Tetrahedron Vol 43, No. 2, pp. 431 to 434, 1987
Printed in Great Britain.

0040-4020/87 $5.00 + .00
Pergamon Journals Ltd

PHOTOOXIDATION OF 5,6-DIHYDROXY-1-METHYL-INDOLE.

Marco d'Ischia and Giuseppe Prota

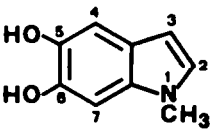
Department of Organic and Biological Chemistry,
University of Naples,
Via Mezzocannone 16, I-80134 Napoli, Italy.

(*Received in UK* 18 *November* 1986)

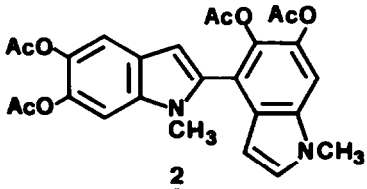
Abstract: Photooxidation of 5,6-dihydroxy-1-methylindole (1) in methanol with Pyrex-filtered UV light led to a complex mixture of fluorescent compounds, the major of which could be isolated as the acetyl derivative and identified as 5,6,5',6'-tetraacetoxy-1,1'-dimethyl-2,4'-biindolyl (2). Five hitherto unknown products of photooxygenation of 1 were also isolated and formulated as the isomeric triacetoxy-1-methyl-indoles 3 and 4, 5,6-diacetoxy-1-methyl-oxindole (5) and the hydroxylated dimers 6 and 7. The relevance of these results to the photochemical processes responsible for light-induced melanin formation and associated processes is discussed.

The increase in the level of melanin pigments which follows exposure of skin to solar radiation or UV light (sun tanning) is known to be the result of two distinct photobiological processes, immediate tanning (IT) and delayed tanning (DT).[1,2] While the biophysical phenomena accompanying these two complex processes have been widely explored,[3] the molecular mechanisms accounting for light-induced stimulation of melanogenesis remain at present obscure. It has been suggested that IT, which is specifically induced by light of wavelength greater than 320 nm, is a manifestation of the photooxidation of melanin precursors present in the skin in a colourless reduced state.[4] The results of ESR studies carried out on skin samples suggest that this photooxidation involves the intermediacy of semiquinone radicals derived from catechol metabolites of the eumelanin pathway.[3] Following this and other observations, studies on the photochemistry of melanogenesis have mainly been concerned with the generation of free radicals during photooxidation of some eumelanin precursors, particularly dopa and related catecholamines.[5,6] Surprisingly, little attention has been focused on the photochemical behaviour of 5,6-dihydroxyindole and related eumelanin metabolites, which represent among the most important products of the melanocyte activity and are commonly found in the skin and physiological fluids.[7]

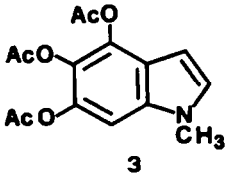
In connection with our interest in the photochemistry and photobiology of melanins[8] we report now the results of a study on the light-induced autoxidation of 5,6-dihydroxy-1-methylindole (1). This compound was preferably used for investigating the photoreactivity of the 5,6-dihydroxyindole system owing to its easy availability by a one step synthesis involving oxidative cyclization of epinephrine.[9] Moreover, 1 is relatively more stable than 5,6-dihydroxyindole and, on oxidation, gives rise to products with more favourable analytical and physical properties.



1

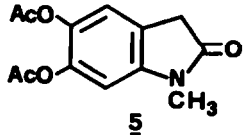


2

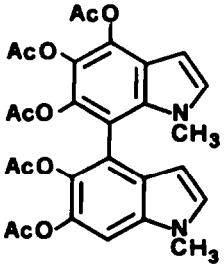


3

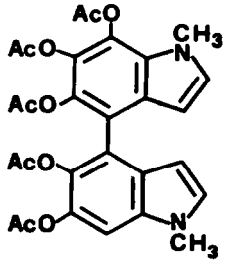
4



5



6



7

When a solution of 1 in methanol was irradiated with Pyrex-filtered UV light, photooxidation proceeded smoothly with progressive formation of a dark brown solution characterized by an ill-defined chromatographic pattern.[10] However, if the reaction was stopped in the early stages by addition of aqueous sodium dithionite and the ethyl acetate-extractable fraction acetylated, TLC analysis of the residue revealed the presence of a very complex mixture of fluorescent products exhibiting chromatographic properties similar to those of the oligomers obtained by enzymic oxidation or autoxidation of 1.[9] Noteworthy, even after prolonged irradiation, large amounts of the starting indole, as the acetyl derivative, were isolated from the reaction mixture, probably owing to a marked inner filter effect of the abundant brown pigments formed which prevent complete photooxidation of 1.

Repeated fractionation of the mixture on silica gel allowed the isolation in very minute amounts of six reaction products.The major of these, which exhibited a typical blue fluorescence, was eventually obtained in crystalline form and identified as 5,6,5',6'-tetraacetoxy-1,1'-dimethyl-2,4'-biindolyl (2) on the basis of spectral analysis. The structural assignment was definitively secured by comparison of the analytical properties with those of an authentic sample of 2.[9]

From the less polar fractions of the photooxidation mixture two closely related compounds were isolated, whose molecular formulas ($C_{15}H_{15}N\,O_6$) and chromophores (λ max = 281 nm) were consistent with isomeric triacetoxy-1-methylindoles. Full structural characterization of the products as 4,5,6-triacetoxy-1-methylindole (3) and 5,6,7-triacetoxy-1-methylindole (4) followed by straightforward analysis of the 1H-NMR spectra, which exhibited the same pattern of resonances

differing mainly for the presence,in the case of the more polar isomer,of the typical long range coupling between the H3 and H7 protons.[11]

Another photooxygenation product of 1 ($C_{13}H_{13}N\ O_5$), which was negative to Ehrlich reagent, was obtained from the more polar fractions of the reaction mixture. This was identified as 5,6-diacetoxy-1-methyl-oxindole (5)by analysis of the 1H-NMR spectrum, showing a singlet at δ 3.51 (2H) for the methylene group, and two singlets (1 H each) in the aromatic region at δ 6.66 and 7.08. Indicative of an oxindole skeleton was also the chromophore, characterized by two absorption maxima at 252 and 281 nm.[12]

The mass spectra of the last two compounds exhibited the same molecular ion peaks at m/e 550($C_{28}H_{26}N_2O_{10}$) and fragment peaks corresponding to subsequent losses of five acetyl groups, suggesting two isomeric pentaacetoxybiindolyls. In line with this view, the 1H-NMR spectra exhibited in the aromatic region two sets of pyrrolic protons H2 and H3 and one H7 signal, this latter appearing as a doublet owing to long range coupling with one of the protons at the 3-position. These data are consistent with only two structures, 4,5,6,5',6'-pentaacetoxy-1,1' -dimethyl-7,4'-biindolyl (6) and 5,6,7,5',6'-pentaacetoxy-1,1'-dimethyl-4,4'-biindolyl (7). The unusually upfield resonance of one of the N-methyl groups (δ 3.05) and the marked difference between the chemical shifts of the two protons at the 3-positions would favour structure 6 for the less polar isomer. On the other hand, the higher symmetry of structure 7 is consistent with the observed similarity between the resonances of the two N-methyl groups as well as of the H3 and H3' protons in the spectrum of the more polar isomer.

The chemical nature of the products 2-7 formed by photooxidation of 1 suggests that the reaction involves a multiplicity of processes which follow a single primary event,i.e. light-induced generation of phenoxyl radicals. These can either undergo coupling at the 2- and 4-positions to give oligomers, e.g.2, or interact with molecular oxygen to give hydroperoxide intermediates, as evidenced by the isolation, after reduction of the mixture, of the oxygenated derivatives 3,4 and 5. To further increase the complexity of the picture, the hydroperoxide intermediates can also partake, along with the phenoxyl species, in the photopolymerization processes to form oxygenated oligomers, e.g. 6 and 7. Thus, although only a small fraction of the photooxidation products of 1 has been characterized, the available evidence suggests that light-induced autoxidation of 1 follows a substantially different course with respect to metal- or alkali-catalysed autoxidation reactions.[9]

Apart from the chemical interest connected with the photoreactivity of the 5,6-dihydroxyindole system, the results of this study may provide an important background for further insight into the molecular mechanisms responsible for light-induced stimulation of melanogenesis.

EXPERIMENTAL

U.V. spectra were recorded with a Perkin Elmer 550 S spectrophotometer. 1H-NMR spectra (200 MHz) were recorded on a Varian XL 200 spectrometer (δvalues are referred to TMS as the internal standard).Electron impact mass spectra were determined with a Kratos MS-50 mass spectrometer. 5,6-dihydroxy-1-methylindole (1) was prepared according to Corradini et al.[9] All solvents were from Carlo Erba and were of the highest purity available. Ethyl ether was freed from peroxides by passage through a column of alumina. Dimethylaminobenzaldehyde was from Fluka. Analytical and preparative thin layer chromatographies were carried out on silica gel F 254 plates from Merck (0.25 and 0.5 mm),proportions given for mixed solvents are by volume. The chromatograms were examined by UV irradiation at 254 and 366 nm and by spraying with an alcoholic solution of p-dimethylaminobenzaldehyde (Ehrlich reagent) followed by exposure to HCl vapours.

Photooxidation of 1.

A solution of 1 (1g) in methanol (1.8 l) was irradiated in a Pyrex immersion apparatus equipped with

a water cooling jacket using a 450 W Hanovia medium pressure mercury lamp and a Pyrex filter sleeve. After 3h, irradiation was stopped and the solvent removed under reduced pressure to a final volume of about 10 ml. An aqueous solution of sodium dithionite was then added and the mixture extracted exhaustively with ethyl acetate. The organic layers were washed twice with a small volume of water, dried over sodium sulphate and evaporated to dryness to give a brownish residue which was acetylated with acetic anhydride (2 ml) and pyridine(100 μl) at room temperature for 12 h. The photooxidation reaction was repeated twice under the same conditions and the combined acetylated extracts were chromatographed on silica gel (150 g, benzene-ethyl acetate 6:4). The first elution gave 5,6-diacetoxy-1-methylindole (800 mg). The subsequent fractions containing products 2-7 were combined in three pools on the basis of TLC examination.

The first pooled fractions were repeatedly chromatographed on silica gel plates (ether) to give 2 (36 mg), Rf = 0.66 in benzene-ethyl acetate 6:4, identical in all respects with an authentic specimen (TLC, UV, MS, NMR),and a less polar fluorescent band. TLC fractionation of this band (CH_2Cl_2) afforded 3 (5mg), Rf = 0.37 in CH_2Cl_2 , λmax (methanol) 281 nm; m/e 305 (M+,$C_{15}H_{15}NO_6$: found 305.0897, requires 305.0899),263,221,179 (base peak); 1H-NMR ($CDCl_3$): δ 2.29 (3Hx2,s,acetyl groups),2.35 (3H,s,acetyl group), 3.74 (3H,s,N-CH_3), 6.34 (1H,dd,J=3.3, 0.8 Hz, H-3), 7.04 (1H,d,J=3.3 Hz, H-2), 7.09 (1H,d,J=0.8 Hz,H-7); and 4 (4 mg), Rf=0.41 in CH_2Cl_2, λmax (methanol) 281 nm; m/e 305 (M+, $C_{15}H_{15}NO_6$: found 305.0895, requires 305.0899), 263, 221, 179 (base peak); 1H-NMR ($CDCl_3$): δ 2.27 (3H,s,acetyl group), 2.28 (3H,s,acetyl group), 2.37 (3H,s,acetyl group), 3.84 (3H,s,N-CH_3), 6.43 (1H,d,J=2.9 Hz, H-3), 6.96 (1H,d,J=2.9 Hz, H-2), 7.30 (1H,s,H-4).

TLC of the second pooled fractions on silica with ether-CH_2Cl_2 95:5 and then CH_2Cl_2-methanol 98:2 gave 6 (4 mg), Rf= 0.53 in benzene-ethyl acetate 6:4, λmax (methanol) 298 nm; m/e 550 (M+,$C_{28}H_{26}N_2O_{10}$: found 550.1596, requires 550.1588), 508, 466, 424 (base peak), 382, 340; 1H-NMR ($CDCl_3$):δ1.92 (3H,s,acetyl group), 1.96 (3H,s,acetyl group), 2.25 (3H,s,acetyl group), 2.30 (3H,s,acetyl group), 2.37 (3H,s,acetyl group), 3.05 (3H,s,N-CH_3), 3.78 (3H,s,N'-CH_3), 6.02 (1H,dd,J=2.8, 0.7 Hz,H-3'), 6.36 (1H,d,J=2.8 Hz,H-3), 6.90 (1H,d,J=2.8 Hz, H-2), 7.01 (1H,d,J=2.8 Hz, H-2'), 7.21 (1H,d,J=0.7 Hz, H-7');and 7 (4 mg), Rf=0.47in benzene-ethyl acetate 6:4,λmax (methanol) 298 nm; m/e550 (M+,$C_{28}H_{26}N_2O_{10}$: found 550.1588, requires 550.1588), 508, 466, 424 (base peak), 382, 340; 1H-NMR ($CDCl_3$): δ 1.97 (3H,s,acetyl group), 2.03 (3H,s,acetyl group), 2.30 (3H,s,acetyl group), 2.31 (3H,s,acetyl group), 2.40 (3H,s,acetyl group), 3.70 (3H,s,N'-CH_3), 3.80 (3H,s,N-CH_3), 6.02 (1H,d,J=2.8 Hz, H-3), 6.10 (1H,dd, J=2.8, 0.7 Hz, H-3'), 6.88 (1H,d, J=2.8 Hz, H-2), 7.00 (1H,d,J=2.8 Hz, H-2'), 7.21 (1H,d,J=0.7 Hz,H-7').

The third fraction, containing mainly 5, was similarly purified by TLC (ether) to give 5 (10 mg), Rf=0.35 in benzene-ethyl acetate 6:4, λmax (methanol) 252, 281 nm; m/e 263 (M+, $C_{13}H_{13}NO_5$: found 263.0798, requires 263.0794), 221, 179 (base peak); 1H-NMR ($CDCl_3$): δ 2.29 (3H,s,acetyl group),2.30 (3H,s,acetyl group), 3.17 (3H,s,N-CH_3), 3.51 (2H,s, CH_2), 6.66 (1H,s,H-7), 7.08 (1H,s,H-4).

ACKNOWLEDGEMENTS.

This work has been supported by grants from CNR, P.F.Chimica Fine e Secondaria, Sottoprogetto Ci, M.P.I. and Lawrence M. Gelb Research Foundation. We thank Drs. A. Chan and L.J.Wolfram (Clairol Research Laboratories, Stamford, CT) for helpful discussions.

REFERENCES AND NOTES.

1. M.R.Chedekel, Photochem. Photobiol., 35, 881, 1982.
2. S.Shono, M.Imura, M.Ota, S.Ono, K. Toda, J. Investigative Dermatol., 84,265, 1985.
3. M.A.Pathak,in Advances in Biology of Skin, Eds. W.Montagna and F.Hu, Pergamon Press, Oxford, 1966, Vol.8,p.397.
4. M.A.Pathak, K.Jimbow,T.B.Fitzpatrick,in Pigment Cell 1981, Phenotypic Expression in Pigment Cells, Ed. M.Seiji, University of Tokyo Press,Tokyo,1981, p.655.
5. C.C.Felix, R.C.Sealy, J.Am.Chem.Soc., 103, 2831, 1981.
6. C.C.Felix, R.C.Sealy, Photochem. Photobiol., 34,423,1981.
7. C.Hansson, Acta Derm. Venereol. (Stockh), 63,147,1983.
8. M.d'Ischia, G.Prota, Gazzetta, 116, 407,1986.
9. M.G.Corradini, A.Napolitano, G.Prota, Tetrahedron,42, 2083,1986.
10. Notably, in aqueous media, e.g. phosphate buffer at pH 6.8,autoxidation processes significantly compete with the primary photochemical reactions, as evidenced by control experiments carried out in the dark.
11. J.A.Elvidge, R.G.Foster, J.Chem.Soc., 981,1964.
12. A.I.Scott,Interpretation of the Ultraviolet Spectra of Natural Products,Pergamon, New York, 1964, p.172.