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# Synthesis and Polymerization Studies of Organic-Soluble Eumelanins†

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## ABSTRACT

**The isolation, structure determination and chemical characterization of eumelanins has been plagued by their very low solubility in organic solvents. To gain insights into the structure and reactivity of these unusual and important biologic macromolecules and to pave the way for their use in electronics, we have prepared soluble melanins *via* the synthesis of monomeric precursors containing lipophilic substituents. Two such monomers derived from 5,6-dihydroxyindole-2-carboxylic acid (DHICA) were prepared, namely the benzyl and octyl ester derivatives. Both benzyl and octyl ester monomers were oxidatively polymerized to yield dark, melanin-like pigments. These polymerization processes were followed by UV–visible, fluorescence and NMR spectroscopy. These studies showed that the polymerizations proceeded by cross-linking at the 4- and 7-positions of the indole nucleus and led to highly heterogeneous polymeric products. Incorporation of a lipophilic benzyl or octyl group resulted in enhanced solubility of the pigments in a wide range of organic solvents. The UV–visible spectra of the organically soluble synthetic melanins were essentially identical to that of natural eumelanin.**

## INTRODUCTION

Melanins are a class of pigmentary macromolecules found nearly ubiquitously throughout nature (1–3). While there are a number of different types of melanins, by far the most widely studied forms are the eumelanins and pheomelanins, responsible for pigmentation in mammals. The eumelanins are macromolecules responsible for brown and black coloration and they are derived from oxidative polymerization of 5,6-dihydroxyindole (DHI, **1**) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA, **2**), which are themselves formed by the oxidation of tyrosine (2). The pheomelanins are red and yellow pigments derived from cysteinyldopa units (**3**) (Fig. 1) (2).

The melanins are thought to possess a high degree of chemical heterogeneity, which results in a diverse range of functions within the body. Indeed, it has recently been proposed that the robust functionality of the melanins *in vitro* is a direct consequence of their extreme heterogeneity (4,5). The roles of these molecules in the body are believed to be as free radical scavengers, antioxidants and as the primary photoprotectants (2). In the human skin and eye, melanin is thought to protect cells by light absorption (6). Its high degree of conjugation renders melanin a powerful radiation absorber with a broadband photon absorption spectrum that extends from the UV into the infrared range. The very low radiative quantum yield measurements reported for the material denote strong excited state-phonon coupling whereby the material has a propensity to relax nonradiatively by vibrational coupling (7). Thus, it is widely believed that the mechanism by which the pigment achieves its biologic photoprotective functions is *via* absorption of harmful radiation and dissipation of this energy nonradiatively.

In 1974 it was shown that a pellet of eumelanin could conduct electricity and it was observed to switch between low conductivity and high conductivity states (8). From the data, it was hypothesized that melanin has the ability to act as an amorphous organic semiconductor. The semiconducting properties of melanin have since been demonstrated in several other studies (9,10). In addition, photoconductivity measurements have shown that a current is produced in a eumelanin film under illumination by white light (11,12). As a result, it has been suggested that this class of molecule may find applications in photovoltaic devices such as dye sensitized solar cells (13,14). Despite this body of evidence (which has led to somewhat of a paradigm in the field), recent more in-depth optical spectroscopy studies have called the amorphous semiconductor model into question (for a review of the current thinking in this respect, see Meredith and Sarna [5]). Current research into electronic devices based on melanin is performed using melanin pellets or electropolymerized films, which are thick, brittle and display a high level of morphologic discontinuity. This greatly increases the likelihood of charge recombination and trapping events and diminishes the efficiency of any electronic or optoelectronic device. Ideally, an extremely thin monolayer should be used for these purposes (15).

Because of its many roles within the body, and its intriguing physicochemical properties, a thorough understanding of the structure–property relationships of melanin is paramount. The eumelanins are intractable, amorphous pigments thought to be of high molecular weight (16), thus rendering their isolation from other cellular components difficult. The pigments are insoluble in organic solvents and only slightly soluble in concentrated alkaline hydroxide solution. Because of these

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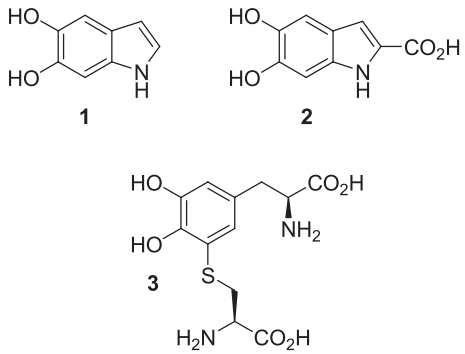



**Figure 1.** Monomeric units of eumelanin: 5,6-dihydroxyindole (DHI, **1**) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA, **2**). Monomer of pheomelanin: cysteinyl-DOPA (**3**).

isolation and handling difficulties, the structure of the pigment remains poorly understood.

Early model studies and theoretical considerations into the oligomerization of these monomeric units suggested that the preferred cross-linking positions for DHI (**1**) were the 3- and 7-positions (17,18). More recently, dimers and trimers linked through the 2-, 4- and 7-positions have been isolated (16,19–21). Panzella *et al*. have characterized DHI tetramers that they obtained by peroxidase/$H_2O_2$-induced oxidative coupling of 2,4′-linked DHI dimers (22). These researchers have also investigated the pulse radiolytic oxidation of 2,2′, 2,4′ and 2,7′-bis indole dimers derived from DHI (23). On the other hand, DHICA (**2**) is believed to undergo polymerization through primarily the 4- and 7-positions (24–26). Both ionic and radical mechanisms for the oligomerization of melanins have been suggested, and two hypotheses have been proposed for the structure of the eumelanin pigment: the first of which suggests that eumelanin is a long chain linear co-polymer of DHI (**1**) and DHICA (**2**) units (25–27), while the second hypothesis proposed for the structure of eumelanin is that the polymer consists of a compilation of discrete, highly conjugated, planar oligomeric units (*i.e*. dimer, trimer, tetramer, *etc*.) (4,28,29). Kaxiras *et al*. have extended this hypothesis, suggesting a structural model of melanin in which four DHI units, linked at positions 2 and 7, form a cyclic tetramer with an inner porphyrin ring (30).

Recently, eumelanin-like materials soluble in DMF and DMSO have been synthesized by oxidative polymerization of DOPA (**4**) using benzoyl peroxide (31–33). Taking a different approach, we chose to investigate the solubility of synthetic melanin containing lipophilic ester groups derived from polymerization of the DHICA benzyl (**6a**) and octyl ester (**6b**). It was expected that the pigment resulting from the oxidative polymerization of such monomers would show increased solubility in a range of common organic solvents, allowing them to be characterized by conventional spectroscopic techniques, thus allowing us to probe the structure of the final melanin pigment, and to investigate the early polymerization products formed during this process. The results reported here show that we can indeed prepare such organic-soluble melanins, capable of being cast into thin films. We have determined reaction rate constants for the oligomerization and we have been able to identify the positions on the indole nuclei at which cross-linking occur.

## MATERIALS AND METHODS

*General*. Nuclear magnetic resonance spectra were measured at 500.13 MHz ($^1$H) and 125.03 MHz ($^{13}$C) using $d_4$-MeOD or $d_6$-DMSO as the solvent and referenced to the residual solvent peak at 3.30 p.p.m. or 2.49 p.p.m. *vs* TMS, respectively. Electronic absorption spectra were measured using 1 cm pathlength quartz cells with solution concentrations of *ca* $5 \times 10^{-6}$ M in DMSO (spectrophotometric grade). Emission spectra were recorded for all samples using a fluorimeter. Emission scans were performed between 370 and 600 nm using an excitation wavelength of 350 nm. A band pass of 4 nm and an integration of 0.5 s were used. The emission spectra were corrected for attenuation of the probe beam and reabsorption of the emission according to a previously published procedure (14). Background scans were performed under identical instrumental conditions using the relevant solvent. Electron-impact mass spectral analyses were performed with a 70 eV ionization potential. Electrospray-ionization (ESI) mass spectral data was performed in positive ESI mode. Melting points are uncorrected.

*3,4-Dihydroxyphenylalanine benzyl ester hydrochloride (**5a**)*. 3,4-Dihydroxyphenylalanine (**4**) (2.0 g, 10 mmol) was stirred with toluene-4-sulfonic acid monohydrate (1.9 g, 10 mmol) in 20 mL benzyl alcohol at room temperature. Toluene-4-sulfonyl chloride (2.3 g, 12 mmol) was added and the solution was heated at 80°C for 2 h. The solution was cooled to room temperature, diluted with 200 mL $CHCl_3$ and washed with saturated sodium bicarbonate solution ($3 \times 100$ mL). The organic layer was dried ($Na_2SO_4$) and concentrated to *ca* 100 mL. A stream of hydrogen chloride was bubbled through the solution and the resulting white precipitate was collected to yield **5a** (1.15 g, 34%), m.p. 178–181°C (lit. [34] m.p. 191–193°C). $^1$H NMR (500 MHz, $CD_3OD$): $\delta$ 7.36–7.3 (5H, m, Bn), 6.70 (1H, d, $J$ = 8.0 Hz, Ar-H5), 6.67 (1H, d, $J$ = 2.1 Hz, Ar-H2), 6.48 (1H, dd, $J$ = 8.0, 2.1 Hz, Ar-H6), 5.22 (2H, m, $CO_2CH_2$), 4.23 (1H, t, $J$ = 7.0 Hz, H$\alpha$), 3.09–3.01 (2H, m, H$\beta$,$\beta$). $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$ 170.1 (C=O), 146.9, 146.3, 136.2, 129.76, 129.72, 129.66, 126.2, 121.8, 117.4, 116.8, 69.1 ($CO_2CH_2$), 55.4 (C$\alpha$), 36.9 (C$\beta$). HRMS calcd for $C_{16}H_{18}NO_4$ $[M+H]^+$ 288.1233, found 288.1235.

*5,6-Dihydroxyindole-2-carboxylic acid benzyl ester (**6a**)*. 3,4-Dihydroxyphenylalanine benzyl ester hydrochloride (**5a**) (162 mg, 0.5 mmol) was dissolved in 130 mL of pH 6 acetate buffer (0.2 M) under a nitrogen atmosphere. Ceric ammonium nitrate (1.096 g, 2 mmol) dissolved in 20 mL of the same buffer was added. After 15 min sodium dithionite (0.68 g, 4 mmol) was added to the red–brown mixture and the solution was stirred for a further 5 min. The reaction mixture was extracted into ethyl acetate ($3 \times 100$ mL). The combined organic layers were dried ($Na_2SO_4$), filtered and concentrated to 5 mL. The crude residue was adsorbed onto silica gel (2 g), loaded onto a pad of silica gel (10 g) and eluted with EtOAc/hexane (3:2). The yellow band was collected and evaporated to yield 3,4-dihydroxyindole-2-carboxylic acid benzyl ester (**6a**) (48.2 mg, 34%), m.p. 168–173°C (lit. [34] m.p. 179–180°C). $^1$H NMR (500 MHz, $(CD_3)_2SO$): $\delta$ 11.27 (1H, br d, $J$ = 1.4 Hz, NH), 9.16 (1H, br s, OH), 8.63 (1H, br s, OH), 7.46–7.33 (5H, m, Bn), 6.94 (1H, dd, $J$ = 1.4, 0.8 Hz, H3), 6.87 (1H, s, H4), 6.79 (1H, s, H7), 5.31 (2H, s, $CO_2CH_2$). $^{13}$C NMR (125 MHz, $(CD_3)_2SO$): $\delta$ 161.0 (C=O), 146.6, 142.3, 136.5, 133.1, 128.5, 127.9, 127.8, 124.4, 119.9, 107.9, 104.9, 96.9, 65.2. HRMS calcd for $C_{16}H_{13}NO_4$ $[M]^+$ 283.0844, found 283.0847. $\lambda_{max}$ 330 nm (log $\varepsilon$ = 4.25) (lit. [34] 315 nm [log $\varepsilon$ = 4.27]).

*3,4-Dihydroxyphenylalanine octyl ester hydrochloride (**5b**)*. 3,4-Dihydroxyphenylalanine (**4**) (1.0 g, 5.0 mmol) was stirred with toluene-4-sulfonic acid (1.7 g, 10 mmol) and toluene-4-sulfonyl chloride (2.3 g, 12 mmol) in 1-octanol (20 mL) at 80°C for 4 h. The solution was cooled to room temperature, diluted with 100 mL $Et_2O$ and washed with saturated sodium bicarbonate solution ($3 \times 100$ mL). Hydrogen chloride gas was bubbled through the solution and the white precipitate collected to yield **5b** (1.5 g, 85%), m.p. 170–173°C (lit. [35] m.p. 180°C). $^1$H NMR (500 MHz, $CD_3OD$): $\delta$ 6.74 (1H, d, $J$ = 8.0 Hz, H5), 6.67 (1H, d, $J$ = 2.1 Hz, H2), 6.56 (1H, dd, $J$ = 8.0, 2.1 Hz, H6), 4.2–4.16 (3H, m, C$\alpha$, $CO_2CH_2$), 3.09–3.01 (2H, m, H$\beta$,$\beta$), 1.62–1.66 (2H, m, $CO_2CH_2CH_2$), 1.30 (10H, br s, $5 \times CH_2$), 0.89 (3H, t, $J$ = 6.8 Hz, $CH_3$). $^{13}$C NMR (125 MHz, $CD_3OD$): $\delta$ 170.2 (C=O), 146.8, 146.2, 126.2, 121.7, 117.3, 116.7, 67.5, 55.4, 37.0, 32.9, 30.27, 30.26, 29.5, 26.9, 23.7, 14.4. HRMS calcd for $C_{17}H_{28}NO_4$ $[M+H]^+$ 310.2018, found 310.2023.

*5,6-dihydroxyindole-2-carboxylic acid octyl ester (**6b**)*. 3,4-Dihydroxyphenylalanine octyl ester hydrochloride (**5b**) (0.162 g, 0.468 mmol) was dissolved in 130 mL of pH 5 acetate buffer under a nitrogen atmosphere. Ceric ammonium nitrate (1.096 g, 2 mmol) dissolved in 20 mL of the same buffer was added. After 15 min sodium dithionite (0.68 g, 4.0 mmol) was added and the solution was stirred for a further 5 min. The reaction mixture was extracted into EtOAc ($3 \times 100$ mL). The combined organic layers were dried ($Na_2SO_4$), filtered and the volume reduced to 5 mL. Upon addition of hexane a precipitate formed which was removed by filtration over Celite. The Celite was washed with warm hexane and the combined filtrates evaporated to dryness to yield 5,6-dihydroxyindole-2-carboxylic acid octyl ester (**6b**) (68 mg, 48%), m.p. 149–152°C. $^1$H NMR (500 MHz, $(CD_3)_2SO$): $\delta$ 11.18 (1H, br s, NH), 9.11 (1H, br s, OH), 8.59 (1H, br s, OH), 6.86 (2H, s H3,4), 6.77 (1H, s, H7), 4.20 (2H, t, J = 6.5 Hz, $CO_2CH_2$), 1.66 (2H, m, $CO_2CH_2CH_2$), 1.36 (2H, br m, $CO_2(CH_2)_2CH_2$), 1.25 (8H, br m, $4 \times CH_2$), 0.84 (3H, t, J = 7.0 Hz, $CH_3$). $^{13}$C NMR (125 MHz, $(CD_3)_2SO$): $\delta$ 161.3 (C=O), 146.5, 142.2, 132.9, 124.7, 119.8, 107.4, 104.9, 96.9, 63.7, 31.2, 28.67, 28.62, 28.3, 25.5, 22.1, 13.9. HRMS calcd for $C_{17}H_{23}NNaO_4$ $[M + Na]^+$ 328.1524, found 328.1531. $\lambda_{max}$ 330 nm (log $\varepsilon$ = 4.22).

*Polymerization of **5a** and **5b***. A solution of the dihydroxyindole (**5a** or **5b**) (40 mg) and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (0.107 mL) in DMSO (3.5 mL) was stirred in a flask open to the air for 7 days. The solution was then diluted with $CHCl_3$ (100 mL), washed with 5% HCl ($2 \times 100$ mL) and brine ($2 \times 100$ mL), dried ($Na_2SO_4$) and evaporated. The solid residue was suspended in water and collected on a frit to yield the polymer product (~37 mg).

## RESULTS AND DISCUSSION

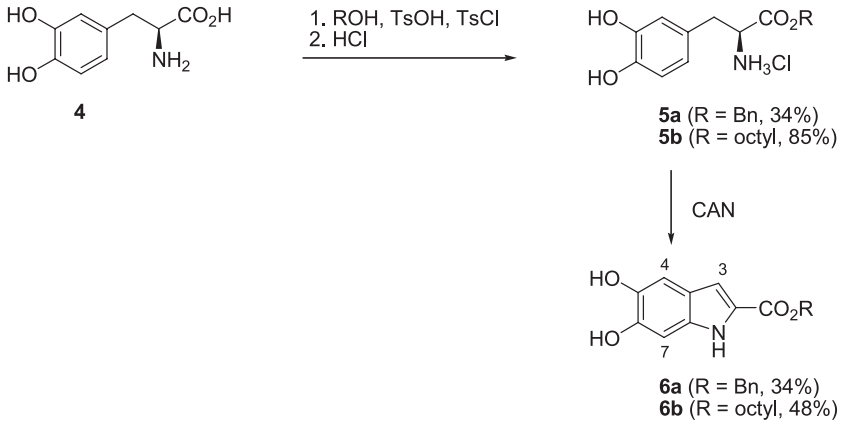
### Synthesis of eumelanin monomers

Initial attempts to synthesize DOPA benzyl ester hydrochloride (**5a**) following the reported procedure (34) were unsuccessful. Mass spectrometry of the crude product isolated under these conditions showed the presence of mainly doubly benzylated species (*m/z* 378) and a small amount of a triply benzylated species (*m/z* 468). These products no doubt arose from benzylation of the electron-rich aromatic ring of **5a**. The $^1$H NMR spectrum of the product confirmed this, displaying a very complicated aromatic region (6–8 p.p.m.) due to the presence of multiple benzyl groups. To synthesize primarily the mono-benzylated species (**5a**), the reaction conditions were modified to those reported (36) for the esterification of tyrosine. This procedure differs from the original (34) only by the reduction in the number of mole equivalents of toluene-4-sulfonic acid and toluene-4-sulfonyl chloride to 1:1:DOPA. Under these conditions, without excess reagents, the mono-benzylated desired product (**5a**) was generated (Scheme 1). The product was isolated as its hydrochloride salt by bubbling hydrogen chloride through the solution; however, the yields remained poor (34%). Conducting the reaction for longer periods again generated the multiply benzylated species. The product (**5a**) was unstable in solution, forming a dark polymer on standing.

Oxidative cyclization of DOPA benzyl ester (**5a**) to the dihydroxyindole (**6a**) was performed using buffered (pH 6) ceric ammonium nitrate (CAN) as the oxidizing agent (34). For successful formation of **6a** it was important that the ester (**5a**) was pure, otherwise the product rapidly degraded upon attempted recrystallization (34) or column chromatography. Optimum yields of **6a** were achieved by rapid filtration of a solution of the crude cyclized product through a pad of silica. It has been reported (18) that the addition of zinc acetate to the oxidative cyclization reaction of DOPA derivatives catalyzes the formation of the dihydroxyindole. In our hands, however, the addition of zinc acetate had no influence on the yields or rate of the reaction.

The $^1$H NMR spectrum of **6a** showed three distinct aromatic signals corresponding to the three indolic protons. A NOESY spectrum of **6a** showed interaction of H7 with the NH. This, in conjunction with the coupling observed between NH and H3 (1.4 Hz) and the 0.8 Hz coupling between H3 and H7 (37) allowed unambiguous assignments of the three indolic aromatic protons.

Unlike the synthesis of DOPA benzyl ester hydrochloride (**5a**), the synthesis of DOPA octyl ester hydrochloride (**5b**) (Scheme 1) proceeded smoothly in good yield (85%). Unfortunately the cyclization procedure used for the benzyl ester (**5a**) could not be directly applied to the octyl ester (**5b**). Under the reported conditions (34) the octyl ester cyclization yielded a dark oily crude material whose $^1$H NMR spectrum showed no evidence of the desired indolic product (**6b**). Potassium ferricyanide was also trialed as this has been used successfully for the oxidative cyclization of DOPA (**5**) to yield DHICA (**2**) (4). However, the $^1$H NMR spectrum of the crude product again showed no evidence of the desired product (**6b**). When CAN was used in unbuffered solution, peaks corresponding to the desired product (**6b**) were seen in the $^1$H NMR spectrum of the crude product. Best results were achieved using CAN in buffered solutions at either pH 4 or pH 5. Like the benzyl indole (**6a**), the octyl indole (**6b**) was found to interact




**Scheme 1.** Synthesis of DHICA esters **6a** and **6b** from DOPA (**4**).

deleteriously with silica gel so alternative purification methods were trialed. Attempts at recrystallization proved unsuccessful. Selective precipitation was attempted, with the observation that a precipitate formed upon addition of hexane to a concentrated ethyl acetate extract of the crude product. Upon removal of this solid it was found that only the desired indole (**6b**) remained in the filtrate and could be isolated by simple removal of the solvent.

### Polymerization studies

Polymerization of the indoles (**6a**) and (**6b**) was accomplished by aerial oxidation in DMSO solution in the presence of 5 molar equivalents of DBU. This base was added to deprotonate one or more of the phenolic groups of the indoles to increase the rate of polymerization. After stirring for 1 week, the polymeric product was isolated almost quantitatively following aqueous workup of the dark solution. Aerial oxidation was our preferred method, which we chose to avoid the inclusion of metal ions in the synthetic melanin products. Solubilities of these synthetic melanins in a range of organic solvents are presented in Table 1.

The solubilities of the polymerized products were similar for both the octyl ester derivative and the benzyl ester derivative and showed that addition of a lipophilic substituent on the DHICA nucleus did indeed lead to synthetic melanins which were soluble in a range of common organic solvents.

Having established that both esters (**6a**) and (**6b**) could be polymerized to form soluble synthetic melanins, we examined the polymerization of these precursors with the aims of establishing rate constants and determining the position(s) on the indole nuclei where cross-linking was occurring. The polymerization processes for both the benzyl ester (**6a**) and the octyl ester (**6b**) were examined in DMSO solution by UV–visible absorption and emission spectroscopy, using an excitation wavelength of 350 nm, for the first 8 h after the addition of 5 molar equivalents of DBU. The time profiles from both these indoles showed a general broadening, leading eventually to relatively featureless broadband absorption spectra essentially identical to that of melanin itself (38), with the benzyl ester (**6a**) appearing to polymerize faster than the octyl ester (**6b**) (Fig. 2). After 24 h under oxidative polymerization conditions both the benzyl and octyl derivatives showed negligible emission and broad monotonic absorption profiles from 280 to 800 nm, consistent with that observed for both natural and synthetic DHI- and DHICA-derived melanins (38).

**Table 1.** Solubilities of synthetic melanins derived from benzyl ester (**6a**) and octyl ester (**6b**).

| Entry | Solvent | Solubility of melanin derived from (**6a**) | Solubility of melanin derived from (**6b**) |
|---|---|---|---|
| 1 | Acetone | High | High |
| 2 | MeCN | Moderate | Low |
| 3 | $CHCl_3$ | High | High |
| 4 | $Et_2O$ | Low | Moderate |
| 5 | DMF | High | High |
| 6 | DMSO | High | High |
| 7 | EtOAc | Moderate | High |
| 8 | Hexane | Low | Low |
| 9 | MeOH | Moderate | Moderate |
| 10 | Pyridine | High | High |
| 11 | THF | High | High |
| 12 | $H_2O$ | Low | Low |

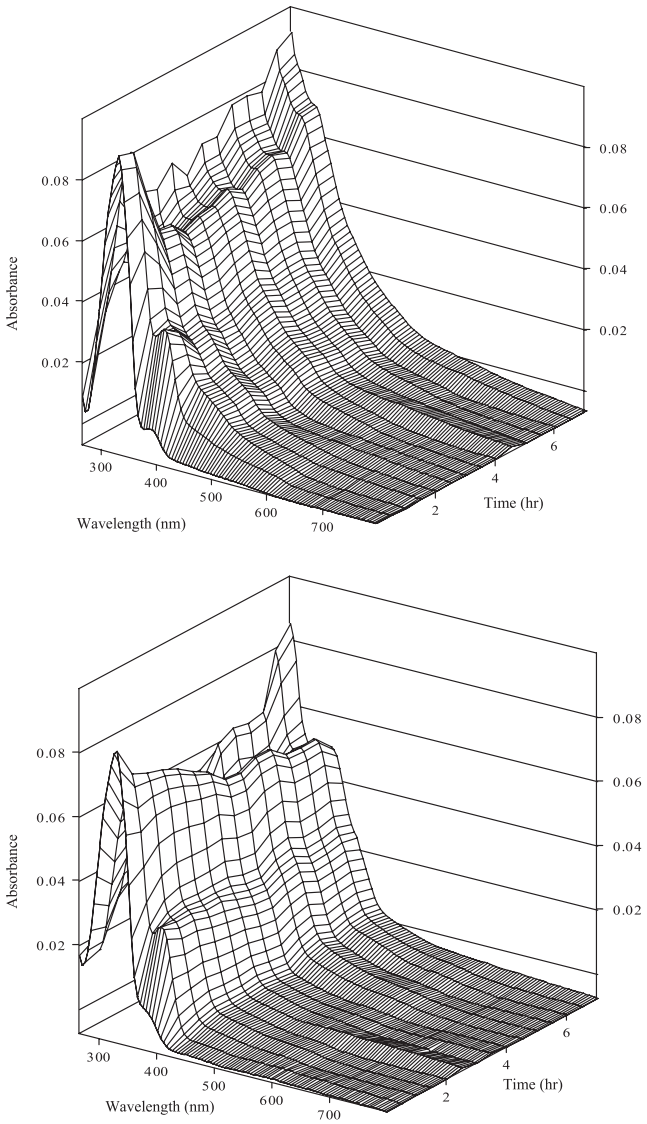



**Figure 2.** Wire mesh plots of absorbance spectra of polymerization of benzyl ester (**6a**) (above) and octyl ester (**6b**) (below) *vs* time (in DMSO solution).

Examination of the emission spectra of these indoles confirmed that the benzyl ester (**6a**) did indeed polymerize faster than the octyl ester (**6b**) (Fig. 3).

By following the decrease in fluorescence at 397 nm for the benzyl ester (**6a**) and at 393 nm for the octyl ester (**6b**), the rate constants for the two polymerization reactions could be determined. Plots of the natural logarithm of the fluorescence intensities *vs* time yielded straight lines (Fig. 4) indicating first-order reactions. This suggested that a simple polymerization was occurring, with no build-up of complex intermediates. The rate constant, $k$, for the polymerization of the benzyl ester (**6a**) was determined to be $2.17 \times 10^{-4}$ $s^{-1}$. For the octyl ester (**6b**), the rate constant for its polymerization reaction was calculated to be $7.42 \times 10^{-5}$ $s^{-1}$.

The polymerization of the benzyl (**6a**) and octyl (**6b**) DHICA derivatives was also followed by $^1H$ NMR spectroscopy for the

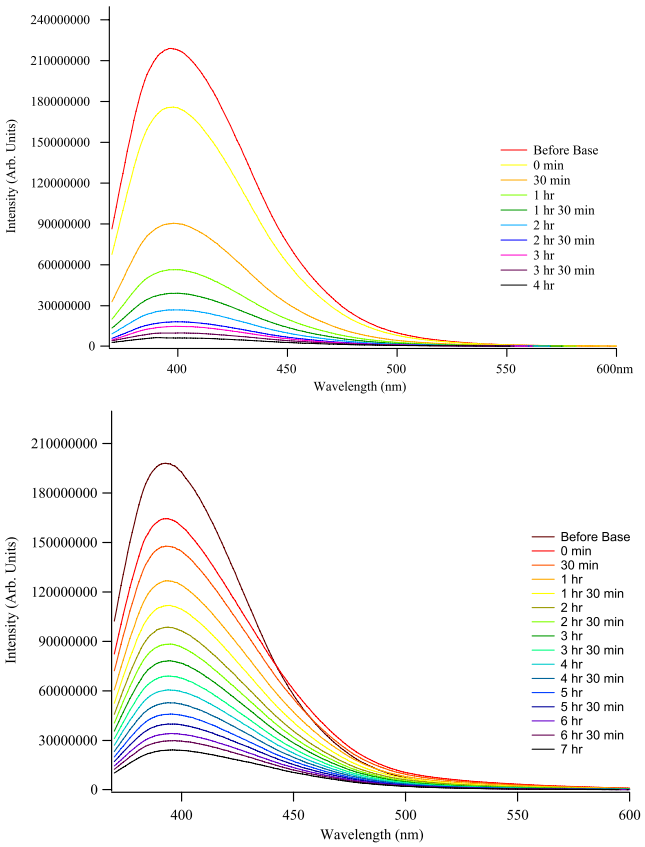



**Figure 3.** Emission spectra of benzyl ester (**6a**) (above) and octyl ester (**6b**) (below) over time (in DMSO solution).

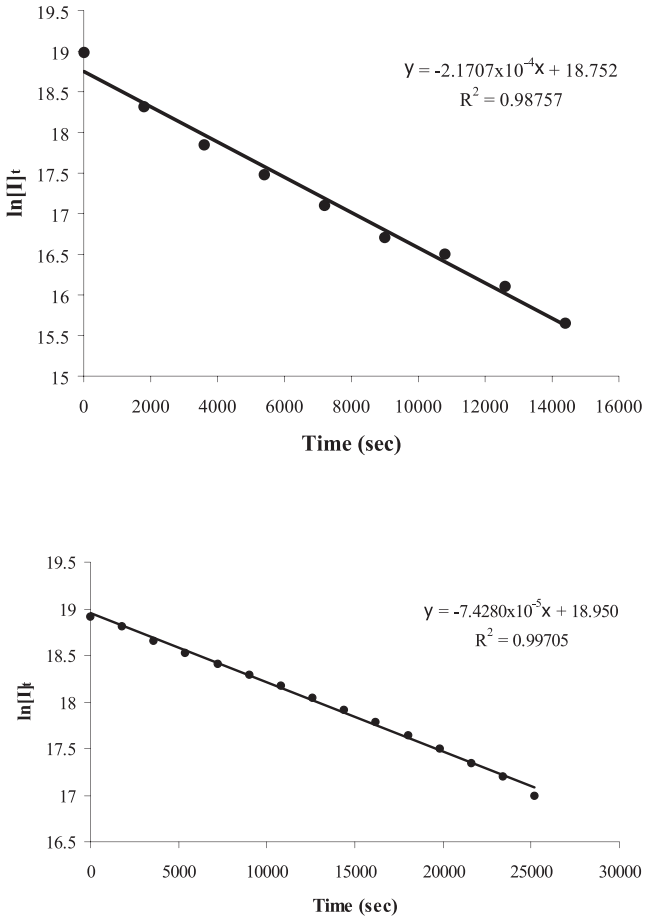



**Figure 4.** Plot of ln[fluorescence intensity] *vs* time for the polymerizations of the benzyl ester (**6a**) (above) and the octyl ester (**6b**) (below).

first 8 h following the addition of base. The polymerizations were performed at a concentration of 20 mg $mL^{-1}$ in $d_6$-DMSO in order to maintain consistency with UV–visible absorption data, and were initiated by the addition of five molar equivalents of DBU. A plot of the aromatic region (7.25–6.25 p.p.m.) of the $^1H$ NMR spectra of the polymerization process for the benzyl indole (**6a**) over time is shown in Fig. 5. This plot shows the disappearance of the peaks attributable to the starting material, and the simultaneous appearance of numerous new peaks due to oligomeric species. The results of the polymerization of the octyl ester (**6b**) were very similar (data not shown).

An expanded region of the spectrum after 8 h can be seen in Fig. 6. Of interest is the presence of numerous doublets ($J$ = 0.7–1 Hz) in the region around the H3 resonance of the monomer, and the absence of such corresponding peaks in the regions near the H4 and H7 resonances of the monomer. We interpret this as arising from oxidative polymerization of **6a** *via* cross-linking at positions 4 and 7, leading initially to a number of oligomers with intact H3 protons, as seen in the expansion in Fig. 6. Thus the benzyl ester (**6a**) would appear to undergo oxidative polymerization predominantly *via* substitutions at positions 4 and 7, with little or any substitution occurring at position 3.

These spectra are the first examples of pH neutral solution NMR studies on a melanin pigment with other studies being conducted in either highly basic aqueous solutions (39,40) or on solid samples (41–47). The spectra recorded in this study show much narrower resonances than those previously reported.

## CONCLUSION

The benzyl and octyl esters of DHICA (**2**) have been synthesized and characterized, with the indole-forming cyclization step being very sensitive to pH. Both esters were subjected to an oxidative polymerization process that was monitored by absorption, emission and $^1H$ NMR spectroscopy. The absorption and emission studies showed that the dark pigments obtained from the polymerization process possessed the two notable and unique characteristics of natural melanin, namely a broadband absorbance profile and low fluorescence quantum yield. Our soluble synthetic melanins thus display spectroscopic characteristics consistent with those observed for both natural and synthetic DHI- and DHICA-derived melanins, allowing comparisons to be drawn between these synthetic melanins and the natural pigment. Both DHICA esters polymerized with first-order kinetics to produce a synthetic melanin soluble in a range of organic solvents, and $^1H$ NMR spectroscopy indicated that the preferred positions for substitution on the indole nucleus were at positions 4 and 7. Future work will focus on the fractionation of these synthetic melanins, with a view to determining their molecular weight distributions.

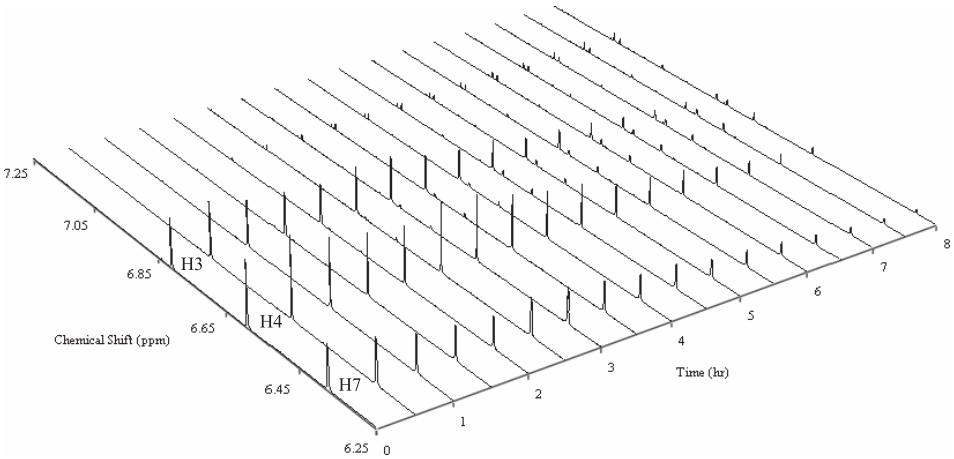



**Figure 5.** $^1$H NMR spectra ($d_6$-DMSO) of the benzyl ester (**6a**) over time.

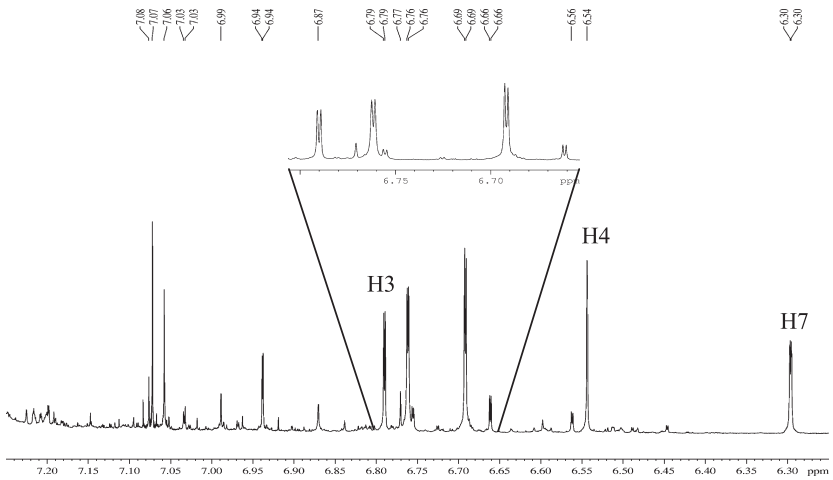



**Figure 6.** Aromatic region (7.25–6.25 p.p.m.) of the $^1$H NMR spectrum of benzyl ester (**6a**) after oxidative polymerization for 8 h. Residual monomer peaks are labeled H3, H4 and H7.

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