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The First 5,6-Dihydroxyindole Tetramer by Oxidation of 5,5′,6,6′-Tetrahydroxy-2,4′-biindolyl and an Unexpected Issue of Positional Reactivity en Route to Eumelanin-Related Polymers

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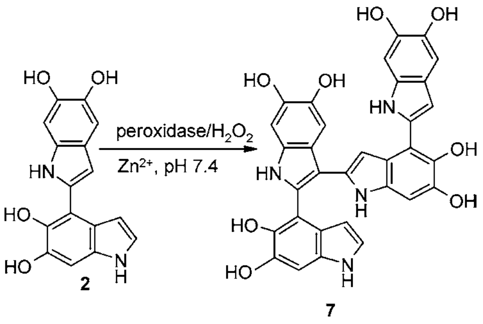
ABSTRACT



The first tetramer of the eumelanin precursor 5,6-dihydroxyindole has been obtained, as the acetyl derivative, by peroxidase/H_2O_2-induced oxidative coupling of 5,5′,6,6′-tetrahydroxy-2,4′-biindolyl (2) in the presence of Zn^{2+} ions. The tetramer, 5,5′,5′′,5′′′,6,6′,6′′,6′′′-octaacetoxy-2,4′:2′,3′′:2′′,4′′′-tetraindolyl (acetylated 7), incorporates an unprecedented 2,3′-biindolyl substructure suggestive of a different positional reactivity of the 5,6-dihydroxyindole system when framed into a dimeric scaffold.

Chemical or enzymatic oxidation of 5,6-dihydroxyindole (**1**) leads to the rapid deposition of a black particulate solid by way of a complex oligomerization/polymerization process that is believed to model the later stages of the buildup of eumelanins, the key functional components of the human pigmentary system.[1] Although the oxidation chemistry of 5,6-dihydroxyindoles has challenged generations of organic chemists,[2] the coupling mechanisms and the major oligomer intermediates have remained largely uncharted. Yet, elucidation of this chemistry is important for unraveling the basic architecture of eumelanin particles and for understanding the origin of their unique physicochemical properties, such as broad band UV−visible absorption, the persistent EPR signal and semiconductor threshold switching.[3−5]

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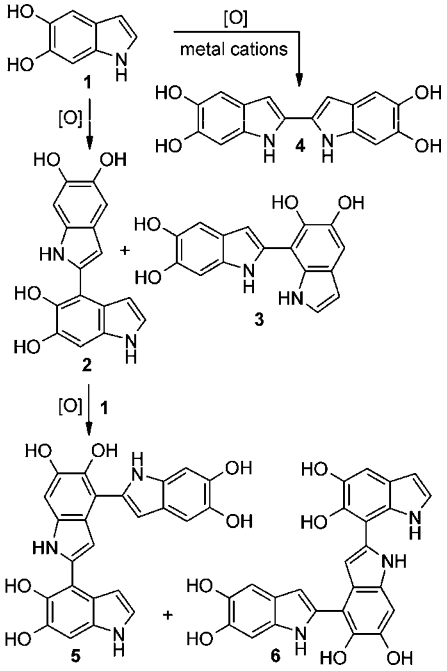
Previous work in our laboratory delineated the early stages of the polymerization process up to the formation of trimers (Scheme 1). The structures of the main oligomers isolated, i.e., **2**−**6**, disclosed a selective mode of coupling of **1** through 2,4′- and 2,7′-linkages.[2,6] These reactivity patterns are proposed to arise from attack of the nucleophilic 2-position of the parent indole to the electrophilic 4- and 7-positions of a transient 5,6-indolequinone intermediate. Symmetric 2,2′-coupling leading to dimer **4** is prevalent in the presence of transition metal ions,[7] e.g., Zn^{2+} and Ni^{2+}, probably as a result of chelate formation with **1** quinone affecting its positional reactivity.

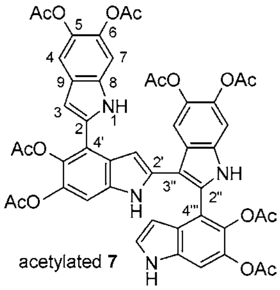
Scheme 1. Main Oligomers Isolated by Oxidation of **1**



Whether the positional reactivity of the parent indole can be extended to the oligomers is unknown and represents a fundamental gap in the present understanding of the secondary structure of the pigment particles. The regiochemistry of 5,6-dihydroxyindole coupling may affect the planarity of the resulting oligomers and may be critical for efficient π-electron delocalization and π-stacking in the final oligomer aggregation process. The isolation and structural characterization of 5,6-dihydroxyindole tetramers and higher oligomers is therefore mandatory for building realistic models for the secondary structural organization of eumelanin-related pigments.

Previous efforts to characterize tetramers of **1** were hindered by the marked complexity of oxidation mixtures, comprising the gradational range of poorly soluble species generated at various levels of oligomerization. A viable strategy to circumvent these difficulties involves oxidation of dimers,[8] e.g., **2**, which can be obtained by oxidation of **1**. The added value of this approach is the possibility to probe whether the observed 2,4′- and 2,7′-mode of coupling of the indole is maintained at the dimer level. After a careful survey of oxidizing agents and reaction conditions, a procedure for the oxidative coupling of **2** was eventually developed. This involved peroxidase/H_2O_2 oxidation of **2** in the presence of Zn^{2+} ions in β-hydroxy-4-(2-hydroxyethyl)-1-piperazinepropanesulfonic acid (HEPPSO) buffer at pH 7.4. Reduction of the mixture with sodium dithionite, acetylation of the fraction extractable with ethyl acetate to improve both stability and chromatographic behavior of the products, and TLC fractionation of the resulting mixture eventually led to the isolation of a single product.

This was obtained as a pale yellow glassy oil, soluble in methanol and acetone, and slightly soluble in chloroform. ESI-MS analysis showed pseudomolecular ion peaks $[M + H]^+$ and $[M + Na]^+$ at m/z 927 and 949, indicating a tetramer of **1**. The UV spectrum showed a broad maximum around 315 nm with a shoulder at 339 nm. The ^{1}H NMR spectrum exhibited 10 signals in the aromatic region, suggesting a nonsymmetric structure. One of these at δ 7.30, correlating with NH and H-3 protons at δ 10.55 and 6.28, respectively, was attributed to an H-2 proton. The presence of only three resonances at δ values lower than 7 coupled with NH protons (COSY evidence), showed moreover that one of the 3-positions was substituted. Close scrutiny of ^{1}H, ^{1}H COSY, ^{1}H, ^{13}C HSQC, ^{1}H, ^{13}C HMBC, and ROESY spectra eventually allowed formulation of the compound as the asymmetric tetramer 5,5′,5″,5‴,6,6′,6″,6‴-octaacetoxy-2,4′:2′,3″:2″,4‴-tetraindolyl (acetylated **7**).



acetylated **7**

NMR data of acetylated **7** with complete resonance assignments are listed in Table 1. The relatively upfield shift of the H-3‴ proton at δ 6.28, as compared to the chemical shift of the analogous H-3′ proton in acetylated **2** (δ 6.75),[6] indicates an average permanence of that proton in the shielding cone of the prospicient indole ring. Similarly, the N-H′ (δ 10.11) and H-7′ (δ 7.17) protons seem to experience the shielding influence of the terminal 4-substituted indole unit.

Confirming structural information came from the ROESY spectrum, showing cross-peaks between the H-3′ signal at δ 6.78 and the H-4″ and N-H proton signals at δ 7.79 and

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Table 1. NMR Data of Acetylated **7**[a,b]

position[c]	^{1}H	^{13}C	position[c]	^{1}H	^{13}C
1	10.45		1″	10.92	
2		135.1	2″		132.3
3	6.74	103.4	3″		108.4
4	7.40	113.9	4″	7.79	113.7
5		137.3	5″		138.0
6		139.0	6″		139.6
7	7.37	106.1	7″	7.43	106.5
8		134.6	8″		134.6
9		126.7	9″		124.7
1′	10.11		1‴	10.55	
2′		135.5	2‴	7.30	127.9
3′	6.78	100.5	3‴	6.28	102.0
4′		118.1[d]	4‴		117.3[d]
5′		138.7	5‴		135.7
6′		139.6	6‴		139.0
7′	7.17	105.8	7‴	7.50	107.4
8′		134.3	8‴		133.7
9′		126.0	9‴		126.7

[a] Spectra run in acetone-d_6, chemical shift values given in ppm. [b] Numbering as shown in structural formula. [c] ^{1}H and ^{13}C resonances of acetyl groups were in the range 1.9−2.4, and 19−20/168−170 ppm, respectively. [d] Interchangeable.

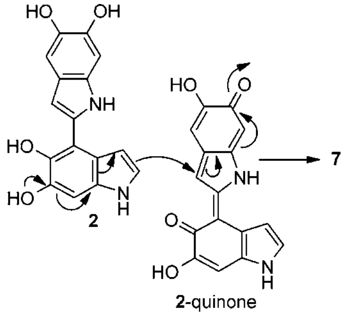
10.45, respectively, and between the H-4″ proton signal and the N-H′ proton signal at δ 10.11.

TLC analysis of oxidation mixtures of **2** in the absence of added Zn^{2+} after acetylation showed the presence of some acetylated **7** along with 3,5,5′,6,6′-pentaacetoxy-2,4′-biindolyl recently described by oxidation of **2**[9] and a number of other species that could not be elucidated.

This observation suggests that metal ions enhance formation of **7** over alternative oxidative coupling channels of **2**. A substantial formation of acetylated **7** was also observed by autoxidation of **2** in HEPPSO buffer at pH 7.4 under a flux of oxygen in the presence of Zn^{2+}, followed by the usual workup.

Recent work based on an integrated chemical, pulse radiolytic, and quantum chemical approach has allowed unprecedented insight into the structures of the transient species formed by oxidation of **2**.[9] An unstable two-electron oxidation product was identified that was assigned a planar extended quinone methide structure (referred to as **2**-quinone) featuring an interring double bond.[9] **2**-Quinone was suggested to exhibit electrophilic reactivity at the 3-position of the 2-substituted indole moiety, as supported by the recent isolation of the 3-hydroxy derivative of **2**.[9] This reactivity was rationalized on the basis of the intrinsic structural properties of the extended quinone methide and a significant LUMO coefficient at that site.[9] Consistent with that view, formation of **7** may now be proposed to reflect a related reaction pathway of **2**-quinone depicted in Scheme 2. In this route, the extended quinone methide of **2** would undergo nucleophilic attack at the 3-position by the reduced dimer **2** via the 2-position of the 4-substituted indole ring. The alternative mechanism, i.e., nucleophilic attack of dimer **2** via the 3-position to the free 2-position of **2**-quinone would be ruled out on the basis of the negligible LUMO coefficient at the 2-position,[9] assuming a frontier orbital-controlled coupling. In this reaction, the role of transition metal cations is probably related to quinone methide stabilization and/or chelate formation at the catechol moiety of **2** enhancing its nucleophilic reactivity at the 2-position.

Scheme 2. Proposed Origin of Tetramer **7** by Oxidation of **2**



Tetramer **7** may represent a new important tool to probe the classic "stacked sheet" model of supramolecular organization of 5,6-dihydroxyindole oligomers in eumelanin-related polymers.[4] This model, which envisages planes of oligomers (4−6 unit protomolecules) stacked in the z-axis with spacing characteristic of graphene sheets, has so far been founded on purely hypothetical tetramer-hexamer structures devoid of experimental support. Alternative eumelanin protomolecules have recently been proposed[10] based on partially oxidized cyclic 5,6-dihydroxyindole oligomers featuring an inner porphyrin ring. However, though in principle attractive, the proposed oligomeric scaffold is largely speculative and awaits direct experimental verification.

Structure **7** raises an unexpected issue relating to the change in the positional reactivity of the 5,6-dihydroxyindole unit when framed in the biindolyl system of **2**. It follows that any realistic model for oligomer chain growth should not be limited to sequential steps of attack of **1** to the growing oligomers but must also take into account the accumulation of **2** as oxidation of **1** progresses and its competing mode of coupling via the 3-position. This increases the degree of complexity expected for the oxidation mixtures of **1** far beyond the early predictions based on sequential 2,4′- or 2,7′-coupling steps and would further support the chemical disorder model for secondary level structures of 5,6-dihydroxyindole-derived pigments.[4,5]

To summarize, we have succeded in isolating and structurally characterizing the first tetramer of **1**, which provides an interesting addition to the currently scanty literature on indole tetramers.[8,11] Despite the relatively low yield (2%) and poor mass balance, it represents a most significant achievement

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in 5,6-dihydroxyindole chemistry because (a) the availability of a tetramer may now make it possible to probe on a more solid ground the classic models of eumelanin structure, based on hypothetic oligomeric structures; (b) the observed coupling of the 2,4′-biindolyl through the 3-position is unusual and must now be considered as a novel possible mode of coupling of indole units en route to eumelanin-like polymers; (c) it is now clear that more realistic models of 5,6-dihydroxyindole polymerization must take into due account the oxidative coupling of dimers, which has so far been overlooked; and (d) the structure of the new tetramer provides additional support to the recently proposed involvement in the polymerization process of the unusual extended quinone methide structure for **2**-quinone.[9]

Availability of tetramer **7** in better yields, a goal currently pursued in our laboratory, might provide a novel building unit for more ordered bioinspired materials with improved optical and electronic properties compared to the highly heterogeneous natural eumelanins. In this regard, the potential of polyindoles as electroconductive materials for various applications, e.g., as sensors, is of relevance.

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Supporting Information Available: Experimental procedures, ^{1}H, ^{13}C, ^{1}H, ^{1}H-COSY, ^{1}H, ^{13}C-HSQC, ^{1}H, ^{13}C HMBC, and ROESY NMR spectra of acetylated **7**. This material is available free of charge via the Internet at http://pubs.acs.org.

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