2. Propylamine (0.2 mole) was added to 2,5-difluoronitrobenzene (0.08 mole) in ethanol (24 ml). Crystals separated on standing, which formed red plates (from ethanol) of 4-fluoro-2-nitro-N-propylaniline (89.5% yield), m.p. 65–66° (Anal.-Found: C, 54.5; H, 5.50; N, 14.0. $C_9H_{11}FN_2O_2$ requires C, 54.5; H, 5.59; N, 14.1%).

This product (0.017 mole) was mixed with granulated tin (3.8 g), concentrated hydrochloric acid (9.0 ml) was slowly added, and then the mixture was warmed to 100 °C for 40 min. After cooling, it was made alkaline, extracted with ether and the combined ethereal extracts extracted with *M* hydrochloric acid (total of 50 ml). Dissolved ether was removed and the combined acidic extracts (containing the diamine) were immediately condensed with mandelic acid.

Preparation of the benzimidazole derivatives. 1. *o*-Phenylenediamine (0.01 mole), *p*-fluoromandelic acid (0.015 mole) and *M* hydrochloric acid (25 ml) were heated under reflux for 6 h. The reaction mixture was cooled, treated with concentrated ammonium hydroxide and the precipitate collected. The latter gave 2-(α-hydroxy-*p*-fluorobenzyl)-benzimidazole (HFBB) (75.4% yield) as microcrystals from benzene, m.p. 105–106°; *M* (mass-spectrum), 242; τ [Me_2SO] −2.20br (1H, NH), 2.4–2.6 (4H, m, aromatic), 2.7–2.9 (4H, m, aromatic), 3.46 (1H, d, *J* 4Hz, OH), 4.06 (1H, d, *J* 4Hz, CH-Ph). The hydrochloride crystallized from ethanol-ether as prisms, m.p. 285° (dec.) (Anal.-Found: C, 60.2; H, 4.43; N, 10.1. $C_{14}H_{12}ClFN_2O$ requires C, 60.3; H, 4.34; N, 10.1%).

2. 5-Fluoro-2-(α-hydroxybenzyl)benzimidazole (5-FHBB) was obtained in a similar manner from 4-fluoro-*o*-phenylenediamine (1 mole) and mandelic acid (1.3 mole), but in 33.3% yield. The hydrochloride separated from the reaction mixture and was collected, dissolved in water, and the solution treated with charcoal. After addition of concentrated ammonium hydroxide, the precipitated base was crystallized from aqueous methanol as plates, m.p. 207.5–208.5° (Anal.-Found: C, 69.2; H, 4.59; N, 11.5; *M* [mass-spectrum], 242. $C_{14}H_{11}FN_2O$ requires C, 69.4; H, 4.58; N, 11.6%; *M*, 242); τ [Me_2SO] −2.45 br (1H, NH), 2.4–2.8 (7H, m, aromatic), 3.03 (1H, td, *J* 9 and 3Hz, H-6), 3.47 br (1H, s, OH), 4.07 (1H, s, CH-Ph).

3. 5-Fluoro-1-propyl-2-(α-hydroxybenzyl)benzimidazole (FPHBB) was obtained from 2-amino-4-fluoro-1-propylamine (1 mole) and mandelic acid (1 mole) using the procedure described for the *p*-fluoro derivative. The yield was 45.5% from the nitropropylaniline. The 5-fluoro-1-propyl compound crystallized as plates from aqueous methanol, m.p. 169–169.5° (Anal.-Found: C, 72.1; H, 5.90; N, 10.0. $C_{17}H_{17}FN_2O$ requires C, 71.8; H, 6.03; N, 9.9%).

Clearly FPHBB is an outstanding compound of unrivalled inhibiting activity and comparatively low cytotoxicity. Its potential is enhanced by its accessibility from a 3-stage synthesis with an overall yield of 40.7%. We are continuing our study of the spectrum of antiviral activity of these fluoro compounds, their mode of action and the possible development of resistant virus strains.

Zusammenfassung. Fluorierte Derivate des 2-(α-Oxybenzyl)-benzimidazol hemmen die Vermehrung des Poliovirus, 1, 2 und 3, sowie diejenige des Coxsackievirus A9 und A21.

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The Major Carotenoid Pigments of Six Species of Barnacle

In the blue oceanic barnacle *Lepas fascicularis* a preponderance of astaxanthin has been found, associated with the characteristic blue carotenoprotein[1,2]. The littoral stalked species *Pollicipes polymerus* also contains large amounts of astaxanthin and its esters, although lutein is the second major pigment, rather than zeaxanthin as in *L. fascicularis*[3]. In contrast the parasitic rhizocephalan species *Sacculina carcini* only contains β-carotene[4]. Three further stalked species, in addition to more specimens of *L. fascicularis*, and 2 sessile species from very different environments have been investigated.

Lepas anatifera, L. fascicularis and *L. pectinata* were obtained during cruises of RRS Discovery in the eastern north Atlantic in 1967, 1968 and 1969 and *Conchoderma virgatum* were removed from the ships hull at the same time. All other animals were deep-frozen at −20 °C on board ship until they could be transported back to the laboratory for analysis. *Chthamalus fragilis* were kindly obtained by Dr. P. Bacon of the University of the West Indies and flown live to England and *Elminius modestus* were grown on settling plates at the Admiralty exposure trials station, Portsmouth, through the courtesy of Mr. D. Houghton.

It proved impracticable to remove gut contents before the pigments were extracted, but the sessile species were scrubbed before removal from their settlement sites in order to remove any adventitious plant material on the external surfaces of their plates. All pigments were extracted, identified, and quantitative determinations made, as described earlier[5]. *Chthamalus* and *Elminius* extracts were saponified before separation in order to remove relatively large amounts of lipid and some chlorophyll products.

The results of the analysis of the various species are given in the Table. Although the stalked species have a carotenoid content some 10 times that of the sessile species when calculated on a fresh weight basis the values would probably be much more nearly equivalent were the weights of the animals' calcareous plates not included. The content of astaxanthin in *L. fascicularis* is rather lower than in the earlier analysis[2], and this is almost certainly correlated with the almost total absence of blue somatic coloration and blue eggs in the animals used in the present investigation. There is a consequent relative increase in the content of zeaxanthin and its esters, and there is a significant proportion of fucoxanthin.

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Concentration and percentage composition of the carotenoid pigments of barnacles

	P. polymerus[3]	*L. fascicularis*[2] Eggs	*L. fascicularis*[2] Gut	*L. fascicularis* Somatic tissues	*L. fascicularis*	*L. anatifera* Shell lip	*L. anatifera* Whole	*L. pectinata*	*C. virgatum*	*E. modestus*	*Chth. fragilis*
Carotenoids (mg/100 g fresh wt.)	–	5.1	2	1.06	1.6	2.0		2.8	1.8	0.15	0.23
% of total											
β-carotene	4	–	6	4	9	11	3	Trace	Trace	8	10
Zeaxanthin esters	–	2	4	4	23	61	10	4	–	–	–
Zeaxanthin	5	–	–	4	14	–	5	10	–	41	29
Astaxanthin esters	26	5	–	–	–	–	–	6	30	{34	{39
Astaxanthin	29	93	56	82	36	23	59	70	44		
Mono-hydroxy xanthophyll	–	–	–	–	–	–	–	–	–	18	23
Canthaxanthin	–	–	–	–	–	–	2	–	–	–	–
Phoenicoxanthin	–	–	–	–	–	3	6	10	10	–	–
Isozeaxanthin?	2	–	–	–	–	–	–	–	–	–	–
Lutein	34	–	–	–	–	–	–	–	–	–	–
Fucoxanthin	–	–	–	–	15	1	11	–	–	–	–
Neoxanthin?	–	–	–	–	–	–	–	–	7	–	–
Peridinin	–	–	12	–	–	–	–	–	–	–	–
Unidentified	–	–	22	4	–	–	4	–	9	–	–

Included in the figures for fucoxanthin content are smaller amounts of an associated very similar but rather more polar pigment, possibly fucoxanthinol, which may be formed during extraction[6]. Faecal pellets produced by living animals were extracted separately and were found to contain astaxanthin as the major pigment and smaller amounts of astaxanthin esters, β-carotene zeaxanthin and fucoxanthin. All the pigments found in this species may therefore be ascribed to similar pigments directly absorbed from the food, with the exception of zeaxanthin esters which are presumably metabolised from ingested zeaxanthin. Similar pigments were found in *L. anatifera*, with the addition of small quantities of canthaxanthin and phoenicoxanthin. The latter pigment was identified by comparison of the properties of the native, saponified and reduced pigment with those reported for phoenicoxanthin[7–9]. The orange lip of this species was removed from several animals and was found to contain a high proportion of zeaxanthin esters. The pigments of *L. pectinata* are similar, though lacking fucoxanthin, and equally high proportions of astaxanthin and its esters were obtained from the dark coloured species *C. virgatum*. The blue-grey colour of this species is not carotenoid in origin. The species contained a much more polar polyhydroxy xanthophyll containing one epoxide group and rather similar to neoxanthin. A non-carotenoid yellow pigment with high UV-absorption was also present.

The 2 sessile species contain lower proportions of astaxanthin and substantial amounts of an unidentified xanthophyll which had a β-carotene-like spectrum, no allylic hydroxyl and no epoxide group. It formed only a single ester on acetylation and was separable on thin layers of silica gel and alumina from cryptoxanthin, but only with difficulty from iso-cryptoxanthin. Virtually the only visibly pigmented tissues in these 2 species were the egg lamellae, which were pale yellow or orange, and the gut. The egg lamellae contained hardly any zeaxanthin, only β-carotene, monohydroxy xanthophyll and astaxanthin. Unsaponified extracts, particularly that of *E. modestus*, showed significant amounts of an orange pigment, with a mobility similar to that of fucoxanthin, when run on thin layers of silica gel. However any such pigment would have been destroyed during saponification.

When the zeaxanthin fraction of each species was acetylated there appeared on thin layer plates, in addition to the main band inseparable from zeaxanthin diacetate, a slightly slower moving band most prominent in the extracts of *Chthamalus* and *Elminius*. It is possible that this minor fraction represents esterified diatoxanthin or alloxanthin, the mono and diacetylenic analogues of zeaxanthin, both of which are accumulated by the mussel *Mytilus*[10], a typical phytoplankton feeder.

Extracts of all species contained additional traces of other pigments, which were not considered further and may have been due in part to undigested food debris. The presence of astaxanthin and other pigments in the faeces of *L. fascicularis* indicates that absorption of pigment from the food is at best a partial process, and the relatively high proportions of astaxanthin in the stalked species may reflect the predominance of animal food in these species[11]. Typical algal xanthophylls such as fucoxanthin are likely to have been absorbed directly from phytoplankton. It has recently been suggested that some fish may synthesize astaxanthin from lutein[12], but there is no evidence that any such isomerism occurs in crustacean carotenoid metabolism.

Résumé. L'astaxanthine (et ses esters) est le caroténoïde principal de 6 espèces de balanes, et la zeaxanthine est le principal xanthophylle neutre. Les différences de composition du pigment des espèces sont probablement liées aux proportions relatives de la matière animale et végétale dans ses aliments.

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