

Carotenoids of Flexibacteria

V.* The Chirality of Zeaxanthin from Different Natural Sources

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The absolute configuration of natural zeaxanthin (1), 3*R*, 3'*R*, was recently elucidated by De Ville *et al.*¹ by means of X-ray crystallographic analysis of a degradation product of fucoxanthin. Fucoxanthin had previously been converted to zeaxanthin under conditions that preserved the stereochemistry at positions 3 and 3'.^{2,3} Bartlett *et al.*⁴ who recently published the optical rotatory dispersion (ORD) of a large number of carotenoids, found that zeaxanthin from various plant sources (pepper, pumpkin, brown alga *etc.*) displayed closely similar curves reflecting identical stereochemistry.

In the present communication we report on the circular dichroism (CD) of zeaxanthin isolated^{5,6} from a blue-green alga, *Arthrospira* sp., and a non-photosynthetic bacterium, *Flexithrix dorotheae*,⁷ previously⁸ named *Flexithrix* strain QQ. The identity of the pigments was established by direct comparison with synthetic (racemic)⁸ and natural (Hoffmann-La Roche, source unknown) zeaxanthin. The latter organism proved to be an excellent source for zeaxanthin (0.08 % of dry weight) as this pigment was the only one produced, and was readily purified.

The CD curves of zeaxanthin ex *Arthrospira* and *Flexithrix* presented in Fig. 1 are analogous. The CD extrema at 225 nm (negative), 243 nm (positive) and 283 nm (negative) correspond well with the values extracted from the figure given by Bartlett *et al.*⁴ for zeaxanthin of plant origin. The present curves cover a somewhat wider wavelength region than

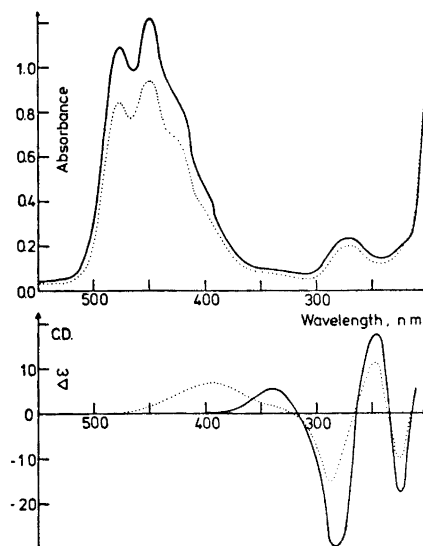
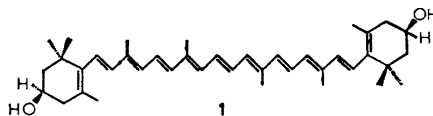


Fig. 1. The electronic spectra and the circular dichroism spectra of — zeaxanthin ex *Flexithrix* strain QQ, and ... zeaxanthin ex *Arthrospira* sp. recorded in EPA solution.

the previous measurements. The discrepancy in the long wave length region may be due to *cis*-isomerization during the registration and is disregarded.

This result might imply that zeaxanthin generally possesses the same absolute configuration 3*R*, 3'*R* irrespective of whether the source is higher plants, algae, or bacteria.



Experimental. The optical properties of 1 ex *Flexithrix* and ex *Arthrospira* were recorded as described elsewhere.⁹

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1. De Ville, T. E., Hursthouse, M. B., Russel, S. W. and Weedon, B. C. L. *Chem. Commun.* **1969** 1311.
2. Bonnet, R., Mallams, A. K., Tee, J. L. and Weedon, B. C. L. *Chem. Commun.* **1966** 515.
3. Bonnet, R., Mallams, A. K., Spark, A. A., Tee, J. L., Weedon, B. C. L. and McCormick, A. J. *J. Chem. Soc.* **1969** 429.
4. Bartlett, L., Klyne, W., Mose, W. P., Scopes, P. M., Galasko, G., Mallams, A. K., Weedon, B. C. L., Szabo, J. and Toth, G. *J. Chem. Soc.* **1969** 2527.
5. Hertzberg, S. and Liaaen-Jensen, S. *Phytochemistry* **5** (1966) 557.
6. Aasen, A. J. and Liaaen-Jensen, S. *Acta Chem. Scand.* **20** (1966) 2322.
7. Lewin, R. A. *Can. J. Microbiol.* **16** (1970) 511.
8. Isler, O., Lindlar, H., Montavon, M., Rüegg, R., Saucy, G. and Zeller, P. *Helv. Chim. Acta* **39** (1956) 2041; **40** (1957) 456.
9. Borch, G., Norgård, S. and Liaaen-Jensen, S. *Acta Chem. Scand.* **26** (1972) 402.

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Purification and Some Properties of a Group of Small Basic Proteins from Rapeseed

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The possibility to obtain high-quality protein for nutritional use from rapeseed, has evoked interest in the chemical and physico-chemical properties of the individual rapeseed proteins. Bhatti *et al.*¹ have isolated three main protein fractions soluble in salt solutions. One of these, containing 17% of the total nitrogen in rapeseed, was shown to be basic and of low molecular weight. Using extraction with neutral buffer, followed by a single gel filtration step, a similar protein fraction has been isolated. This fraction consists of several basic proteins, which appear to be closely related.

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Isolation of a low-molecular weight basic protein fraction. Rapeseed (*Brassica napus* L. var. *oleifera*), strain Panter, was a gift of Dr. Ragnar Olsson, Karlshamns Oljefabriker, Sweden. The seeds were put through a roll-mill and the flaky product was extracted 10 times on a Büchner funnel with an equal volume (w/v) of hexane (b.p. 66–69°). The hexane was removed by vacuum distillation in a Büchi Rotavapor at 50°, 2 h. 15 g dry rapeseed meal was extracted with 450 ml ice-cold 0.1 M sodium phosphate pH 7.0 in centrifuge bottles. The bottles were shaken vigorously for 5 min and the insoluble material was removed at 23 500 g, 10 min, 0°. The extract, containing 6520 mg protein, was immediately applied to a Sephadex G-50 column. The elution pattern is shown in Fig. 1. The basic protein fraction is collected with the second peak. The yield is 2760 mg.

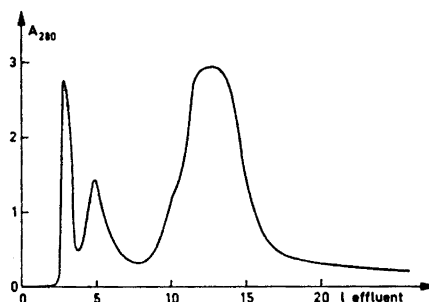


Fig. 1. Rapeseed meal extract on a Sephadex G-50 column. Column dimensions 95 × 12.0 cm. Sample volume 350 ml (6520 mg protein). Elution with 0.1 M sodium orthophosphate pH 7.0. Flow rate 0.84 l/h.

Extraction with neutral buffer solubilizes between 50 and 60% of the total protein in rapeseed meal. The small basic proteins are completely solubilized. Thus the initial extraction in itself constitutes a purification step.

The short time and the low temperature used in the extraction were chosen in order to minimize the action of myrosinase, which is known to be present in rapeseed.² Myrosinase splits thioglucosides present in the extract and the products of this splitting may possibly form complexes with the proteins. Longer extraction times and higher temperatures only led to slight increases in yield.

Separation of individual basic proteins by ion exchange chromatography. The basic protein fraction from the gel filtration step was