column. Neoisorenieratene C<sup>5</sup> cannot be separated from *trans*  $\beta$ -isorenieratene on kieselguhr paper. However, on aluminium oxide paper a satisfactory separation of the stereoisomeric sets of (I), (II), and (III) is obtained. The chromatographic properties of these carotenes are summarized in Table 3.

The predominance of 1,2,5-trimethyl-aryl-carotenes in *Phaeobium* is of interest for classification purposes. The distribution pattern of carotenoids in these organisms lends some support to the postulated formation of aryl-carotenes by dehydrogenation of and methyl migration in the corresponding carotenes with so-called  $\beta$ -end groups.<sup>6,2</sup>

## EXPERIMENTAL

*Materials and methods* have been described in earlier papers of this series.<sup>11,2</sup> Visible light absorption spectra were, in the present study, recorded on a Beckmann DB automatic recording spectrophotometer, and melting points (uncorrected) were determined on an Electrothermal melting-point apparatus.

*Cultures and cultural conditions.* Cell material from 1.5 l cultures of four isolates of *Phaeobium*, referred to as *Phaeobium* Polden, *Phaeobium* Blankvann, *Phaeobium* Langvik 1 B, and *Phaeobium* Langvik 2 A, was kindly provided by N. Pfennig.

*Pigment extraction.* To 25 ml aqueous cell suspension was added 500 ml of an acetone-methanol (1:1) mixture. The extraction was carried out at  $-20^{\circ}\text{C}$  overnight.

*Saponification* was conducted in 5% KOH-methanol for 8 h in the usual manner.<sup>7</sup>

*Chromatographic separation* was performed on deactivated alumina columns<sup>7,2</sup> and on various paper.<sup>8,10</sup>

*Quantitative carotenoid content* was estimated after column chromatography and subsequent paper-chromatographic examination of the column fractions, using  $E_{1\text{ cm}}^{1\%} = 2500$  for  $\beta$ -carotene, 3000 for chlorobactene, 2600 for isorenieratene and 2550 for  $\beta$ -isorenieratene at the highest absorption peak in petroleum ether. The result is presented in Table 1.

## Isorenieratene (I)

From all isolates needles of isorenieratene (I) crystallized in clusters from ether-petroleum ether; total yield 0.95 mg. The crystals were collected as previously described;<sup>2</sup> m.p. 195.5°C. Synthetic isorenieratene showed the same melting point and mixed m.p.; 195°C. Natural isorenieratene had abs. max. (425), 452, and 480  $m\mu$ , % III/II<sup>7</sup> = 38;  $E_{1\text{ cm}}^{1\%} = 2560$  at 452  $m\mu$  in petroleum ether. According to the method described<sup>12</sup> the IR-spectrum of 0.20 mg natural isorenieratene in 0.2 g KBr and of 0.25 mg synthetic isorenieratene in 0.2 g KBr were recorded. The spectra are given in Fig. 1.

On co-chromatography on circular kieselguhr paper the *trans*, neo A and neo B isomers of natural isorenieratene were not separated from those of synthetic isorenieratene (cf. Ref. 5).

 $\beta$ -Isorenieratene (II)

The paper-chromatographically purified *trans* isomers exhibited an absorption spectrum analogous to that described above for isorenieratene, with somewhat reduced fine structure. The composition of the iodine-catalyzed equilibrium mixture of natural and synthetic  $\beta$ -isorenieratene was determined in the usual manner.<sup>7,8</sup> Upon co-chromatography on aluminium oxide paper of the two stereoisomeric sets there was complete agreement in the four stereoisomers. The result is presented in Table 2. The true nature of the *cis* isomers as members of the  $\beta$ -isorenieratene stereoisomeric set was proved by reversed stereoisomerization in light, followed by paper-chromatographic examination.

 $\beta$ -Carotene (III)

The absorption spectrum agreed with that of isorenieratene. On aluminium oxide paper iodine catalyzed  $\beta$ -carotene from the present isolation and authentic  $\beta$ -carotene both exhibited a yellow zone ( $R_F = 0.33$ ) and an orange zone ( $R_F = 0.29$ ) when petroleum ether was used as developer. Co-chromatography tests indicated identity of the two stereoisomeric sets. In this system members of the  $\beta$ -isorenieratene stereoisomeric set exhibited lower  $R_F$ -values.

## Chlorobactene (IV)

The *trans* isomer had abs. max. at 434, 460, and 491  $m\mu$ , % III/II (7) = 60 in petroleum ether. In light *cis*-isomerization occurred. The *trans*, neo A and neo B isomers co-chromatographed, on kieselguhr paper (petroleum ether), with those of authentic chlorobactene (cf. Ref. 3).

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