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5,6-Dihydroxyindole Tetramers with "Anomalous" Interunit Bonding Patterns by Oxidative Coupling of 5,5′,6,6′-Tetrahydroxy-2,7′-biindolyl: Emerging Complexities on the Way toward an Improved Model of Eumelanin Buildup

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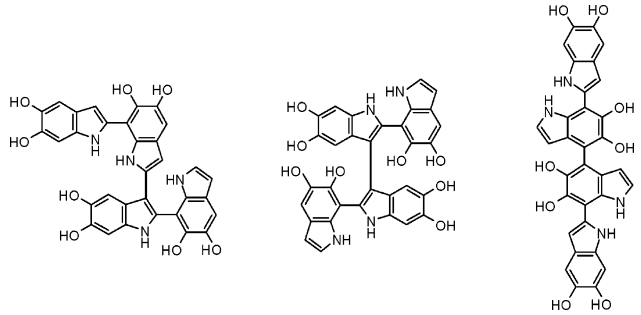

Chemical or enzymatic oxidation of 5,6-dihydroxyindole (**1**) leads to the rapid deposition of a black solid resembling eumelanin pigments by way of a complex oligomerization/polymerization process that proceeds in the early stages via dimers **2**−**3** and trimers **5**−**6** characterized by 2,4′- and 2,7′-couplings. Despite extensive efforts, the structures of the higher oligomers, which define the structural architecture and physicochemical properties of the eumelanin particles, have so far defied elucidation. Using a dimer−dimer coupling strategy that has recently allowed the first successful entry to a tetramer of **1**, we report now three additional tetramers obtained by oxidation of 5,5′,6,6′-tetrahydroxy-2,7′-biindolyl (**3**) with the peroxidase/H_2O_2 system. On the basis of extensive 2D NMR and mass spectrometric analysis, the products were identified as 5,5′,5″,5‴,6,6′,6″,6‴-octaacetoxy-7,2′:3′,3″:2″,7‴-tetraindolyl (acetylated **8**, 3%), 5,5′,5″,5‴,6,6′,6″,6‴-octaacetoxy-2,7′:4′,4″:7″,2‴-tetraindolyl (acetylated **9**, 4%), and 5,5′,5″,5‴,6,6′,6″,6‴-octaacetoxy-2,7′:2′,3″:2″,7‴-tetraindolyl (acetylated **10**, 5%), in which the inner units are linked through unexpected 3,3′-, 4,4′-, and 2,3′-linkages. If verified in further studies, the newly uncovered coupling patterns would entail important consequences for current models of eumelanin structure based on one-dimensional structural chains with extended π-electron conjugation or π-stacked flat oligomer aggregates.

Introduction

5,6-Dihydroxyindole (**1**) belongs to a unique group of naturally occurring indole compounds which have intrigued generations of organic and natural product chemists[1] because of their central role in the biosynthesis of eumelanins, the major determinants of human skin, hair, and eye pigmentation.[2]

Indole **1** is synthesized within epidermal melanocytes by oxidative cyclization of L-tyrosine promoted by the copper enzyme tyrosinase. Just formed, **1** takes part in a complex oxidative polymerization process that leads eventually to the deposition of the black eumelanin pigments.[3]

The unique status of eumelanins among natural pigments is due to their socio-economic and biomedical relevance, encom-

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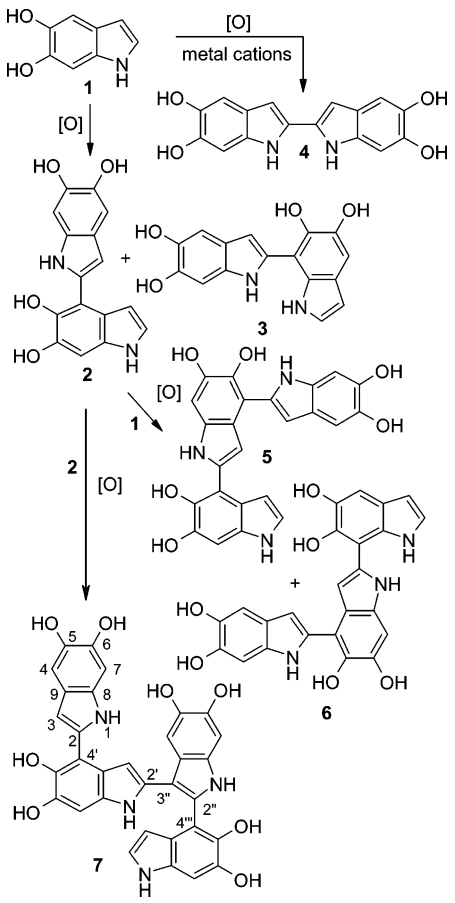
passing racial pigmentation, skin photoprotection, sun tanning and pigmentary disorders such as albinism, vitiligo, and melanoma.[4] Moreover, they display a quite unusual set of physicochemical properties[5] including insolubility in all solvents, broadband monotonic absorption in the UV−visible range,[6] a persistent free radical character,[7] metal and drug-binding properties,[8−10] susceptibility to redox changes,[11] strong excited state-phonon coupling,[12] and amorphous electrical switch behavior.[13] These properties have provided a basis to propose the potential application of eumelanin-related materials in the field of molecular electronics and photon-harvesting systems.[14]

Despite many decades of work within the organic chemistry, biophysics, and pigment cell communities, eumelanin structure remains virtually unknown, because of the marked chemical heterogeneity and the lack of well-defined physicochemical properties, preventing successful application of spectroscopic techniques. While there is general agreement that at the primary level eumelanins are made up of 5,6-dihydroxyindole units at various degrees of oxidation and linked together in a covalent fashion, at the secondary level the situation is far less clear. According to an early view, eumelanins are highly heterogeneous linear polymers arising by random coupling of the monomer indole units. Subsequently, an alternate model largely deduced from X-ray scattering and scanning tunneling data has gained credit, suggesting that eumelanins are not polymers but rather consist of supramolecular aggregates of 4−6 oligomers stacked in the z-plane in a graphene-like fashion.[15] In most cases, however, hypothetical structural models have been used as a working basis to fit experimental data, because of the lack of knowledge of the mechanisms of oxidative polymerization of **1** and its congeners. Extensive studies carried out over the past two decades have elucidated the early stages of the oxidation of **1** leading to dimers **2**−**4** and trimers **5** and **6** as the main isolable oligomers.[16] These structures underscored a dominant mode of coupling of **1** involving nucleophilic attack through

SCHEME 1



the 2-position to the 4- and 7-positions of a transient 5,6-indolequinone. Symmetric 2,2′-coupling leading to dimer **4**, on the other hand, prevails in the presence of transition metal ions, e.g., Zn^{2+} and Ni^{2+}, and is probably dictated by formation of chelate complexes affecting the positional reactivity of **1** or its quinone.[17]

The reactivity patterns of **1** exemplified by **2**−**6** (Scheme 1) have provided a convincing ground to postulate that the higher oligomer structures ultimately involved in the supramolecular aggregation processes were generated by sequential oxidative coupling of **1** through the 2-, 4-, and 7-positions. This mechanism, however, has remained so far unverified because, as the size of the oligomers increases, their isolation becomes increasingly complex, due to the gradational range of species of increasing mass which typify the oxidation mixtures of **1** and the consequent need to contend with very complex mixtures containing myriads of species.

Knowledge of high oligomer structures is yet essential for predicting their planarity and, hence, the efficiency of π-electron delocalization and π-stacking in the final aggregation process. Moreover, it would offer a valuable background for the purposeful design of eumelanin-like materials for potential practical applications.

With these goals in mind, we have recently been prompted to investigate the oxidation of 5,6-dihydroxyindole dimers as a straightforward access route to tetramers of **1** enabling a direct insight into their basic structural features. Against all expecta-

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tions, a new scenario was uncovered when the first tetramer of **1**, 5,5′,5″,5‴,6,6′,6″,6‴-octahydroxy-2,4′:2′,3″:2″,4‴-tetraindolyl (**7**), was obtained as the acetyl derivative by oxidation of the 2,4′-biindolyl **2**.[18] Structure **7** featured an "anomalous" 2,3′-biindolyl linkage that was unprecedented in 5,6-dihydroxyindole chemistry, and that was suggested to reflect nucleophilic attack of **2** to an extended quinone methide intermediate.[19] Whether this peculiar mode of coupling denoted a general modification of the 5,6-dihydroxyindole reactivity pattern in dimers and higher oligomers was an important issue raised by that study. In this paper we report the structures of three new tetramers of **1** obtained by oxidation of the biindolyl **3** and a discussion of how the emerging pathways of dimer oxidation fit with currently held schemes of oxidative polymerization of **1**.

Results and Discussion

The oxidation of **3** was carried out essentially as described,[18] using the peroxidase/H_2O_2 system. The dimer, stored as the acetyl derivative, was deprotected by an established methodology involving treatment with 0.1 M phosphate buffer, pH 12 for 1−2 min under an Ar atmosphere, to prevent oxidation of the deacetylated dimer by air. pH was then brought to 8, to ensure sufficient solubility of the dimer at 1 mM concentration.

A critical parameter affecting the product composition and yields was the reaction time, with too short times resulting in much unreacted substrate and prolonged oxidation times leading to abundant eumelanin-like materials with little isolable oligomers. Accordingly, after several trials, a reaction time of 20 s was adopted for all experiments. The purple-blue reaction mixture thus obtained was treated with excess sodium dithionite, and the clear ethyl acetate-extractable fraction was acetylated to improve both stability and chromatographic behavior of the products. TLC analysis ($CHCl_3$:CH_3OH = 98:2) of the resulting mixture indicated little residual **3** and a number of chromatographically distinct fluorescent products, two of which could be isolated by TLC fractionation and were characterized by extensive spectral analysis, including ^{1}H,^{1}H COSY, ^{1}H,^{13}C HSQC, ^{1}H,^{13}C HMBC, and ROESY spectra.

The product at R_f 0.42 gave a pseudomolecular ion peak in the ESI(+)-MS spectrum at *m*/*z* 949 $[M + Na]^+$, indicating a tetramer of **1**, and was formulated as the symmetric 5,5′,5″,5‴,6,6′,6″,6‴-octaacetoxy-7,2′:3′,3″:2″,7‴-tetraindolyl (acetylated **8**, 3% isolated yield), in which the 2,7′-biindolyl units were linked through a 3,3′-bond.

The ^{1}H NMR spectrum of **8** (Table 1) exhibited five signals in the aromatic region at δ 6.02, 6.94, 6.95, 7.34, and 7.49. Of the two NH protons, only that at δ 9.02 gave cross-peaks in the COSY spectrum with protons resonating at δ 6.95 (H-2) and 6.02 (H-3), while the N−H resonance at δ 10.54 did not show apparent correlation peaks. The ROESY spectrum revealed a distinct NOE contact between the H-7 proton at δ 7.34 and the N−H proton resonating at δ 10.54, allowing definitive assignment of this resonance to the disubstituted indole moiety, and between the two N−H protons, suggesting that they are in close proximity in the most populated conformations. It is also worth noting that a number of protons, i.e., H-3_a (δ 6.02), H-4_a (δ 6.94), H-2_a (δ 6.95), and N−H_a (δ 9.02), resonate significantly upfield relative to acetylated dimer **3**, indicating that outer ring **a**/**a**′ falls in the shielding cone of the inner ring **b**/**b**′.

TABLE 1. Salient ^{1}H and ^{13}C NMR Resonances of Acetylated 8[a,b].

position[c]	^{1}H	^{13}C	position[c]	^{1}H	^{13}C
1	9.02		1′	10.54	
2	6.95	128.5	2′		133.8
3	6.02	101.6	3′		110.1
4	6.94	114.9	4′	7.49	114.1
5		135.1	5′		137.5
6		136.1	6′		139.6
7		110.1	7′	7.34	106.7
8		133.8	8′		133.8
9		125.1	9′		125.6

[a] Spectra run in acetone-d_6, chemical shift values given in ppm. [b] Numbering as shown in the structural formula. [c] ^{1}H and ^{13}C resonances of acetyl groups were 2.12 (3H × 2), 2.28 (3H × 3), 2.32 (3H × 3) ppm, and 20.2 ($COCH_3$), 169.5 ($COCH_3$) ppm, respectively.

TABLE 2. Salient ^{1}H and ^{13}C NMR Resonances of Acetylated 9[a,b].

position[c]	^{1}H	^{13}C	position	^{1}H	^{13}C
1	10.78		1′	10.71	
2		132.4	2′	7.39	127.0
3	6.84	103.8	3′	6.16	103.9
4	7.44	114.3	4′		121.4
5		137.7	5′		138.0
6		139.9	6′		139.5
7	7.40	106.5	7′		111.8
8		134.9	8′		134.9
9		126.7	9′		126.8

[a] Spectra run in acetone-d_6, chemical shift values given in ppm. [b] Numbering as shown in the structural formula. [c] ^{1}H and ^{13}C resonances of acetyl groups were 2.04 (3H × 2), 2.21 (3H × 2), 2.31 (3H × 2), 2.32 (3H × 2) ppm, and 20.1 ($COCH_3$), 168.3 ($COCH_3$) ppm, respectively.

The product eluted under the band at R_f 0.27 was another symmetric tetramer (pseudomolecular ion peak at *m*/*z* 949 $[M + Na]^+$, ESI(+)-MS; five aromatic proton resonances and four acetyl signals in the ^{1}H NMR spectrum). Closer spectral analysis eventually allowed its formulation as 5,5′,5″,5‴,6,6′,6″,6‴-octaacetoxy-2,7′:4′,4″:7″,2‴-tetraindolyl (acetylated **9**, 4% isolated yield) featuring a 4,4′-linkage between the inner units. The ^{1}H NMR spectrum (Table 2) displayed typical resonances for two H-3 protons and one H-2, H-4, and H-7 proton. The COSY spectrum indicated coupling of the NH_b proton resonating at δ 10.71 with the signals at δ 7.39 (H-2_b) and 6.16 (H-3_b), and coupling of the NH_a (δ 10.78) with the resonance at δ 6.84, attributed to the H-3_a proton.

The ROESY spectrum showed moreover cross-peaks between H-3_a and H-4_a, H-7_a and NH_a, and H-3_a and NH_b allowing complete assignment of all resonances. The marked upfield shift of the H-3_b proton (δ 6.16) is attributable to the diamagnetic anisotropy of the adjacent indole ring.

The results so far described are a significant achievement when considered in the light of the notorious difficulties in isolating higher oligomers of **1**. Unfortunately, attempts to isolate other oxidation products of **3** were unsuccessful due to the marked complexity of the reaction mixture and the very low formation yields of the products. During the recent investigation of the oxidation chemistry of **2**[18] it was found that transition metal cations, particularly Zn^{2+} and Ni^{2+} ions, directed the reaction course toward the formation of a more defined pattern of products, allowing the isolation of tetramer **7** in sufficient yields for structural characterization. It seemed therefore of interest to extend the study of the role of transition metal cations to the oxidative coupling of **3**.

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TABLE 3. Salient ^{1}H and ^{13}C NMR Resonances of Acetylated 10[a,b]

position[c]	^{1}H	^{13}C	position[c]	^{1}H	^{13}C
1	10.18		1″	10.97	
2		135.2[d]	2″		133.3[d]
3	5.96	102.8	3″		108.4
4	7.44	114.2	4″	7.35	113.4
5		137.3	5″		137.3
6		139.2	6″		139.6
7	7.30	106.1	7″	7.43	106.6
8		134.7	8″		134.7
9		126.3	9″		124.6
1′	9.26		1‴	10.39	
2′		134.5 [d]	2‴	7.18	127.7
3′	6.84	100.5	3‴	6.47	102.6
4′	7.81	113.9	4‴	7.64	116.0
5′		137.3	5‴		137.3
6′		139.9	6‴		137.8
7′		105.8	7‴		107.4
8′		134.7	8‴		134.7
9′		127.0	9‴		127.7

[a] Spectra run in acetone-d_6, chemical shift values given in ppm. [b] Numbering as shown in the structural formula. [c] ^{1}H and ^{13}C resonances of acetyl groups were 2.17 (3H), 2.27 (3H), 2.30 (3H × 2), 2.31 (3H), 2.32 (3H), 2.33 (3H), 2.34 (3H) ppm, and 20.1 ($COCH_3$), 169.9 ($COCH_3$). [d] Interchangeable.

A preliminary screening of the effect of various divalent cations showed that Zn^{2+} ions could lead to a simpler reaction mixture. Accordingly, the oxidation of **3** (1 mM) was repeated in the presence of 3 mM Zn^{2+} in 0.5 M TRIS buffer, pH 8, using peroxidase/H_2O_2, the latter at 4.9 mM concentration.

TLC fractionation after the usual workup revealed two main products, one of which proved to be chromatographically and spectrally indistinguishable from **8**, while the other was a new tetramer that was present in much smaller amounts in the reaction mixture obtained under metal-free conditions (TLC and HPLC evidence).

On the basis of a pseudomolecular ion peak at *m*/*z* 949 [M + Na]$^+$ (ESI(+)-MS) and extensive 2D NMR analysis, the new product (R_f 0.20, $CHCl_3$:CH_3OH = 98:2, isolated yield 5%) was eventually assigned the structure of the asymmetric tetramer 5,5′,5″,5‴,6,6′,6″,6‴-octaacetoxy-2,7′:2′,3″:2″,7‴-tetraindolyl (acetylated **10**).

The ^{1}H NMR spectrum (Table 3) exhibited ten resonances in the aromatic proton region, with only one H-2-type proton (δ 7.18), showing coupling with the H-3 (δ 6.47) and NH (δ 10.39) protons on the same terminal unit, designated *d* in the structural formula. The COSY spectrum allowed unambiguous assignment of the four NH protons and the H-3 protons to each of the remaining units, designated *a* and *b*.

Distinct cross-peaks were observed in the ROESY spectrum between the following resonances: H-3_b (δ 6.84) and H-4_b (δ 7.81); H-3_d (δ 6.47) and H-2_d (δ 7.18) and H-4_d (δ 7.64); H-7_a (δ 7.30) and NH_a (δ 10.18); H-7_c (δ 7.43) and NH_c (δ 10.97). These data, coupled with ^{1}H,^{13}C HMBC correlations, allowed straightforward assignment of all protons to the relevant units, and provided conclusive evidence for the 2,3′-mode of coupling of the dimer.

Structures **8**−**10**, along with the previously isolated tetramer **7**, allowed an unprecedented insight into oligomers of **1** past the trimer stage. Notably, for all isolated tetramers markedly nonplanar structures would be anticipated, due to the presence of sterically congested 2,3′- and 3,3′-disubstituted indole units in **8** and **10**, or the 4,4′-linkage with a high torsional barrier in **9**.[20] It is noted, and will be addressed in more detail elsewhere, that the absorption maxima of the acetylated tetramers **8**−**10** are not red-shifted by much compared to the acetylated dimer **3**, suggesting partially interrupted electronic delocalization through the dimer−dimer bonds possibly due to such steric effects. The same arguments can reasonably be applied to predict the geometry of tetramers **8**−**10** in their oxidized state(s), e.g., in their quinonoid forms, with important consequences on the efficiency of π-electron delocalization. Given the central relevance to eumelanin structure, the steric and electronic structures of the oxidized (quinonoid) derivatives of indole oligomers are a crucial issue deserving of special attention in future studies.

The novel bonding modes that derive from the dimer−dimer coupling pathways would accommodate a much greater degree of structural complexity and a broader variety of molecular shapes for higher oligomers than previously believed, supporting the chemical disorder model (but not a true statistical randomness) for the secondary level structures of eumelanins.[21] Clearly, only a few tetramers have been isolated in small amounts, whereby other oligomerization pathways and coupling mechanisms are possible.[22]

Mechanistically, formation of tetramers **7**−**10** discloses unexpectedly different patterns of reactivity of the 5,6-dihydroxyindole system when framed into the dimeric scaffolds **2** and **3**. Oxidation of **2** has recently been shown to proceed via a quinone intermediate featuring a planar extended quinone methide structure with an interring double bond.[19] A similar intermediate, **3**-quinone, may be involved in the oxidative coupling of **3** leading to **8**−**10** (Scheme 2). DFT calculations on **3**-quinone indicated a planar structure with a high LUMO coefficient on the 3-position of the 2-substituted indole unit,[19] suggesting that such a position may display electrophilic reactivity and may be attacked by **3** in a frontier orbital-controlled fashion leading to tetramers **8** and **10**. Whereas formation of **10** would thus resemble the previously described formation of **7**,[18] in the symmetric tetramer **8** the 3-position would serve both as an electrophilic site in **3**-quinone and as a nucleophilic site in the reduced counterpart **3**.

Tetramer **9** may arise by an alternate reaction mode of **3** and its quinone via the 4-positions, but the factors underlying this regiochemistry are less clear. It may be worth noting that the LUMO of **3**-quinone bears a modest coefficient at the reactive 4-position,[19] but whether this has any role remains to be determined.

An overview of possible oxidation pathways of **3** leading to **8**−**10** is given in Scheme 2. The ability of Zn^{2+} to favor

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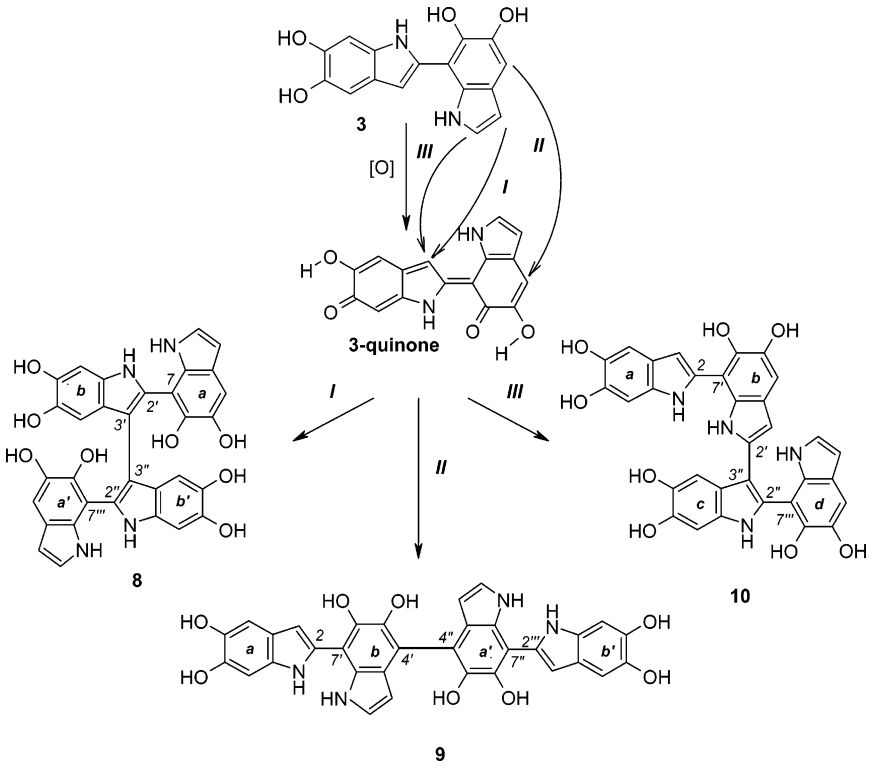
(22) The workup procedure used, based on ethyl acetate extraction, ensured a virtually quantitative recovery of the low molecular weight oligomers. This was checked in each experiment after ethyl acetate extraction by subjecting the residual insoluble fraction of the mixture to the acetylation procedure, leading invariably to ill-defined materials difficult to characterize, with no further isolable species. Accordingly, it can confidently be argued that the workup procedure does not inherently favor linear oligomers over other possible species, e.g., extended aromatic oligomers or bi-coupled species. These latter, if present in sufficient amounts, should not have escaped isolation, as shown in previous studies demonstrating that planar diindolocarbazole cyclotrimers obtained from **1** are easily isolated by the same workup (Manini, P.; d'Ischia, M.; Milosa, M.; Prota, G. *J. Org. Chem.* **1998**, *63*, 7002−7008). Nonetheless, a note of caution about the actual significance of the isolated oligomers is in order until a more complete picture of the oligomerization process and product patterns is available.

SCHEME 2. Proposed Mechanism of Formation of Tetramers 8−10



formation of **10** (route III) over **9** probably reflects an enhanced nucleophilic reactivity at the 2-position of the 7-substituted unit of **3**. This effect is analogous to that observed in the case of dimer **2** and would be the consequence of the partial ionization of the OH group at C6 following chelate formation. It should be emphasized that the role of the metal is only to direct more dimer toward route III, since the same route is operative also under metal-free conditions, albeit to a lesser extent.

An alternative mechanism for **10** based on nucleophilic attack of **3** through the 3-position to the free 2-position of **3**-quinone would be ruled out on the basis of the negligible LUMO coefficient at the 2-position.[19]

Tetramers **7**−**10** offer hints for an improved mechanistic view of the oxidative polymerization of **1**, in which the gradational sequence of oligomeric indole units would be populated at the tetramer level both by species arising by attack of trimers to **1** quinone, likely via 2,4′-and 2,7′-linkages, and by species derived from oxidative coupling of **2** and **3** via 2,3′-, 3,3′-, and 4,4′-linkages. The relative involvement of the oligomer−monomer versus the oligomer−oligomer coupling pathways would depend on several factors, including the rate of substrate oxidation and the extent of the reaction, since it is likely that the dimer coupling route becomes especially significant in the later phases of the process when most of the monomer has been consumed and sufficient amounts of dimers begin to accumulate. The tetramer construction process via dimer−dimer coupling offers significant opportunities to engineer the structures of 5,6-dihydroxyindole oligomers beyond the limits explored to-date and might allow the design and preparation of innovative bioinspired polyindole-based materials for various applications.[14]

A brief comment seems in order here about the relevance of these results to eumelanin structure and properties. Discussion of this issue requires consideration, in addition to **1**, of 5,6-dihydroxyindole-2-carboxylic acid, which plays an important role in melanogenesis.[1−5] The presence of the carboxylic group dramatically affects the reactivity and oxidative coupling patterns of the 5,6-dihydroxyindole system, hindering reactivity at the 3-position. Indeed, an array of tetramers of the 2-carboxylic acid have been described in a previous paper,[20] which exhibit only 4,4′-, 7,7′-, and 4,7′-inter-unit bondings, implying atropisomerism, at least in their reduced forms. It will be of interest, and an important goal for future studies, to investigate how the different coupling behaviors of the indole monomers and their oligomers affect the geometry/planarity of eumelanin building blocks, both in their reduced and oxidized states, and concur to determine the final properties of natural eumelanins also in terms of chemical disorder.

Conclusions

This study represents an important step forward toward an improved model of eumelanin structure. One important outcome is the recognition of the potential role of dimer coupling as an alternative to the trimer−monomer reaction in the mechanisms of tetramer formation. This issue has been overlooked in traditional models of 5,6-dihydroxyindole polymerization and should now be taken into due consideration when attempting to draw consistent models of oligomer chain growth.

Another relevant outcome is the availability of structurally characterized tetramer structures to probe currently held models of supramolecular organization of oligomers in eumelanin-related polymers.[15] A variety of fully planar structures for 5,6-dihydroxyindole oligomers have been proposed[23] which envisage either bicoupling (two bonds between adjacent monomer units) or cyclic scaffolds featuring an inner porphyrin ring.

(23) (a) Kaxiras, E.; Tsolakidis, A.; Zonios, G.; Meng, S. *Phys. Rev. Lett.* **2006**, *97*, 218102/1−218102/4. (b) Stark, K. B.; Gallas, J. M.; Zajac, G. W.; Eisner, M.; Golab, J. T. *J. Phys. Chem.* **2003**, *107*, 3061−3067. (c) Stark, K. B.; Gallas, J. M.; Zajac, G. W.; Eisner, M.; Golab, J. T. *J. Phys. Chem.* **2003**, *107*, 11558−11562.

Though in principle attractive, these structures remain so far hypothetical and have not yet been supported by direct experimental evidence. While waiting for additional oligomers to be isolated and characterized, to depict a more detailed scenario of the later stages of 5,6-dihydroxyindole polymerization, it is advisable that only the structures of tetramers **7**−**10** be used when elaborating predictive or interpretative models of supramolecular oligomer layer aggregation and particle buildup.

Experimental Section

5,6-Dihydroxyindole (**1**)[24] and 5,5′,6,6′tetraacetoxy-2,7′-biindolyl (**3**)[19] were synthesized as previously reported. For general experimental methods and other materials see the Supporting Information.

Oxidation of 3: **Isolation of Tetramers 8**−**10.** A solution of **3** (280 mg, 0.60 mmol) in acetone (5 mL) was taken under a flux of argon and treated with 0.1 M phosphate buffer pH 12 (0.5 L) that had been thoroughly purged with argon. After 2 min the pH of the solution was taken to 8 by addition of 3 M HCl and peroxidase (4.9 U/mL) and 30% H_2O_2 (135 μL, 1.3 mmol) were added sequentially. The reaction mixture turned immediately from pale yellow to deep violet. After 20 s the oxidation was halted by addition of a 5% w/w aqueous solution of sodium dithionite and acidified to pH 4 with 3 M HCl. The reaction mixture was extracted repeatedly with ethyl acetate (3 × 250 mL), and the combined organic layers were dried over sodium sulfate and taken to dryness. The residue was acetylated with acetic anhydride−pyridine 95:5 (v/v) and fractionated by preparative TLC ($CHCl_3$/MeOH 98:2) to give besides residual **3** (R_f 0.70, 50 mg), acetylated **8** (R_f 0.42; R_t 24.5 min; 8 mg, 3% yield, >95% pure by ^{1}H NMR analysis) and acetylated **9** (R_f 0.27, R_t 26.7 min; 10 mg, 4% yield, >95% pure by ^{1}H NMR analysis). In other experiments the oxidation reaction was carried out in the presence of zinc sulfate (534 mg, 1.8 mmol) in 0.5 M TRIS buffer at pH 8.0 as the reaction medium. Workup and fractionation as above afforded besides unreacted **3** (60 mg), acetylated **8** (R_f 0.42; 6 mg, 2% yield) and acetylated **10** (R_f 0.20; R_t 23.8 min; 15 mg, 5% yield, >95% pure by ^{1}H NMR analysis) as colorless oils.

Acetylated **8**: HR ESI$^+$-MS *m*/*z* 949.2186 [M + Na]$^+$, calcd for $C_{48}H_{38}N_4O_{16}Na$ 949.2180; UV λ_{max} (EtOH) 301, 320 (s) nm. ^{1}H and ^{13}C NMR data are reported in Table 1.

Acetylated **9**: HR ESI$^+$-MS *m*/z 949.2175 [M + Na]$^+$, calcd for $C_{48}H_{38}N_4O_{16}Na$ 949.2180; UV λ_{max} (EtOH) 313, 337 nm. ^{1}H and ^{13}C NMR data are reported in Table 2.

Acetylated **10**: HR ESI$^+$-MS *m*/z 949.2178 [M + Na]$^+$, calcd for $C_{48}H_{38}N_4O_{16}Na$ 949.2180; UV λ_{max} (EtOH) 313 nm. ^{1}H and ^{13}C NMR data are reported in Table 3.

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Supporting Information Available: General methods, and ^{1}H, ^{13}C, ^{1}H, ^{1}H COSY, ^{1}H, ^{13}C HSQC, ^{1}H, ^{13}C HMBC, ROESY NMR spectra for acetylated **8**, **9**, and **10**. This material is available free of charge via the Internet at http://pubs.acs.org.

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