

pounds<sup>7</sup> (originally prepared by Backer and co-workers) were also examined. They all gave good polarographic waves over the whole pH range indicating a two electron reduction. Especially in acid solution the agreement between their half-wave potentials and that of *racem*-(I) is excellent. — In this connection the polarographic method has been used to further emphasize the improbability of coordinated sulphur-sulphur bonds<sup>8</sup>.

This general polarographic character of the five-membered cyclic disulphide has also been found to include that of 6,8-thioctic acid.

*Experimental.* The investigation was performed at 25° C with a Leybold Polarograph model 54. In order to minimize the influence of the cathodic mercury on the solution a special type of polarographic cell according to Schwabe and Berg was used.

In connection with a current polarographic investigation of a number of aliphatic diseleno dicarboxylic acids a closer examination of the above subject will be undertaken.

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## Carotene Isomers in some Red Algae

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According to previous investigations,  $\beta$ -carotene is the only carotene isomer present in most red algae (Table 1). An investigation of the unsaponifiable matter of *Rhodymenia palmata*, carried out at the Institute of Organic Chemistry at the Norwegian Technical University in Trondheim, seemed to indicate the presence of both  $\alpha$ - and  $\beta$ -carotene in this alga. As this was contrary to previous observations of Heilbron *et al.*<sup>1</sup> (Table 1), it was decided to carry out a further investigation of the carotene isomers in red algae.

Samples of the more common red algae were collected in the Trondheimsfjord during the months of May to August 1955. The samples were blanched with steam at 100° C as soon as possible after harvesting and dried at approximately 35° C overnight. This proved to be the best procedure, since quantitative extraction of the carotene from fresh material was difficult to accomplish and no carotene breakdown was observed during the steam-treatment and drying<sup>2</sup>. After having been ground to pass a 60 mesh sieve, 1 g of the sample was moistened with 5 ml of water, and extracted overnight with 25 ml of acetone. The extraction was repeated until the extract was colourless. The carotene was extracted from the acetone solution, after addition of more water, with light petroleum, and the light petroleum extract concentrated to a small volume on a water bath. The carotenes were separated from chlorophylls and other carotenoids by chromatography on magnesium oxide — Hyflo Super-Cel (1:3). The carotene zones were washed through the column with light petroleum containing 0.5 % acetone, the eluate made up to 50 ml and the carotene concentration determined by reading the optical density at 436  $\mu$  in a Beckman spectrophotometer.

In the case of *Rhodymenia palmata*, two zones were observed on the column, apart from the stationary chlorophyll and carotenoid zones: A fast moving yellow zone, and a slower moving brick-red zone. Complete separation of the two pigments was

Table 1.

	$\alpha$ -Carotene	$\beta$ -Carotene	Refs.	Total amount of carotene, p.p.m.
<i>Ahnfeltia plicata</i> (Huds.) Fries	+ (+?)	+ (+)	3	69
<i>Ceramium rubrum</i> (Huds.) Ag.	+ (+)	+ (+)	5, 6, 3	326
<i>Chondrus crispus</i> Stackh.	+ (+?)	+ (+)	3	84
<i>Delesseria sanguinea</i> (L.) Lamour.	+ (-)	+ (+)	4	270
<i>Dumontia incrassata</i> (Müll.) Lamour.	+	+		222
<i>Furcellaria fastigiata</i> (Huds.) Lamour.	+ (-)	+ (+)	4	135
<i>Gigartina stellata</i> (Stackh.) Batt.	+ (+?)	+ (+)	3	86
<i>Membranoptera alata</i> (Huds.) Stackh.	+	+		67
<i>Ptilota plumosa</i> (L.) Ag.	+	+		65
<i>Phycodrys sinuosa</i> (Good. et Woodw.) Kütz.	+	?		
<i>Rhodymenia palmata</i> (L.) Grev.	+ (-)	+ (+)	1	360
<i>Callithamnion arbuscula</i> (Dillw.) Lyngb.	-	+		200
<i>Corallina officinalis</i> L.	- (-)	+ (+)	3, 5	
<i>Polysiphonia fastigiata</i> (Roth.) Grev.	- (-)	+ (+)	3	245
<i>Polysiphonia urceolata</i> (Dillw.) Grev.	-	+		
<i>Porphyra</i> sp.	- (-)	+ (+)	3, 4	85

The signs in brackets refer to earlier investigations.  
References are given in the third column.

obtained by chromatography on calcium hydroxide from light petroleum, and developing with the solvent mentioned above. The two zones were cut out of the column and the pigments extracted with acetone. After filtering off the adsorbent, the acetone was removed by evaporation *in vacuo* and the pigments redissolved in carbon disulphide (spectroscopic grade). The absorption spectra were read in a Beckman spectrophotometer. The pigment from the yellow zone showed an absorption pattern in carbon disulphide identical with that of  $\alpha$ -carotene, with the peaks at 509 and 477  $m\mu$  (Fig. 1). Further identification was carried out by means of carotene isolated from carrots. This well known mixture of  $\alpha$ - and  $\beta$ -carotene showed exactly the same picture as that from *Rhodymenia* when chromatographed on the same adsorbent. Both isomers were isolated from carrots and a "misch"-chromatogram with the corresponding zones from *Rhodymenia* was run on calcium hydroxide. Both mixtures behaved as homogeneous substances. In order to rule out the possibility of the first zone being a *cis-trans* isomer of the second, the pigments from the yellow zone of *Rhodymenia* and carrots were isomerised by boiling under reflux in light petroleum. Chromatography of the isomerised pigments showed exactly the same zones in

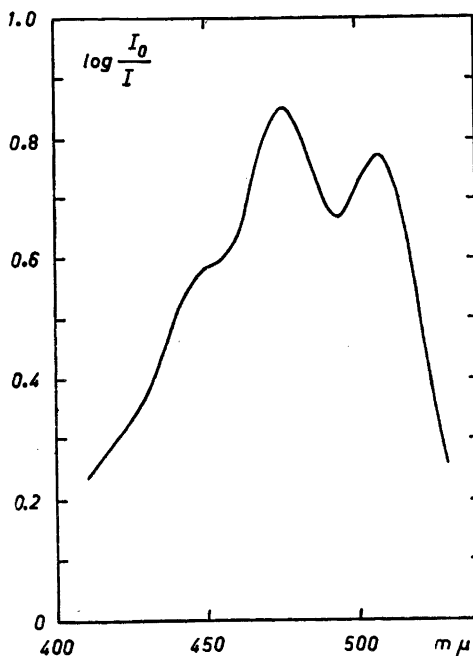


Fig. 1. Absorption spectrum of the pigment from the yellow zone.

both cases, and no brick-red zone could be observed above the yellow one.

Since Carter *et al.*<sup>3</sup> and Kylin<sup>4</sup> state that *Ceramium rubrum* contains both  $\alpha$ - and  $\beta$ -carotene, we also carried out a comparison between this alga and *Rhodymenia*. Chromatography of the light petroleum extract on magnesium oxide and calcium hydroxide showed exactly the same picture for both species, and the isolated pigments were indistinguishable with respect to their absorption spectra in carbon disulphide. According to these experiments, therefore, we find it safe to conclude that *Rhodymenia palmata* contains both  $\alpha$ - and  $\beta$ -carotene.

Several other species of *Rhodophyceae* were investigated along the same lines. The results are given in Table 1. The results from earlier investigations are shown in the same table. As the table shows, 11 of the 16 investigated species were found to contain  $\alpha$ -carotene.

The table also shows the total amounts of carotene ( $\alpha + \beta$ ) given as mg per 1 000 g dry matter. The amounts were found to depend strongly on the season and the treatment of the samples. The figures given here should, however, give a fairly correct picture of the variation between the species during the summer months.

The relative amounts of  $\alpha$ - and  $\beta$ -carotene were determined in 6 species. The results are given in Table 2 as the amount of  $\alpha$ -carotene divided by the amount of  $\beta$ -carotene. The amounts are calculated from the optical densities in carbon disulphide and the molar extinction of  $\alpha$ - and  $\beta$ -carotene in the same solvent. As the table shows, the amount of  $\alpha$ -carotene in the investigated species was of the same order of magnitude as that of  $\beta$ . *Delesseria sanguinea* was found to be particularly rich in  $\alpha$ -carotene. Contrary to the total amounts of carotene, the relative amounts seemed to be approximately constant, independent of the season (samples from the winter months were not investigated) and the treatment of the samples.

*Phycodrys sinuosa* differed markedly from the other investigated species, as in

this alga we were not able to show the presence of  $\beta$ -carotene. Samples from different localities and collected at different seasons gave the same result. We are thus forced to conclude that *Phycodrys sinuosa* either has a specific mechanism for a rapid destruction of  $\beta$ -carotene, or that the amount of  $\beta$ -carotene, if this is present at all, was very small compared with  $\alpha$ -carotene. Whatever the explanation may be, this species seems to deserve a further investigation.

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### Carotene Breakdown in *Rhodymenia palmata* (L.) Grev.

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During an investigation of the carotene breakdown in some Norwegian seaweeds and seaweed meals an extraordinary rapid loss of carotene was observed in the red alga *Rhodymenia palmata*. This alga, therefore, seemed to be a convenient object for further studies of the breakdown mechanism. The carotene present in *Rhodymenia palmata* was found to consist of about equal amounts of the  $\alpha$ - and  $\beta$ -isomers<sup>1</sup>.

Table 2.

	$\alpha/\beta$
<i>Delesseria sanguinea</i>	3.1
<i>Rhodymenia palmata</i>	1.4
<i>Furcellaria fastigiata</i>	1.3
<i>Ceramium rubrum</i>	0.5
<i>Gigartina stellata</i>	0.5
<i>Dumontia incrassata</i>	0.4