The Isolation and Structure of Trichosiderin B

The trichosiderins[2] are a group of phaeomelanic pigments occurring in human and mammalian red hair and in the feathers of many species of birds.

In previous reports[3–5] we have shown that a suitable source for the extraction of these pigments are the feathers of the New Hampshire chicken, from which 3 homogenous trichosiderins (C, E and F) have been isolated and characterized as $\Delta^{2,2'}$-bi(2H-1,4-benzothiazine) derivatives (I, II, III)[6]. From the same source we have now isolated an isomer of trichosiderin C which has been called trichosiderin B (IV), since it corresponds to the fraction designated B2 in earlier chromatographic work[7].

Alkaline extracts (0.1 *N* NaOH) of feathers (2.5 kg), cooled to 4 °C, were adjusted to pH 1 with 6 *N* HCl and centrifuged to remove the acid-insoluble gallophaeomelanins[8] and proteins. The yellow supernatant was then passed on to a column (2.5×10 cm) of Dowex 50W-X2 (100–200 mesh, H+ form), kept at 4 °C. After washing with 1 *N* HCl and distilled water, trichosiderins were eluted from the resin with 0.5 *N* NaOH and separated into the individual components by column chromatography on Sephadex G-25 (5×94 cm), using as the eluent phosphate buffer at pH 11. The fraction containing trichosiderin B, which emerged from the column after about 3 l, was re-chromatographed on Sephadex twice and eventually concentrated by resin treatment as described above. Acidification of the concentrated solution to pH 3 gave the pigment (65 mg) as an amorphous red-orange precipitate, insoluble in water and in any organic solvent.

The microanalyses suggested that the molecular formula was isomeric with that of trichosiderin C (Found: C, 49.51; H, 4.02; N, 9.70; S, 11.31. $C_{23}H_{20}N_4O_9S_2$ requires: C, 49.28; H, 3.57; N, 10.00; S, 11.42). Moreover, like trichosiderin C, the pigment showed aminoacidic properties and gave 4.7% Van Slyke nitrogen, corresponding to 2 primary amino groups.

Further structural similarity of the pigment to trichosiderin C was revealed by their absorption spectra which coincide in the UV and visible region (Table), indicating that the same chromophore must be present in both pigments.

Absorption spectra λ_{max} nm (log ε_{max}) in 0.2 *N* NaOH

Trichosiderin B	454	329	243	(3.98	4.01	4.60)
Trichosiderin C	452	327	240	(4.13	4.04	4.56)

On brief heating with 0.1 *N* HCl the pigment lost CO_2 (1 mole) to give descarboxytrichosiderin B (V; scheme I), obtained as an amorphous red powder, $C_{22}H_{20}N_4O_7S_2$ (Found/calc. C, 51.06/51.16; H, 4.03/3.87; N, 10.90/10.85; S, 12.07/12.40), by column chromatography of the reaction mixture on Sephadex G-25 (eluent 0.1 *N* NaOH). The UV-spectrum of V (in 0.2 *N* NaOH) displayed absorption maxima at 462, 313 and 245 nm (log 4.19, 4.15 and 4.49) with a large bathochromic shift on acidification ($\lambda^{H^+}_{max}$ 533, 358 and 299 nm), as expected for a $\Delta^{2,2'}$-bibenzothiazine chromophore without a carboxyl group at C-3[4].

Treatment of descarboxytrichosiderin B with HI and red phosphorus under mild conditions (30 min at 100 °C) gave β-7-(3-oxo-5-hydroxy-3,4-dihydro-2H-1,4-benzothiazinyl)-alanine (VI) and a mixture of 3-hydroxy-4-aminophenylalanine (VII, minor component) and 3-amino-4-hydroxyphenylalanine (VIII), identified[9] by comparison of their chromatographic properties with those of authentic samples[10,11].

Considering that 3-hydroxy-4-aminophenylalanine is a secondary product arising from further degradation of the amide VI[4], the results of the degradative experiment prove unequivocally the nature and the relative positions of the aromatic substituents attached to the $\Delta^{2,2'}$-bibenzothiazine chromophore of descarboxytrichosiderin B, which accordingly can be formulated as V. Hence structure IV can be derived for trichosiderin B.

Therefore it appears that trichosiderin B differs from trichosiderin C only in the position of attachment of an alanine residue to the $\Delta^{2,2'}$-bibenzothiazine chromophore. This structural difference is consistent with recent biosynthetic studies[12] according to which trichosiderins are considered as a new group of animal pigments deriving from tyrosine and cysteine by the intermediacy of either IX or X or of both.

Riassunto. Un nuovo pigmento feomelanico, denominato tricosiderina B, è stato isolato dalle piume di pollo di razza New Hampshire. Sulla base delle proprietà chimiche e spettrali a tale pigmento, $C_{23}H_{20}N_4O_9S_2$, è stata assegnata la struttura $\Delta^{2,2'}$-bibenzotiazinica IV.

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13 May 1971.

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[1] This work was supported by a grant from Laboratorio per la Chimica e Fisica di Molecole di Interesse Biologico del C.N.R., Napoli.

[2] P. Flesch, J. invest. Derm. *51*, 337 (1968) and references cited therein.

[3] G. Prota and R. A. Nicolaus, in *Advances in Biology of Skin* (Eds. W. Montagna and F. Hu; Pergamon Press, New York 1967), vol. 8, p. 323.

[4] R. A. Nicolaus, G. Prota, C. Santacroce, G. Scherillo and D. Sica, Gazz. chim. ital. *99*, 323 (1969).

[5] G. Prota, G. Scherillo, O. Petrillo and R. A. Nicolaus, Gazz. chim. ital. *99*, 1193 (1969).

[6] The stereochemistry of the $\Delta^{2,2'}$-bibenzothiazine skeleton in I, II and III has not yet been defined. However, studies of model compounds (F. Giordano, L. Mazzarella, G. Prota, C. Santacroce and D. Sica, J. chem. Soc., in press) have shown that the interconvention of geometrical isomers in this series occurs rather readily and the isolated pigments may well be equilibrium mixtures of the 2 forms.

[7] G. Prota and R. A. Nicolaus, Gazz. chim. ital. *97*, 665 (1967).

[8] Under these conditions trichosiderin C, which is only slightly soluble in aqueous dilute acids, co-precipitates in part with gallophaeomelanins.

[9] The identification was carried out using a Beckman Amino Acid analyzer (mod. 116) with a column (22 cm) of PA 35 resin operating under the standard conditions for the analysis of basic amino acids.

[10] E. Fattorusso, L. Minale, S. De Stefano, G. Cimino and R. A. Nicolaus, Gazz. chim. ital. *98*, 1143 (1968).

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[12] G. Prota, S. Crescenzi, G. Misuraca and R. A. Nicolaus, Experientia *26*, 1058 (1970).