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A REINVESTIGATION OF THE ANAEROBIC CONVERSION OF ADRENOCHROME TO "ADRENALINE BLACK".

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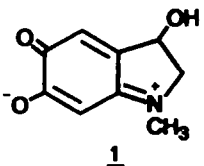
Abstract: In contrast to previous work, which suggested that "adrenaline black", derived from adrenochrome under acid conditions, was a 3,4-linked polymer of 1-methyl-5,6-dihydroxyindole, we have now isolated (after derivatization) two 2,3'-linked indole dimers (9 and 10) and a 4,7'-3',3''-linked trimer (7). In the light of these results the transformation of adrenochrome to "adrenaline black" appears to be an uncorrected model for the study of melanogenesis.

Eumelanins are widely occurring natural pigments found in skin, hair and melanomas, as well as in the eye and substantia nigra (neuromelanin).[1] Despite intensive studies over nearly a century, the chemistry of these pigments is still little understood.[2,3] The main difficulty arises from the intractable nature of these insoluble materials which are non-homogeneous from the viewpoint of molecular uniformity. In consequence, the normal approach of NMR and X-ray crystallographic analysis, which has been so successful in the structural elucidation of complex natural products, is not applicable. Chemical, biosynthetic and isotope labelling studies[4] have led to the view that eumelanins are irregular polymers consisting mainly of 5,6-dihydroxyindole units derived biogenetically from tyrosine via dopa and related metabolites, e.g. dopamine and adrenaline. However, the conclusions drawn from these studies are largely speculative owing to the lack of definite information regarding the nature and the mode of polymerization of the reactive species involved.

In a study of melanin formation from adrenaline, Harley-Mason[5] suggested that the process involved the intermediacy of adrenochrome (1), and that the subsequent polymerization steps are



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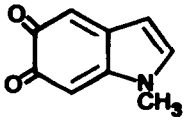

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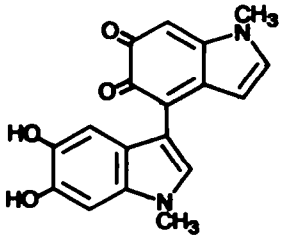

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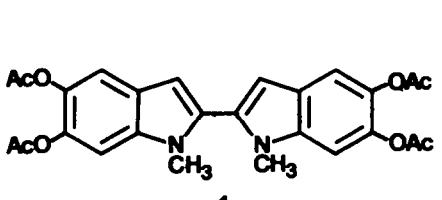

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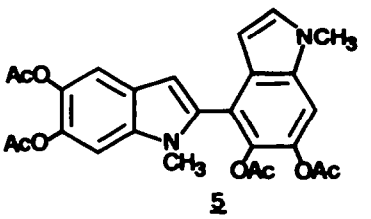

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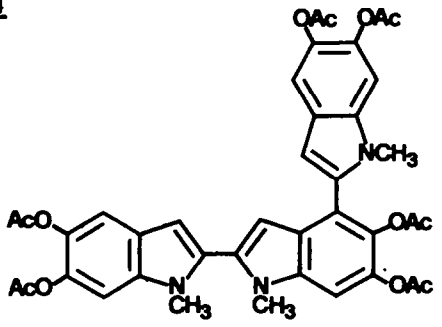

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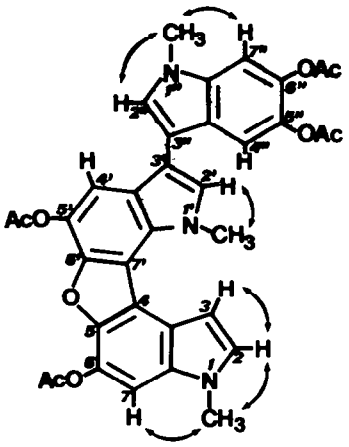
strongly catalyzed by acid. Moreover, he found that the pigment, named "adrenaline black", could also be obtained directly from adrenochrome by a non-oxidative process, by merely keeping an aqueous solution of 1 under N_2 for 48 hours. Based upon this and other experiments, Bu'Lock[6] later suggested that the formation of "adrenaline black" involved the self-combination of 1-methyl-5,6-indolequinone (2), derived by acid catalyzed dehydration of 1, to give a 3,4'-linked dimer (3) and related oligomers. In contrast with this view, we have recently found[7] that oxidative polymerization of 5,6-dihydroxy-1-methylindole proceeds mainly by radical coupling at 2- and 4-positions to give dimers and trimers such as 4,5 and 6. These divergent results prompted us to reinvestigate the conversion of adrenochrome to melanin.

When an aqueous solution of adrenochrome was allowed to decompose , under anaerobic conditions, a dark brown amorphous precipitate formed, accounting for about 90% of the starting material. As reported[6], the product was insoluble in most organic solvents with the exception of pyridine, but soluble in aqueous alkali to give a dark greenish-brown solution. On treatment with $Na_2S_2O_4$, the precipitate was reduced to give an ethyl acetate soluble fraction which was acetylated with Ac_2O-pyridine (18 hours at room temperature). TLC analysis of the product revealed the presence of a complex mixture, but the major component could be isolated in crystalline form, after flash chromatography on silica, and was identified as 7 on the basis of the following evidence.

The mass spectrum showed the molecular ion peak at m/e 635 corresponding to the formula $C_{35}H_{29}N_3O_9$ (requires 635.1904, found 635.1930) and diagnostic fragments at m/e 593, 551, 509, 467, consistent with the presence of four acetyl groups.

The ^1H NMR spectrum exhibited, besides the signals of acetyl and methyl groups, two doublets at δ 6.49 and 7.04 due to H-3 and H-2, respectively, and six singlets,two of which at δ 7.35 and 7.39 correspond to H-2' and H-2'' and the remaining four at δ 7.61, 7.62, 7.74[8] and 7.95 to H-7, H-7', H-4'' and H-4', respectively.



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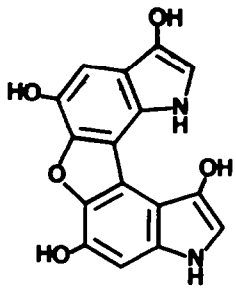

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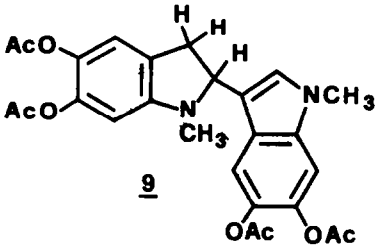
These assignments were substantiated by the results of the NOED experiments, as illustrated in 7. Notably, significant nuclear Overhauser enhancement difference (NOED) effects were measured between the N-methyl groups and the proximal H-7 and H-2 protons but not between the H-4 and H-3 protons showing that the coupling of the two indole mojeties forming the dibenzofuran ring involved the 4- and 7'-positions.

Consistent with the proposed structure was the ^{13}C NMR spectrum which exhibited, besides eight doublets for the unsubstituted indole carbons, two singlets at δ151.29 and 154.90, typical for carbons adjacent to oxygen in the dibenzofuran ring.[9]

Notably, the possible formation of dibenzofuran intermediates, e.g. 8, in the conversion of 5,6-dihydroxyindole to melanin was suggested in 1948 by Burton[10] on purely theoretical grounds based upon the analogy with some examples of dehydrogenative coupling quoted by Erdtman[11] for hydroxyquinols. A considerable number of similar examples of dehydrogenative coupling of natural and synthetic quinones has since been reported.[9] It should be noted, however, that this type of reaction is unlikely to occur in melanogenesis as it requires an acid catalysis. Indeed, when the conversion of 5,6-dihydroxyindole to melanin is performed at neutral pH, the formation of oligomers containing the dibenzofuran ring is not observed,[12] the reaction proceeding without concomitant oxidation at position 3 to give 2,2'- and 2,4'-linked dimers, in close analogy with the oxidative behaviour of 5,6-dihydroxy-1-methylindole.

Repeating the conversion of adrenochrome to "adrenaline black" but in water acidified to ca. pH 2, and workup as before, yielded, besides 7, two more oligomers which were identified as 5,6-diacetoxy-1-methyl-3-(5',6'-diacetoxy-1'-methyl-2'-indolinyl)-indole (9) and 5,6,5',6'-tetraacetoxy-1,1'-dimethyl-2,3'-biindolyl (10).

The mass spectrum of 9 showed the molecular ion peak at m/e 494, an intense M^+-2 peak at m/e 492 due to the dehydrogenation of the indoline system, and other diagnostic ions at m/e 452, 410, 366 and 324 (base peak). The ^1H NMR of 9 exhibited, besides an ABX system of the $-CH_2-CH-$ grouping, five singlets, three of which at δ 7.46, 7.14 and 7.07 were attributable to H-4 ,H-7 and H-2, respectively, and the remaining two at δ 6.86 and 6.24 due to the H-4' and H-7',



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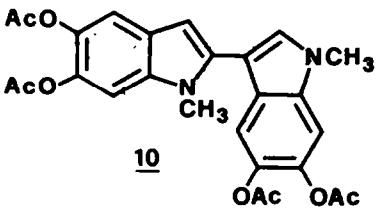

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respectively. Significant NOED effects were measured between the signals at δ 2.56 ($N'CH_3$) and the H-7' singlet at δ 6.24 and between the NCH_3 signal at δ 3.73 and that of the H-2 and H-7 protons at δ 7.07 and 7.14, respectively.

The mass spectrum of 10 showed the molecular ion peak at m/e 492 and diagnostic fragments at m/e 450, 408, 366 and 324 (base peak) due to the subsequent losses of four acetyl groups. The ^1H NMR spectrum showed, besides the aliphatic signals of the acetyl and methyl groups, two signals at δ 6.53 and 7.15 of the H-3 and H-2' protons, and four singlets in the aromatic region at δ 7.15, 7.21, 7.36 and 7.46, attributable to the H-7, H-7', H-4 and H-4', respectively.

The formation of dimers 9 and 10 is likely to take place by acid catalyzed dimerization of 5,6-dihydroxy-1-methylindole which is known to be formed in the conversion of adrenochrome to "adrenaline black"[5]. In line with this view, when 5,6-dihydroxy-1-methylindole was allowed to polymerize in acid medium, fractionation of the incubation mixture, after derivatization as above, led to the isolation of two dimers, identified as 9 and 10.

In conclusion, the anaerobic transformation of adrenochrome to "adrenaline black" appears to be an uncorrected model for the study of melanogenesis since under the reported conditions[5] polymerization of the intermediary indoles is accompanied by side reactions, such as acid catalyzed ring closure of hydroxyquinol intermediates, as well as enamine-imine type dimerization[13] of 5,6-dihydroxy-1-methylindole.

EXPERIMENTAL

UV spectra were recorded with a Perkin Elmer 550 S spectrometer. ^1H NMR (200 MHz) and ^{13}C NMR (50 MHz) were recorded on a Varian XL 200 spectrometer (δ values are referred to TMS as the internal standard). The electron impact mass spectra were determined with a Kratos MS-50 mass spectrometer. For flash chromatography, silica gel 60 Merck 9385 was used. Analytical TLC was carried out on precoated silica gel F-254 plates (E. Merck), the proportion given for mixed solvents are by volume. The chromatograms were examined by UV irradiation at λ 366 nm and 254 nm. Adrenochrome[14] and 5,6-dihydroxy-1-methylindole[15] were prepared as previously reported.

Formation of "adrenaline black" from 1 in water.

Adrenochrome (1.2 g) was dissolved in air-free distilled water (20 ml), and the solution transferred to a tightly-stoppered flask under nitrogen. After 48 hours at room temperature, the precipitate (collected by filtration) was treated with a solution of sodium dithionite and the mixture so obtained was extracted exhaustively with ethyl acetate. The organic layers were washed with water, dried over sodium sulphate and evaporated to dryness to give a brownish residue which was acetylated with acetic anhydride (10 ml) and pyridine (500 µl) at room temperature for 12 hours. After evaporation to dryness, the residue was fractionated by flash chromatography over silica gel (CH_2Cl_2:MeOH, 97.5:2.5) yielding 24 mg of 7, white prisms from

methanol , m.p. 210 °C;λ max (CH_2Cl_2) 332, 312 and 265 nm (logε 4.32, 4.36 and 4.84); m/e 635 (M^+,$C_{35}H_{29}N_3O_9$: found 635.1930, requires 635.1904), 593, 551, 509, 508, 467, 466; ^{1}H NMR (acetone-d_6): δ (ppm) 2.25 (3H, s, acetyl group), 2.28 (3H, s, acetyl group), 2.30 (3H, s, acetyl group), 2.32 (3H, s, acetyl group), 3.84 (3H, s, NCH_3), 3.96 (3H, s, N''CH_3), 4.00 (3H, s, N'CH_3), 6.50 (1H, d, J=3.1 Hz, H-3), 7.22 (1H, d, J=3.1 Hz, H-2), 7.35 (1H, s, H-2'), 7.39 (1H, s, H-2''), 7.61 (1H, s, H-7), 7.62 (1H, s, H-7''), 7.74 (1H, s, H-4''), 7.95 (1H, s, H-4'); ^{13}C NMR ($CDCl_3$):δ(ppm) 20.71 (q), 20.79 (q), 28.46 (q), 32.97 (q), 33.06 (q), 101.10 (d), 101.16 (d), 104.05 (d), 104.50 (d), 110.61 (s), 112.30 (d), 113.59 (d), 119.57 (s), 120.15 (s), 120.39 (d), 124.74 (s), 126.76 (s), 130.03 (d), 130.34 (s), 134.26 (s), 135.77 (s), 135.95 (s), 136.79 (s), 136.90 (s), 137.37 (s), 137.51 (s), 151.29 (s), 154.90 (s), 169.21 (s), 169.06 (s), 169.41 (s).

Formation of "adrenaline black" from 1 in acid medium (pH 2).

Adrenochrome (200 mg) was dissolved in a solution of water (100 ml) and acidified to pH 2 with hydrochloric acid under nitrogen. After 15 minutes,the reaction was stopped by addition of $Na_2S_2O_4$,and the mixture was repeatedly extracted with ethyl acetate. The organic layers were washed with water, dried over Na_2SO_4 and evaporated to dryness to give a residue which was acetylated with Ac_2O (2 ml) and pyridine (100 µl) at room temperature for 12 hours. After evaporation to dryness, the residue was fractionated by flash chromatography over silica gel (Et_2O: MeOH, 98:2) to give, besides the trimer 7, the dimer 9 (8 mg),λmax ($CHCl_3$) 285 nm; m/e 494 (M^+,$C_{26}H_{26}N_2O_8$), 492, 452, 450, 410, 408, 368, 366 and 324 (base peak); ^{1}H NMR ($CDCl_3$):δ(ppm) 2.26 (3H, s, acetyl group), 2.28 (3H, s, acetyl group), 2.30 (3H,s, acetyl group), 2.31 (3H, s, acetyl group), 2.56 (3H, s, N'CH_3), 3.16 (1Hx2, m, H-3a', H-3b'), 3.73 (3H, s, NCH_3), 4.58 (1H, dd, Jax= 9 Hz, Jbx=11 Hz), 6.24 (1H, s, H-7''), 6.86 (1H, s, H-4''), 7.07 (1H, s, H-2), 7.14 (1H, s, H-7), 7.46 (1H, s, H-4); ^{13}C NMR ($CDCl_3$):δ(ppm) 20.61 (q), 32.98 (q), 33.79 (t), 37.13 (q), 65.16 (d), 101.59 (d), 103.85 (d), 113.87 (d), 114.65 (s), 118.77 (d), 124.06(s), 127.01 (s), 129.13 (d), 133.14 (s), 135.10 (s), 135.73 (s), 138.19 (s), 141.50 (s), 151.73 (s), 168.73 (s), 169.13 (s), 169.34 (s); and the dimer 10 (2 mg), λmax ($CHCl_3$) 303 nm; m/e 492 (M^+, $C_{26}H_{24}N_2O_8$), 450, 408, 366, 324 (base peak), 163; ^{1}H NMR ($CDCl_3$):δ(ppm) 2.29 (3H, s, acetyl group), 2.32 (3H, s, acetyl group), 2.33 (3Hx2, s, acetyl groups), 3.64 (3H, s, NCH_3), 3.78 (3H, s, NCH_3), 6.53 (1H, s, H-3), 7.15 (1Hx2, s, H-7, H-2'), 7.21 (1H, s, H-7'), 7.36 (1H, s, H-4), 7.46 (1H, s, H-4'); ^{13}C NMR ($CDCl_3$): δ(ppm) 20.57 (q), 20.69 (q), 31.13 (q), 33.25 (q), 101.79 (d), 103.70 (d), 104.08 (d), 107.27 (s), 113.28 (d), 113.72 (d), 125.31 (s), 125.82 (s), 129.68 (d), 134.26 (s), 135.31 (s), 135.82 (s), 136.16 (s), 136.99 (s), 137.38 (s), 138.55 (s), 169.03 (s), 169.12 (s), 169.19 (s), 169.28 (s).

If the reaction was stopped after 45 minutes by addition of $Na_2S_2O_4$, work up of the mixture as above gave the trimer 7 in better yields (20 mg).

Acid catalyzed dimerization of 5,6-dihydroxy-1-methylindole.

5,6-dihydroxy-1-methylindole (200 mg) was dissolved in a solution of water (100 ml) acidified to pH 2 with hydrochloric acid under nitrogen. After 30 minutes the reaction mixture was extracted exhaustively with ethyl acetate, and the organic layers were washed with water, dried over sodium sulphate and evaporated to dryness to give a residue which was acetylated with Ac_2O and pyridine at room temperature for 12 hours. After evaporation to dryness, the residue was fractionated by flash chromatography over silica gel (Et_2O:MeOH, 99:1) to give 9 (12 mg) and 10 (17.4 mg).

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