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Cyclic Structural Motifs in 5,6-Dihydroxyindole Polymerization Uncovered: Biomimetic Modular Buildup of a Unique Five-Membered Macrocycle

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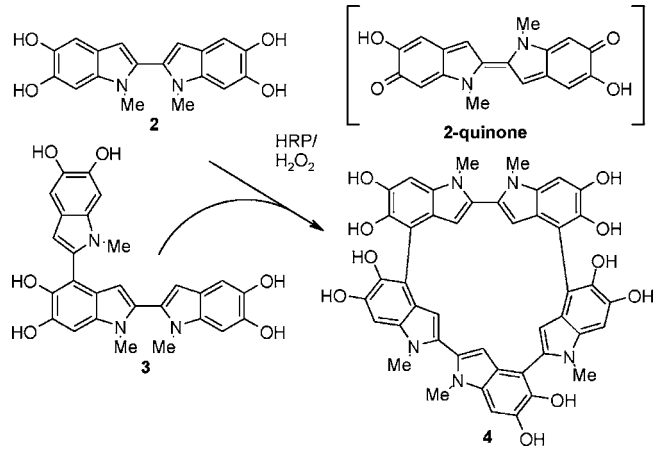
ABSTRACT



An unprecedented 5,6-dihydroxyindole macrocycle (4) featuring a rigid twisted backbone was obtained by biomimetic oxidative cross-coupling of the 2,2′-biindole 2 and triindole 3. A putative reaction intermediate, 2-quinone, was detected and characterized by pulse radiolysis and DFT calculations. Discovery of 4 indirectly supports for the first time theoretically predicted cyclic structural motifs as potential eumelanin building blocks.

Indole-based macrocyclic systems attract increasing interest in relation to their conformational and self-association properties, stacking interactions, spectroscopic features, cavity shape, and performance as ligands or ion-sensing scaffolds.[1] Surprisingly, however, no macrocyclic structure built solely on indole units appears to have yet been described.

The cyclic 5,6-dihydroxyindole oligomer **1**, consisting of four units in an arrangement that contains an inner porphyrin ring,[2] has recently been proposed on a theoretical basis to account for the structural and spectral properties of eumelanins, the black insoluble semiconductor biopolymers of

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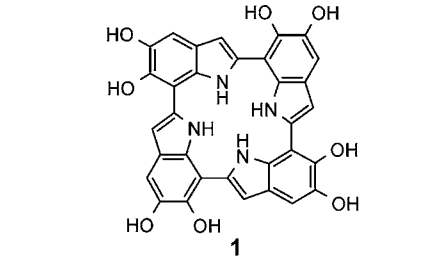
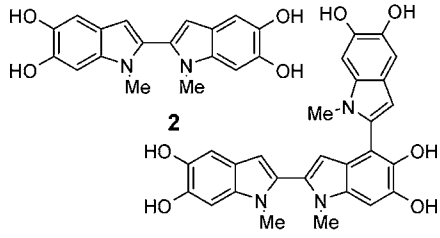
human skin, hair, and eyes.[3] Unfortunately, verification of this hypothesis has been hampered by the notorious difficulties in the investigation of the oxidative polymerization of 5,6-dihydroxyindoles, the key step in eumelanin synthesis.[1,4] The characterization of cyclic 5,6-dihydroxyindole based oligomers has remained therefore a theoretical curiosity as well as an experimental challenge in an intriguing field of research at the crossroads of biology and materials science.[3c,4] Herein, we report the discovery of the first 5,6-dihydroxyindole macrocycle.



1

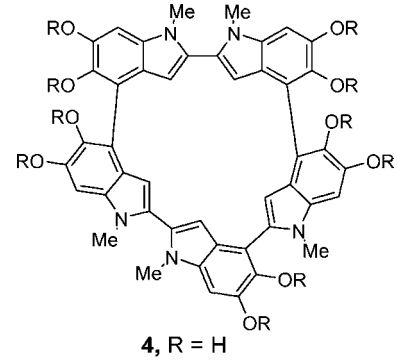


2

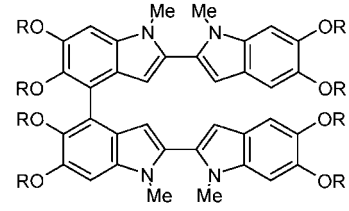
3

This remarkable finding was the outcome of a study aimed at extending our oligomer coupling strategy toward higher 5,6-dihydroxyindole-based scaffolds[5] to dimer **2** and trimer **3**,[4,6] which share the hitherto unexplored 2,2′-biindole system. Compounds **2** and **3** are easily accessible from 5,6-dihydroxy-1-methylindole, a major oxidation product of adrenaline (epinephrine),[1,4] and exhibit chromatographic properties better than those of most 5,6-dihydroxyindole oligomers.[4,6]

Quite interestingly, biomimetic oxidation of an equimolar mixture of **2** and **3** with peroxidase/H_2O_2 at pH 7.4 followed by reductive treatment and acetylation of the ethyl acetate extractable fraction led to the formation of a single isolable product, along with abundant eumelanin-like material. Repeated chromatographic steps eventually afforded small amounts of the product, which displayed pseudomolecular ion peaks (ESI-MS(+)) at *m*/*z* 1226 ($[M + H]^+$) and 1248 ($[M + Na]^+$), i.e., two mass units lower than those expected for a pentamer. The ^{1}H NMR spectrum indicated only 10 singlets in the aromatic region, and the ^{13}C NMR spectrum displayed consistently 10 sp^2 C−H signals. These resonances were attributed to the presence of five H3-type protons and five H7-type protons on the basis of HSQC and HMBC spectra and comparison with available NMR data for 5,6-dihydroxyindole oligomers[4−6] (see Supporting Information). Accordingly, the product was unambiguously identified as the cyclopentamer **4** (decaacetyl derivative **4**-*Ac*). Compound **4** is the highest 5,6-dihydroxyindole oligomer so far isolated and, to the best of our knowledge, the first macrocycle composed of five *N*-methyl-5,6-dihydroxyindole units.[7] Its isolated yield (ca. 5%) is comparable to that of most 5,6-dihydroxyindole oligomers[5a,b] and is significant considering that the building of cyclic structures, though entropically favored, has to compete against the distribution of the linear oligomers.



4, R = H
4-*Ac*, R = OAc



5, R = H
5-*Ac*, R = OAc

The structural features of **4** were explored at the DFT level of theory,[8] using the hybrid PBE0 functional[9] in conjunction with the 6-31+G(d,p) basis set.[10] All structural minima are

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(3) For basic information on eumelanin chemistry, see: Prota, G. *Melanins and Melanogenesis*; Academic Press: San Diego, CA, 1992. Recent advances are summarized in (a) Simon, J. D.; Hong, L.; Peles, D. N. *J. Phys. Chem. B* **2008**, *112*, 13201–13217. (b) Simon, J. D.; Peles, D. N.; Wakamatsu, K.; Ito, S. *Pigm. Cell Melanoma Res.* **2009**, *22*, 563–579. (c) d'Ischia, M.; Napolitano, A.; Pezzella, A.; Meredith, P.; Sarna, T. *Angew. Chem., Int. Ed.* **2009**, *48*, 3914–392. (d) Watt, A. A. R.; Bothma, J. P.; Meredith, P. *Soft Matter* **2009**, *5*, 3754–3760.

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(6) For a detailed spectral characterization of **1** and its oligomers, see: Corradini, M. G.; Napolitano, A.; Prota, G. *Tetrahedron* **1986**, *42*, 2083–2088.

(7) The only known cyclic structures containing solely indole units are star-shaped diindolocarbazoles. For 5,6-dihydroxyindole-derived cyclotrimers, see: Manini, P.; d'Ischia, M.; Milosa, M.; Prota, G. *J. Org. Chem.* **1998**, *63*, 7002–7008.

of C_1 symmetry and therefore occur as pairs of conformational enantiomers. Equilibration of the enantiomeric pairs would require a concerted inversion at the C4(A)−C4(E) and C2(E)−C4(D) interring dihedrals, which cannot be achieved without severe strain of the macrocyclic ring (as a matter of fact, steric interactions at the biphenyl-type 4,4′-bonds suffice to induce atropisomerism even in linear biindoles[11]). Within each series, two main conformational basins can be identified: in the first one, the A and B indole rings assume an almost perpendicular disposition (and the C and D units are much less skewed); the opposite holds in the second basin. Additional conformational complexity is connected to the details of the pattern of intramolecular O−H···O hydrogen bonds (see Supporting Information). Such a complex conformational behavior suggests an inherent difficulty to efficient supramolecular packing, which may well account for our failure to grow crystals suitable for X-ray analysis. Figure 1 shows the most stable conformer identified.

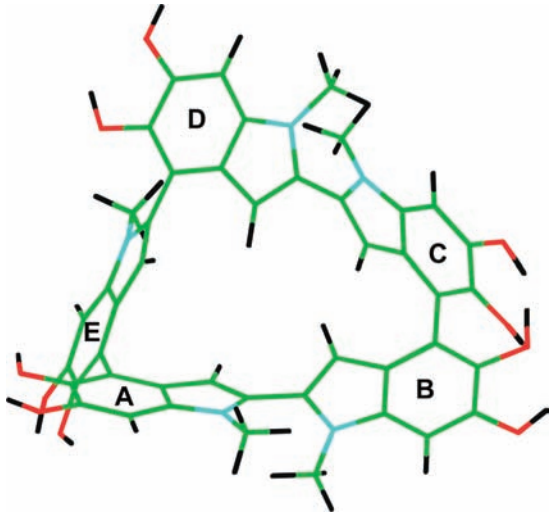
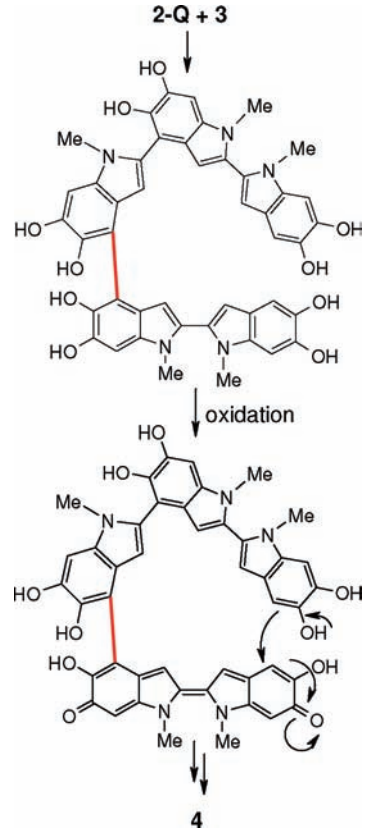


Figure 1. Most stable conformer of **4**.

Compound **4** conceivably arises by sequential cross-coupling−cyclization of **2** and **3** via catechol−quinone interactions.[4,5c] A DFT investigation was therefore carried out on **2** and **3**, both in their reduced and oxidized (quinonoid) forms. The most stable conformation of **2**-quinone (**2Q**, Figure 1) features an interunit double bond with a N−C2−C2′−N′ dihedral angle of 165°, consistent with previous results on the *N*-unsubstituted 2,2′-dimer.[12] On the other hand, the most stable tautomer of the quinone trimer (**3Q**) possesses only one inter-ring double bond on the 2,2′-biindole moiety (see Supporting Information). The free energy change for the equation **2** + **3Q** ⇆**2Q** + **3** was estimated as −1.19 kcal/mol (in vacuo) and as −0.12 kcal/mol following introduction of solvent effects by means of the polarizable continuum model (PCM)[13] in its united atom for Hartree−Fock (UAHF) parametrization.[14]

On this basis, it was suggested that formation of **4** involves generation of **2**-quinone and its coupling with **3** via a 4,4′-type bonding (Scheme 1). The rigid 2,2′-biindole moieties in the resulting pentamer would bring the two termini into close proximity upon folding, thus favoring intramolecular cyclization after an additional oxidation step. Support to the main mode of reaction of **2** via the 4-position was obtained in separate experiments in which dimer **2** was found to give on oxidation the novel 2,2′:4′,4″:2″,2‴-tetraindole **5**, isolated

Scheme 1. Representative Mechanism of Formation of **4**[a]



[a] Newly formed bonds are in red.

(8) All quantum-mechanical calculations were carried out with *Gaussian, Development Version, Revision F.01*; Frisch, M. J. et al.; Gaussian, Inc.: Wallingford, CT, 2006 See full reference in Supporting Information.

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as the acetyl derivative (**5**-*Ac*). Preferential reactivity of **2** via the 4-position may be largely determined by steric factors, including the effect of the *N*-Me groups hindering the 7-position relative to the parent 5,6-dihydroxyindole.[4]

Some insights into the **2**-quinone was gained by pulse radiolytic oxidation of **2** (Figure 2), using the same methods

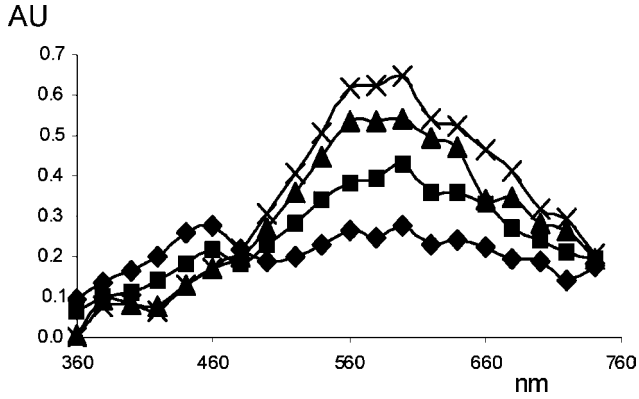


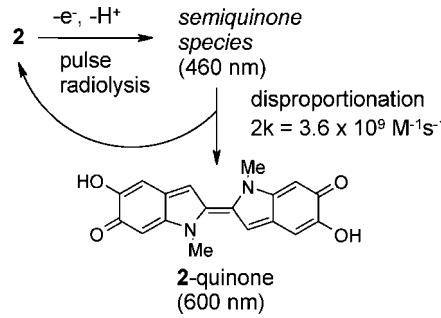
Figure 2. Changes in absorption at various times after pulse radiolysis of an N_2O-saturated aqueous solution of dimer **2**: (◆) 200 μs, (■) 1060 μs, (▲) 4060 μs, (×) 8980 μs.

as previously applied to the corresponding unmethylated 2,2′ dimer.[12] The initial product formed 200 μs after the pulse was the semiquinone, with a maximum around 460 nm (for the reaction of **2** with $Br_2^{-\bullet}$, $k = 3.5 \times 10^8$ M^{-1} s^{-1}). The transient species decayed by second order kinetics ($2k = 3.6 \times 10^9$ $M^{-1}s^{-1}$) to a strongly absorbing chromophore ($\lambda_{max} = 600$ nm, $\varepsilon = 5.3 \times 10^4$ M^{-1} cm^{-1}) which was attributed to the quinone based also on the good agreement with the computed absorption maximum (584 nm) of the most stable conformer of **2Q** in water (see Supporting Information). Quinone formation apparently involves disproportionation of the semiquinone, as suggested by the isosbestic point at 480 nm and second order kinetics (Scheme 2).

No detectable self-coupling products (e.g., **5** or hexamers) were formed by co-oxidation of equimolar amounts of **2** and **3**. This would point to prevalent cross-coupling pathways of **2** and **3**, with the necessary caution imposed by the poor mass balance and the abundant polymeric material interfering with product analysis.

In conclusion, we have disclosed the generation under biomimetic conditions of the first cyclic 5,6-dihydroxyindole oligomer. The importance of this finding lies mainly in the

Scheme 2. Generation of **2**-Quinone by Disproportionation of the Semiquinone Radical



discovery and characterization of an unprecedented indole-based structural motif of potential academic and practical interest. For example, the modular assembly of **4** by 2,2′-biindole-containing scaffolds highlights the bonding patterns and geometries required to construct a five-membered indole macrocycle. The spontaneous oligomer−oligomer coupling/cyclization pathway suggests, moreover, that macrocyclic structures like those recently hypothesized[2] are not entirely devoid of foundation and may contribute to the patterns of structural diversity generated during 5,6-dihydroxyindole polymerization.[3c,15] The great versatility of 5,6-dihydroxyindole in terms of positional reactivity would in principle allow for the formation of a diverse range of cyclic structures during eumelanin buildup.

Acknowledgment. M.dI. acknowledges support from Italian MIUR, PRIN 2008 project. All computations were performed on the large-scale computer facilities of the VILLAGE network (http://village.unina.it.)

Supporting Information Available: Experimental procedures, ^1H and ^{13}C NMR, ^1H,^{13}C HSQC, ^1H,^{13}C HMBC, ROESY of **4**-*Ac* and **5**-*Ac*; inter-ring dihedrals, relative energies and optimized structures of the main conformers/tautomers of **4** and of the fully reduced (QH_2) and quinonoid (Q) forms of **2** and **3**. Computed electronic spectrum of the quinonoid form of **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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