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Acid-soluble Pigments of Molluscan Shells

4. IDENTIFICATION OF SHELL PORPHYRINS WITH PARTICULAR REFERENCE TO CONCHOPORPHYRIN

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(*Received* 13 *December* 1948)

Particular interest has been attached to the nature of the porphyrins present in shells since Fischer & Jordan (1930) reported the occurrence in the marine mollusc *Pteria radiata* of a porphyrin, containing five carboxyl groups, which they named conchoporphyrin, and which was the only pentacarboxylic porphyrin known in nature. Fischer & Holt (1934) later compared it with a synthetically prepared pentacarboxylic porphyrin. The findings of other workers (Fischer & Haarer, 1932; Waldenström, 1937; Tixier, 1945) indicate, however, that only uroporphyrins I and III and traces of coproporphyrin are responsible for the pink fluorescence of molluscan shells.

One of us (Comfort, 1949) has recorded an extensive survey of the pigments present in molluscan shells, and has noted the phylogenetic distribution of porphyrin.

Having at our disposal a method of separating porphyrins, by partition chromatography, into groups depending upon the number of carboxyl functions in the molecule (Nicholas & Rimington, 1949; Rimington, 1949), we have examined as many porphyrin-containing shells of different species as possible, including *Pteria radiata* Lk., in the hope of confirming and extending Fischer & Jordan's (1930) observations. We have also had access to some of the original porphyrin material prepared by these authors (m.p. 270–273°) kindly provided by Prof. J. Waldenström of Uppsala, but we have obtained no evidence for the existence of a pentacarboxylic porphyrin.

METHOD

Porphyrins and other acid-soluble shell pigments are quantitatively adsorbed on talc; satisfactory separation of the individual porphyrins was not, however, achieved by this method. The acetic acid-ether technique for the extraction of ether-soluble porphyrins is difficult to apply to shell extracts on account of the large quantities of calcium salts.

Talc adsorption chromatography was used in order to separate the porphyrin as far as possible from the other shell pigments present, and finally the paper-partition method (Nicholas & Rimington, 1949) for the separation of the individual groups of porphyrins.

Technique of talc chromatography

Preparations of columns. Talc powder (pharmaceutical) was shaken with excess of 2N-HCl, and the suspension poured through a funnel containing some glass beads (to remove bubbles) into glass tubes 20 × 2 cm., the lower end of each being closed by a cotton-wool plug resting on a ceramic insulator. The suspension was then packed with the aid of suction.

Extraction of shells. The shells were broken and decalcified for 24 hr. in conc. HCl, or in a mixture of phosphoric acid (sp.gr. 1·75) with an equal volume of water. The extracts were filtered through glass wool to remove debris, adjusted to contain between 1 and 2·5N-acid by dilution with water, and poured into the tubes.

Chromatography. The extracts were drawn through the columns under suction. The chromatograms were either developed with increasing strengths of acetone in aqueous 3 N-HCl, or dried for several hours over suction and extruded by air pressure from below, the fluorescent zones being cut out with a cover slip, or with a specially made glass knife.

Precautions. The adsorptive power of various samples of talc showed considerable variation, and the acidity of the solvent should not exceed 2·5 N; stronger acids lead to blurring of bands and reversal of their order. Packing from acid suspension was found necessary, even with 'acid-washed' talc, to avoid cracking of the column when suction was applied.

The detailed appearance of the chromatograms and the spectral character of the non-porphyrin bands has been described in a previous paper of this series (Comfort, 1949). The porphyrin present was localized to one narrow pink fluorescent band, except in the case of *Pteria vulgaris*, where division of the porphyrin was obtained during the separation of a blue pigment; spectroscopy of the porphyrin fractions in acid solution showed in each, however, a Soret band consistent with uroporphyrin ($\lambda = 405$ mμ. in 2 N-HCl).

Preparation of porphyrin for partition chromatography

The section of the talc columns containing the porphyrin, as shown by ultraviolet light, was cut out and placed in acetone containing 1 % by volume of 3 N-HCl. This eluted all the porphyrin together with some of the other pigments adjacent to it on the original talc column. The eluate was filtered through a sintered-glass funnel to remove the talc and the latter washed with HCl-acetone solution. After combining the washings with the first eluate, the acetone was removed under reduced pressure at room temperature. The solution, which was highly fluorescent in ultraviolet light, but varied in colour with individual shells from brown to violet, was then passed through a small talc column to remove the remaining non-porphyrin pigments and the inorganic salts which interfere with the partition chromatography.

This column, 3 × 0·5 cm., was packed from a suspension of talc in distilled water; under these conditions the porphyrin is adsorbed on the top of the column with the other pigments beneath it. The column was then washed with distilled water until the eluate was free from Cl^-. Any remaining non-fluorescent pigments were then removed by washing the column with increasing concentrations of aqueous NH_3, beginning with 0·01 N, the porphyrin being finally eluted with 10 N-NH_3 in which it descends as a narrow band and can be collected in approximately 0·1 ml. of solution which was applied to the paper chromatogram.

The specimen of Fischer's conchoporphyrin ester (m.p. 270–273°; Fischer & Jordan, 1930) was prepared for chromatography by hydrolysing it in approximately 7 N-HCl for 24 hr. at room temperature. The HCl was removed in a vacuum desiccator over KOH and the porphyrin residue dissolved in 0·1 ml. of aqueous 10 N-NH_3.

Partition chromatography

The partition chromatograms were run on strips of Whatman no. 1 filter paper (6 × 40 cm.) at a temperature of 21°, using a mixture of the 2:4- and 2:5-dimethylpyridines ('lutidine') as solvent, and in an atmosphere saturated with the solvent and water vapour.

Porphyrins are separated under these conditions into spots clearly visible by their pink fluorescence in ultraviolet light. It has been shown that the R_F values of the spots bear an inverse linear relationship to the number of carboxyl functions in the porphyrin molecule which they represent (Nicholas & Rimington, 1949).

The R_F values at 21° for representative porphyrins are recorded in Table 1.

Table 1. *R_F values of porphyrins in 'lutidine' at* 21°

Porphyrin	R_F values	No. of carboxyl functions
*Uroporphyrin	0·07 (0·12)	8
Coproporphyrin	0·5	4
Protoporphyrin (and other 2-carboxyl porphyrins)	0·75	2
Phylloerythrin	0·87	1
All esters	0·98	0

* Uroporphyrin characteristically gives two spots.

In order to avoid misinterpretation through slight changes in R_F due to variation in conditions, known markers were run on the strip in every case alongside the material under investigation.

RESULTS

The results which we have obtained with eight different types of shell and with Fischer's conchoporphyrin (Fischer & Jordan, 1930), stated to be derived from *Pteria radiata*, are recorded in Table 2.

Table 2. *R_F values and identification of porphyrins present in materials examined*

(The R_F value in brackets represents the additional uroporphyrin spot.)

Shell	R_F values of spots obtained	Porphyrin present
Fischer's 'conchoporphyrin' (*Pteria radiata*)	0·06 (0·11) 0·49	Uroporphyrin and coproporphyrin
Pteria radiata from S. Carolina	0·07 (0·12)	Uroporphyrin
Pinctada vulgaris (*Pteria vulgaris*)	0·07 (0·12) 0·49	Uroporphyrin and coproporphyrin
Trivia europœa	0·07 (0·12)	Uroporphyrin
Bulla sp.	0·08 (0·14)	Uroporphyrin
Placuna sella	0·05 (0·1)	Uroporphyrin
Umbonium australe	0·08 (0·13)	Uroporphyrin
Malleus vulgaris	0·06 (0·11)	Uroporphyrin
Gibbula cineraria	0·07 (0·11)	Uroporphyrin

DISCUSSION

The results confirm earlier evidence that the porphyrin present in shells is uroporphyrin, accompanied in some cases by smaller quantities of coproporphyrin. No evidence has been found for the existence of a penta- or hepta-carboxylic porphyrin.

The fact that the uroporphyrin does not behave as an entity on the chromatogram would point to the possibility of a seven carboxyl porphyrin (R_F 0·12) being present. This has been excluded on the grounds: first, that other samples of uroporphyrin of non-molluscan origin, considered pure by all other standards, behave identically; and secondly that the 'spot' of R_F 0·07, which represents the majority of the porphyrin, can be shown to decompose again into two spots (R_F 0·07 and 0·12) by rotating the chromatogram through 90° and re-running in the new direction with the same solvent.

There exists an element of doubt surrounding Fischer's identification of his shell material (Fischer & Jordan, 1930); his published papers deal specifically with two species of *Pteria*, viz. *Pteria radiata*, described as a Venezuelan species from which Fischer & Jordan (1930) obtained conchoporphyrin, and *Pteria vulgaris*, now called *Pinctada vulgaris*, which is widely distributed in the Indian Ocean and used in the button and fancy goods trade under the name of Persian lingah shell.

The specimens of *Pteria radiata* Lk.* which we have examined were from South Carolina and their identity was kindly established by Dr John Burch of Los Angeles who supplied them. We have also examined carefully identified *Pinctada* (*Pteria*) *vulgaris* from Bahrein on the Persian Gulf. These were obtained from the dealer who supplied Fischer with his specimens of *P. vulgaris*.

In view of the uncertainty, both chemical and zoological, surrounding Fischer's material, it would be difficult to express a definite opinion as to the existence of his 'conchoporphyrin', but the fact that we found his ester material, m.p. 270–273°, to be a mixture of uroporphyrin and coproporphyrin, casts doubts upon his claims. Certainly no pentacarboxylic porphyrin is present in any of the species of shell we have examined.

The uniformity which we find between the porphyrins of different groups of shells might have been predicted if it is assumed that they are end products of a closely similar or identical metabolic cycle. It is of interest that no porphyrins of the chlorophyll series have been encountered.

Although we have not been able to confirm the existence of conchoporphyrin, it may be mentioned that the technique of partition chromatography has revealed in other materials the existence of porphyrins presumably containing five and seven carboxyl groups respectively (Rimington, personal communication).

* A photograph of the shell may be obtained from the authors.

SUMMARY

1. Extracts of porphyrin-containing molluscan shells have been examined by adsorption and paper-partition chromatography.

2. The presence of uroporphyrin and, in one species, of coproporphyrin has been shown.

3. No evidence has been found for the existence in these shells of the pentacarboxylic porphyrin described by Fischer & Jordan (1930) under the name of 'conchoporphyrin'.

We wish to express our thanks to Prof. C. Rimington for his help and encouragement and for access to unpublished data, to Prof. J. Waldenström of Uppsala for a specimen of Fischer's conchoporphyrin, to Dr E. M. Jope, Medica Research Council Spectrographic Unit, London Hospital, for assistance with spectrographic measurements and to Miss A. Benson, for technical assistance. The work was financed by the Yarrow Research Fund (London Hospital) and by a grant from the Medical Research Council. The substance of this, and the foregoing three papers on molluscan pigments in this series, has been approved as part of a Thesis for the degree of Ph.D. in the University of London.

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