

THE STRUCTURE OF SEPIOMELANIN

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Sepiomelanin was obtained in a high degree of purity; the analytical study of this natural pigment gives an interesting confirmation of the indolequinone theory.^{1,2} The black pigment from Sepia officinalis was purified by several washings with 1% HCl, exhaustive extraction by hot acetone, prolonged boiling with concentrated HCl (about 150 hours). Finally the pigment was washed first with water and then with acetone; after drying in vacuo at 80°C over P₂O₅ it was obtained as an ashless black powder, with a very small sulphur content (0.2%).

For analysis, samples were dried in vacuo at 110°C over P₂O₅ (17 hours) and weighed in sealed tubes. The following results were obtained:

C, 64.08 H, 3.00 N, 8.52

¹ W.D.Bu'Lock and J.Harley-Mason, J.Chem.Soc. 703(1951).

² H.S.Mason, Pigment Cell Biology, Academic Press, New York (1959).

Very similar results were obtained with samples isolated in the same way from different specimens of Sepia officinalis.

Sepiomelanin, suspended in ether, reacted with diazomethane; this reagent was added until it was no more absorbed. The pigment was then washed with methanol, dried in vacuo at 110°C over P₂O₅ (17 hours), and analyzed. The following results were obtained:

C, 65.57 H, 4.75 N, 7.82 O, 22.00 -OCH₃, 17.43

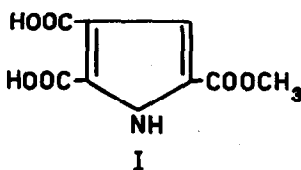
The analytical results of samples from different specimens of Sepia officinalis were found to be in a surprisingly good agreement. ³ Methylated sepiomelanin gave a positive test for -O-CH₂O- group (gallic acid in conc. H₂SO₄).

The examination of the analytical results of sepiomelanin, before and after reaction with diazomethane, showed an oxygen content higher than that calculated for a polymer of indole-5,6-quinone. Since no water was contained in the analyzed sepiomelanin, because of its exhaustive drying, presence of carboxyl groups was suspected. In order to give a definite proof of their existence, sepiomelanin was treated with saturated solution of HCl in methanol at room temperature: an ester was so obtained with a 4% methoxyl content. This value indicated the presence of

³ When considering the fact that all the sepiomelanin samples are very difficult to be burnt.

one carboxyl group about every fifth indolequinone nucleus.

The pigment after reaction with diazomethane was suspended in cold 2N K_2CO_3 and oxidized with $KMnO_4$: pyrrole-2,3-dicarboxylic acid, pyrrole-2,4,5-tricarboxylic acid and pyrrole-2,3,4,5-tetracarboxylic acid, normal oxidative degradation products of sepiomelanin,^{4,5,6} could not be detected by paper chromatography. Nevertheless, after alkaline hydrolysis of crude oxidation product, pyrrole-2,4,5-tricarboxylic acid was identified. The result showed that partial methyl-ester of pyrrole-2,4,5-tricarboxylic acid, almost certainly the 2-carbomethoxy-pyrrole-4,5-dicarboxylic acid(I)



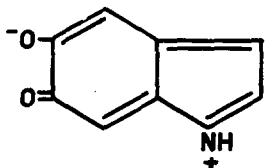
was contained in the degradation products mixture of methylated sepiomelanin. Therefore, pyrrole-2,4,5-tricarboxylic acid must derive from a 2-carboxy-indole-5,6-quinone unit, and sepiomelanin can be formulated as a copolymer of indole-5,6-quinone and 2-carboxy-indole-5,6-quinone.

⁴ L.Panizzi and R.A.Nicolaus, Gazz.Chim.Ital. 82,435(1952).

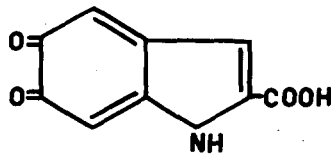
⁵ R.A.Nicolaus and L.Mangoni, Gazz.Chim.Ital. 85,1397(1955).

⁶ R.A.Nicolaus, A.Vitale and M.Piattelli, Rend.Acc.Sc.fis. e mat.vol.XXVI, 3(1959).

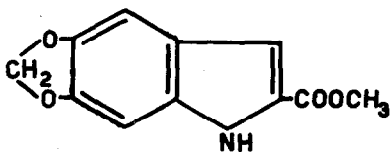
All the analytical data are in good agreement for a polymer in which the units (II) and (III) are present in the ratio 4:1, respectively. Similarly, for the methylated sepiomelanin the units (IV) and (V) should be in the ratio 1:4.



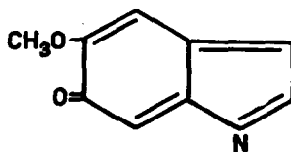
II



III



IV



V

On the basis of the analytical data, the presence of some reduced indolequinone unit cannot be excluded. It is not yet determined which positions of the indolequinone units play a part in the building up of the polymer : useful indications shall be desumed from the study of degradation products of methylated sepiomelanin.

Full details on this investigation will be published in the Rendiconto dell'Accademia delle Scienze Fisiche e Matematiche della Società Nazionale di Scienze, Lettere ed Arti in Napoli.