

Identity of the Shell Pigment of *Haliotis cracherodii* Leach

IN 1903 Schultz directed attention to the blue, acid-soluble pigment which occurs in the shells of the Californian abalone. Subsequent studies by Schultz, Schultz and Becker¹, Kodzuka² and Lemberg³ have further subdivided the pigments present; but there has been no agreement as to their identity. Schultz, in his later papers, pointed to the similarity between the visible absorption band of the main pigment (λ_{\max} . 622 $m\mu$) and that of the known indigos, and Lemberg identified the pigment provisionally as a pyrrole body, since its solubility in dilute acids is greater than that of most indigoids. The pigment is known to give a strong colour reaction with nitrites and with fuming nitric acid. There has been doubt about the exact nature of this reaction, and about the number of pigments responsible for it.

Using a technique of chromatography on talc, and tarting with acid extracts (in 2N hydrochloric acid) of shells of *Haliotis cracherodii*, of which Schultz's *H. californiensis* is a variety, we have obtained clear-cut separation of the pigments on the column into a lower green, brown and violet series, some of which give reactions typical of bilitrienes, and a main blue reaction corresponding to Lemberg's pigment B. We further purified this main pigment by chromatography of amyl alcohol extractives from the original acid solution, and produced a homogeneous fraction which could not be resolved on the column. Details of this preparation will be published elsewhere.

The reaction of the blue pigment with nitrite is not typical of Gmelin's bilitriene reaction; it is, however, closely similar to that given by indolyacetic acids (Nencki-Sieber test), and the amyl alcohol-soluble product of the reaction gives a visible absorption, plotted on the Beckmann spectrophotometer, which resembles that recorded for the urosein pigments by Homer⁴, by Watson⁵, and by Rimington, Holiday and Jope⁶.

<i>Haliotis</i> derivative	Urosein (Watson ⁵)	
Ammonium hydroxide :	522-492	555-557 $m\mu$
Hydrochloric acid :	559-550, 540-490	544, 511
Urosein from indolyacetic acid (Homer ⁴)		
	552-531, 505-492	
from indolyacetic acid		
	579-539, 502-485	

In view of these findings, a specimen of the blue pigment in chloroform-cyclohexane was examined for fine structure in the ultra-violet region, using a Hilger medium quartz spectrograph and a hydrogen arc source, and the resulting bands compared with those of indigotin. The following results were obtained :

	<i>Haliotis</i> blue	Indigotin
Bands	3570	3560
λ_{\max} . (A.)	3370	3370
	3226	321
	3070	3070
	—	2856
	—	2745

These results confirm the view of Schultz and Becker¹ that the pigment is closely related to indigotin, and is not a pyrrole; in its solubilities it differs considerably from the known indigos, being considerably more soluble in aqueous acids even than the N-N' dialkyl indigos. Details of its behaviour, and of the concomitant pigments, will appear elsewhere.

The finding is of interest in view of the occurrence of substituted indigos in the eggs and hypobranchial gland of many *Stenoglossa*. Pigments of this type

have not previously been detected in the lower gastropods.

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² Kodzuka, T., *Tōkoku J. Exp. Med.*, **2**, 287 (1921).

³ Lemberg, R., *Hoppe-Seyler's Z. phys. Chem.*, **200**, 173 (1931).

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⁴ Homer, A., *J. Biol. Chem.*, **22**, 345 (1915).

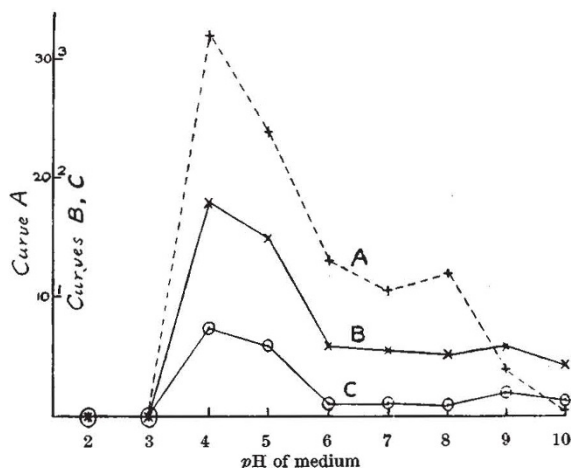
⁵ Watson, C. J., *Proc. Soc. Exp. Biol. N.Y.*, **39**, 514 (1938); **41**, 591 (1939).

⁶ Rimington, C., Holiday, E. R., and Jope, E. M., *Biochem. J.*, **40**, 669 (1946).

Germination and Development of Heather and the Hydrogen Ion Concentration of the Medium

SEVERAL workers¹⁻³ have shown that, in the field, heather (*Calluna vulgaris* Salisb.) is found most frequently and attains its maximum development on soils the reactions of which are about pH 4. I have examined the germination and the subsequent seedling development of *Calluna* seeds planted on agar media adjusted to give a range of reactions. A quantity of 1 per cent British agar was prepared with a form of Knop's solution. This was divided equally between nine flasks. The contents of these were adjusted to pH 2, 3, 4, 5, 6, 7, 8, 9 and 10, respectively, by the addition of the requisite quantities of sulphuric acid or caustic soda. After adjustment, the agar in each flask was poured into six 1-in. boiling tubes to give a depth of agar of about 1½ in. The tubes were plugged and the agar allowed to set. A liberal sowing of *Calluna* seeds was then made in all tubes.

After six weeks, germination was found to have occurred in all the cultures, although, in most cases,



Number and dimensions of seedlings of *Calluna vulgaris* in relation to pH. (A) Mean number of seedlings; (B) average of mean shoot-lengths (cm.); (C) average of mean root-lengths (cm.)