Tetrahedron Letters,Vol.26,No.23,pp 2805-2808,1985 0040-4039/85 $3.00 + .00
Printed in Great Britain

A REINVESTIGATION OF THE STRUCTURE OF MELANOCHROME

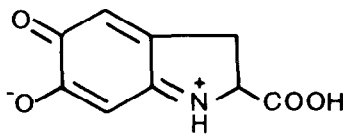
Alessandra Napolitano, Maria Grazia Corradini and Giuseppe Prota*
Dipartimento di Chimica Organica e Biologica, Università di Napoli
Via Mezzocannone 16, 80134 Napoli, ITALY

SUMMARY: Reduction and subsequent acetylation of melanochrome, the last UV detectable intermediate in the biosynthesis of eumelanins, leads to the isolation of a crystalline derivative, leucomelanochrome A acetate, identified as 5,6,5',6'-tetraacetoxy-2,2'-biindolyl (5).

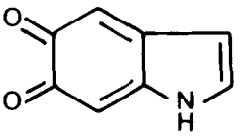
Most of our knowledge concerning the chemistry of eumelanins[1,2,3] arises from biosynthetic works on the enzymic oxidation of tyrosine or 3,4-dihydroxyphenylalanine. Spectroscopic studies[4] of this process showed that it takes place in three chromophoric phases, the first corresponding to the formation of a red pigment (λ_{max} 475 nm), dopachrome (1), the second to a purple intermediate, designated melanochrome[5], with a broad absorption maximum at 540 nm, and a third characterized by a general absorption due to melanin.

The nature of the purple pigment has been the subject of controversial publications[6,7] since 1948 when Mason[4] first suggested that melanochrome could be the monomeric quinone 2 arising by the oxidation of 5,6-dihydroxyindole (3). This structure was later questioned by Beer *et al.*[5] who showed that the pigment did originate by oxidation of 3, but pointed out that the absorption maximum of melanochrome was more consistent with that of a dimer or an oligomer of 2. Subsequent direct investigation of the structure of melanochrome was hampered by the intractable nature of the material. However, extensive model studies[9] on the chemistry of variously substituted 5,6-dihydroxyindoles led to the conclusion that the oxidative coupling of 3 involved the 3- and 7-positions as shown in formula 4.

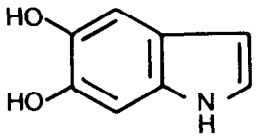
We have now reexamined the problem anew and have found that, in the presence of metallic cations (e.g. Ni^{2+}, Zn^{2+}), the formation of melanochrome is markedly accelerated. Accordingly, we have developed a procedure which afforded a sufficient amount of melanochrome for chemical investigation. In a typical experiment, aereal oxidation of 3 (8 mM) in 4-(2-hydroxyethyl)-piperazine-1-ethanesulfonic acid (HEPES) buffer at pH 6.8 with $Zn(OAc)_2$ or $NiSO_4$ (1 eq.)



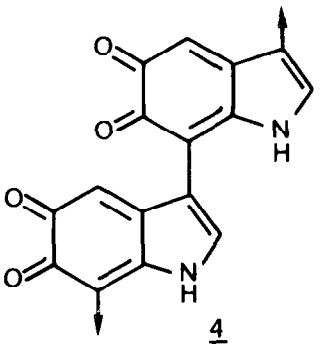
1



2



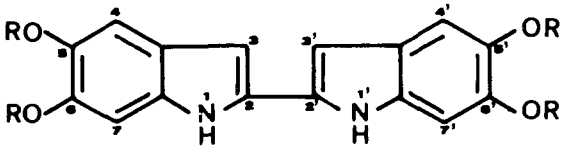
3



4

and mushroom tyrosinase[10] (214 units/ml) leads in a few minutes to a purple-violet reaction mixture which deposits melanochrome as a deep violet precipitate becoming darker on being kept further. As obtained, the product is highly insoluble in all organic solvents including DMSO, DMF and pyridine. On treatment with dithionite, melanochrome is instantaneously reduced to give an ethyl acetate soluble fraction (λ_{max} 367, 348, 335 nm) which is acetylated with Ac_2O-pyridine (24 h at room temperature). After evaporation to dryness, the residue is taken up in acetone and the major component of the mixture, leucomelanochrome A acetate, is collected (in 10% yield) as white prisms, ($C_{24}H_{20}N_2O_8$[11], m.p. 308-310°C (dec.)). The product was identified as 5,6,5',6'-tetraacetoxy-2,2'-biindolyl (5) on the basis of the following evidences.

The UV spectrum (DMSO) exibits absorption maxima at 325 (shoulder), 342 and 361 nm (logε 4.40, 4.64, 4.64), consistent with a 2,2'-biindolyl chromophore[12]. In addition to molecular



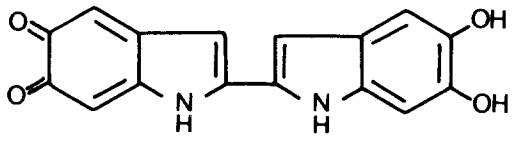
5 R = Ac

6 R = H

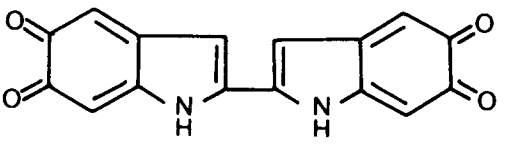
ion peak at m/e 464, the mass spectrum of 5 shows diagnostic fragments at m/e 422, 380, 338, 296 and a prominent ion peak at m/e 149 due to the deacetylated monomer. The ^{1}H-NMR spectrum is consistent with the highly symmetrical structure 5 showing, beside two signals at δ2.29 (3Hx2, s) and at δ2.28 (3Hx2, s) for the four acetyl groups, two singlets at δ7.41 (1Hx2, s, H-4, H-4') and δ7.25 (1Hx2, s, H-7, H-7') due to the aromatic protons and a pair of doublets at δ11.76 (1Hx2, d, J=1.47 Hz) and δ6.94 (1Hx2, d, J=1.47 Hz). These latter, exchanged by D_2O[13], are attributed to the indolic N-H and to the proton at position 3, respectively. Further evidence that the coupling between the two indole moieties occurs at position 2 is provided by ^{13}C spectrum[14] which exibits a signal at δ99.8 (d) typical of the carbon at position 3[15].

Mild alkaline hydrolysis under reductive conditions ($Na_2S_2O_4$) leads to the parent tetrahydroxy derivative (λ_{max} 369, 350 nm) which, in alkaline medium, rapidly autoxidizes to give a blue pigment, melanochrome A, which exibits the same chromophore (λ_{max} 560 nm) of the original oxidation mixture of 5,6-dihydroxyindole.

Unfortunately, the high instability of melanochrome A and its complete insolubility have prevented any direct investigation. However, the structure of the leucoderivative acetate 5 suggests that melanochrome A is a quinone derived from 5,6,5',6'-tetrahydroxy-2,2'-biindolyl (6) probably corresponding to 7 or 8 or a mixture of both. In this connection, it is noteworthy that, in the spectroscopic course of melanochrome formation, the initial maximum at 540 nm undergoes a subsequent shift to 560 nm[8]. These absorption maxima could represent two different levels of oxidation of 6 corresponding to the formation of the quinonoid forms 7 and 8.



7



8

However, in spite of these uncertainties regarding the precise structure of melanochrome, the isolation of the dimer 5 is of interest in relation to the biosynthesis of eumelanins since it reveals for the first time an unexpected tendency of 5,6-dihydroxyindole to undergo oxidative coupling at position 2, in contrast with all other modes of dimerization previously suggested[5,6,9].

Appropriate experiments are underway to characterize other components of melanochrome mixture in order to draw a more complete picture of the reactivity of 3 under the conditions of

melanogenesis *in vitro*.

Acknowledgements. We thank the M.P.I. and the Lawrence M. Gelb Research Foundation for financial supports and the *Centro di Spettrometria di Massa del CNR e dell'Università di Napoli*.

References and notes

1. R.A. Nicolaus, Melanins (Ed. E. Lederer), Hermann, Paris, 1968.
2. G.A. Swan, Fortsch. Chem. Org. Naturst. (Eds. W. Herz, H. Grisebach and G.W. Kirby), Springer-Verlag, Wien, 1974, vol. 31, p. 521.
3. G. Prota and R.H. Thomson, Endeavour, 35, 32 (1976).
4. H.S. Mason, J. Biol. Chem., 172, 83 (1948).
5. R.J.S. Beer, T. Broadhurst and A. Robertson, J. Chem. Soc., 1947 (1954).
6. J.D. Bu'Lock, J. Chem. Soc., 52 (1960).
7. J.D. Bu'Lock, Arch. Biochem. Biophys., 91, 189 (1960).
8. H.S. Mason and E.W. Peterson, Biochem. Biophys. Acta, 111, 134 (1965).
9. J.D. Bu'Lock and J. Harley-Mason, J. Chem. Soc., 703 (1951).
10. Notably, the reaction does not require a specific enzymic assistence since melanochrome is also obtained in comparable yield by aereal oxidation of 3 at higher pH (e.g. 7.5) in the presence of the sole metallic ions as catalyst.
11. Satisfactory analytical data were obtained for this compound.
12. S.A. Faseeh and J. Harley-Mason, J. Chem. Soc., 4141 (1957).
13. R.A. Heacock, O. Hurtzinger, B.D. Scott, J.W. Daly and B. Witkop, J. Am. Chem. Soc., 85, 1825 (1963).
14. ^{13}C-NMR (DMSO-d_6, 270 MHz) δ ppm: 21.3 (q), 21.4 (q), 99.8 (d), 106.3 (d), 114.4 (d), 126.8 (s), 133.6 (s), 135.1 (s), 137.2 (s), 139.0 (s), 169.8 (s), 170.0 (s).
15. V. Bocchi and G. Palla, Tetrahedron, 40, 3251 (1984).

(Received in UK 1 March 1985)