# Effect of Dimerization on Vibrational Spectra of Eumelanin Precursors<sup>†</sup>

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#### ABSTRACT

We have synthesized a compound ideally suited to the study of structure-function relationships in eumelanin synthesis. N-methyl-5-hydroxy-6-methoxy-indole (MHMI) has key functional groups strategically placed on the indole framework to hinder binding in the 2, 5, 6 and 7 positions. Thus, the dimer bound exclusively in the 4-4' positions was isolated and characterized. In order to study the difference in vibrational structure between the MHMI monomer and dimer, Raman spectra were acquired of both compounds, as well as indole, indole-2-carboxylic acid and 5,6-dihydroxyindole-2-carboxylic acid (DHICA). Peaks were assigned to particular vibrational modes using B3LYP density functional theory calculations, and experimental and theoretical spectra displayed good agreement. Addition of functional groups to either benzene or pyrrole rings in the indole framework impacted vibrational spectra attributed to vibrations in either ring, and in some cases, peaks appearing unchanged between two compounds corresponded to different contributing vibrations. Dimerization resulted in an expected increase in the number of vibrational modes, but not a significant increase in the number of apparent peaks, as several modes frequently contributed to an individual observed peak. Comparison of spectral features of the monomer and dimer provides insight into eumelanin photochemistry, but final conclusions depend on the planarity of oligomeric structure in vivo.

#### INTRODUCTION

Eumelanin is a crucial skin pigment responsible for camouflage and photoprotection. Its photoprotective role is largely due to its broadband absorption profile with high absorptivity in the UV region and its extremely low radiative quantum yield (1). Because of its prevalence in the animal world and its importance in protection against solar radiation, the pathways responsible for the nonradiative dissipation of energy have been of great interest (2-5). These pathways are likely linked to aggregation and increased coupling between the electronic and vibrational states. Although eumelanin has long been understood to be composed of the indolic compounds, 5,6-dihydroxyindole (DHI) and 5,6-dihydroxyindole-2-carboxylic acid (DHICA), only recently has it become apparent that their arrangement is not uniform. Several oligomeric structural arrangements have been shown to be consistent with observed phenomena (6-8)and recent studies of DHICA oligomers have provided great insight into possible intermediates involved in eumelanin formation in vivo (9,10). Indeed, many of the optical properties of eumelanin have been explained by the presence of differentsized aggregates of oligomers, each having different structural arrangements, within the eumelanin compound (11-13), and this chemical disorder model has gradually become the dominant paradigm for understanding eumelanin structure (14).

As a result of the inherent disorder in eumelanin, its vibrational spectra are broad and without narrow, distinct peaks (15,16). Thus, most studies of the vibrational structure of eumelanin have been aimed at determination of eumelanin content in tissue samples and *in vivo* diagnosis of cancerous states, rather than understanding of eumelanin structure (16–18). Comparison of absorbance and emission spectra to those of its constituent oligomers has been instructive toward understanding the structure of eumelanin, and comparison of corresponding vibrational spectra can be equally instructive.

A number of studies have characterized the vibrational structure of indole, indole-2-carboxylic acid (ICA) and DHICA using infrared and Raman spectroscopy as well as inelastic neutron scattering (19–21). However, there has been very little attention paid to the vibrational structure of intermediate compounds—that is, indolic dimers and oligomers. A comparison of indolic monomers and dimers can be useful because it sheds light on changes in vibrational spectra during eumelanin synthesis. The current work builds on previous studies by examining changes in vibrational spectra during dimerization. Using DHICA in such a study is problematic because DHICA is able to bind in the 4, 7 and

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possibly even the 3 positions, yielding several structurally and possibly optically distinct compounds (9). Therefore, we have synthesized a new compound, *N*-methyl-5-hydroxy-6methoxy-indole (MHMI), which is ideally suited to such studies (22). Functional groups in MHMI have been strategically placed on the indole framework, sterically hindering binding to the 2 and 7 positions and allowing dimerization via 4-4' coupling alone. We examine the near-IR Raman spectra of MHMI and MHMI dimer, as well as DHICA, indole and ICA for comparison. Comparing spectra with calculations of the vibrational modes will provide insight into the effect of dimerization on the vibrational spectra of indolic compounds.

#### MATERIALS AND METHODS

*Compound synthesis.* 5-Benzyloxy-6-methoxyindole purchased from Sigma Aldrich was *N*-methylated in dry DMF using sodium hydride as the base and methyl iodide as the alkylating agent. The reaction was carried out under an atmosphere of argon for 2 h. The *N*-methyl-5-benzyloxy-6-methoxyindole thus obtained was subjected to hydrogenolysis for 3 h in ethyl acetate-acetic acid solution, using 5% palladium on carbon as catalyst to furnish *N*-methyl-5-hydroxy-6-methoxyindole (MHMI) monomer. The crude product thus obtained was purified over a column of silica using chloroform as eluant, and then crystallized from dichloromethane-hexane.

To synthesize the MHMI dimer, 0.65 g of MHMI was dissolved in ethanol (45 mL) and stirred under argon. Potassium carbonate (1 g in 6 mL H<sub>2</sub>O and 30 mL EtOH) was added and the contents left stirring overnight under argon, then cooled in ice. The red-black solution was acidified with HCl and extracted with chloroform. The organic layer was washed with brine, dried and the solvent removed to obtain 0.66 g dark solid. The solid was washed with toluene, and dried under vacuum to furnish 0.6 g dark solid. This solid was treated with ~40 mL warm ethyl acetate, stirred under argon for 0.5 h, filtered and the solvent removed to obtain 0.52 g of a red foam. Column purification (SiO<sub>2</sub>, CHCl<sub>3</sub>) afforded the pure monocoupled dimer as an off-white solid in 13% yield. Recrystallization from ethanol-chloroform afforded yellow micro crystals: mp 200°C (decomposes); <sup>1</sup>H NMR (500 MHz, CD<sub>3</sub>OD)  $\delta$  3.77 (6 H, s, NCH<sub>3</sub>), 3.98 (6 H, s, OCH<sub>3</sub>), 5.82 (2H, m, H-3,3', 6.86 (2H, d, J = 3.0, H-2,2'), and 6.99 (2H, s, H-7,7'). <sup>13</sup>C NMR (75 MHz, CD<sub>3</sub>OD)  $\delta$ 33.05, 56.94, 93.19, 102.32, 115.74, 123.36, 127.74, 132.39, 139.24, and 147.28; m/z [ESI<sup>+</sup>] 353 (MH<sup>+</sup>, 40%), 375 (MNa<sup>+</sup> 100%) C<sub>20</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub>Na requires 375.1321. Found: 375.1323; Anal. Calcd. for C<sub>20</sub>H<sub>20</sub>N<sub>2</sub>O<sub>4</sub>: C 68.17%, N 7.95%, H 5.72%. Found: C 67.99%, N 7.86%, H 5.75%. This dimer is a monocoupled species from MHMI, coupled in the 4,4' positions, and the two rings are staggered because of steric interactions. The full crystal structure and other characterization details will be published separately.

Experimental Raman spectroscopy. Raman spectra were acquired for indole, ICA, MHMI monomer and dimer, and DHICA in the solid state. Raman spectra were acquired for the compounds in solid form using a Raman system built in-house (Ontario Cancer Institute, Toronto). A titanium:sapphire laser (Spectra Physics model 3900S) pumped by 5 W CW DPSS green laser at 532 nm (diode-pumped solid-state Verdi V5 laser; Coherent) was used as an excitation source tuned at 785 nm. Excitation light was passed through a holographic bandpass filter (Kaiser Optical Systems, Ann Arbor, MI) with greater than 90% transmission of s-polarized light at 785 nm and a bandwidth of less than 2 nm. The rejected light was used to monitor the wavelength of the excitation laser using a wavemeter WA-1600 (Burleigh Instruments, Inc., Fishers, NY). Scattered light was collected through a collection system designed in-house and coupled into a custom-made fiber optic "Spot to Slit line" type bundle assembly consisting of 43 CeramOptec P/N UV105/115/140P28 fibers with NA of 0.28 ending in a line of approximately 0.14 mm × 6.02 mm in the active area (CeramOptec Industries, Inc., East Longmeadow, MA). The scattered light was analyzed with a high-throughput holographic spectrograph (Holospec f/1.8; Kaiser Optical Systems).

The spectrograph was equipped with a liquid N<sub>2</sub> cooled CCD detector array (Series 200; Photometrics Ltd., Tuscon, AZ based on EEV CCD15-11 1024×256 CCD chip [Marconi Applied Technologies, Chelmsford, Essex, UK]). The Holospex grating we used had a range from 35 to 3556 cm<sup>-1</sup>. A holographic notch filter was placed in the spectrometer to reject the laser line (bandwidth of 300 cm<sup>-1</sup>, optical density of >6 at the excitation wavelength and >80% transmission for scattered light). Acquired spectra were corrected for variations in detector sensitivity and a baseline correction was applied.

Calculated Raman spectra. Minimum energy geometries, harmonic vibrational frequencies and Raman intensities for the MHMI monomer and dimer, DHICA, indole and ICA were computed using B3LYP hybrid density functional theory (23) and a 6-31(d) basis set (24) within the Gaussian03 software suite (25). Frequencies were scaled by a factor of 0.97, which is within the range of scaling factors suitable for the methods used (26). A uniform Lorentzian broadening of 5 cm<sup>-1</sup> was applied to generate the simulated spectra, which were then normalized by visual inspection against the experimental data. Cartesian coordinates, B3LYP/6-31(d) ground state energies and complete lists of frequencies and Raman intensities are provided as Supplemental Material.

# RESULTS

Validation of the spectrometer used here was performed by comparison of indole and ICA Raman spectra (Fig. 1a,b) with those available from the literature (27,28). The frequency positions of peaks shown in the spectra acquired by the current system matched those of previously acquired Raman spectra precisely (data not shown). Comparison of the Raman spectra with previous neutron scattering studies of indole (21) showed that only the in-plane vibrations were evident in the Raman spectra. This is to be expected, as only those vibrations corresponding to a change in the molecular polarizability are Raman active. Out-of-plane vibrations would require a large displacement from the molecular plane in order to exhibit a detectable change in the polarizability.

Addition of the carboxylic acid group to the 2 position of the indole results in significant shifting of vibrational peaks. Recent inelastic neutron scattering studies together with ab initio calculations have shown that virtually all peaks between 400 and 1000 cm<sup>-1</sup> in both indole and ICA are combination bands incorporating several vibrational modes, and that the carboxylic acid group primarily adjusts the bands corresponding to vibrations in the pyrrole ring (21). Similarly, addition of hydroxy and methoxy groups to the 5 and 6 positions can be expected to affect vibrations in the benzene ring.

Raman spectra for the MHMI monomer and dimer are shown in Fig. 1c,d. In order to attribute these peaks to molecular vibrations, B3LYP density functional theory calculations were performed for all compounds, and only the dominant contributing modes are listed in Tables 1-3. Calculated spectra to which a broadening term has been applied are shown in the figures above the experimental data, and correspond excellently with the experimentally acquired data. The dominant peak in indole at  $762 \text{ cm}^{-1}$  is due to stretching of the benzene ring primarily and its position is largely unaffected by the addition of the hydroxy and methoxy groups. The peaks between 1300 and 1400 cm<sup>-1</sup> become much more intense in MHMI relative to indole, and are well fit by the calculations. The calculations here attribute them to benzene ring stretches primarily, contradicting older data attributing them to the pyrrole ring (29). The peak at  $1520 \text{ cm}^{-1}$ , which is also largely unmoved



Figure 1. Raman spectra of (a) indole, (b) indole-2-carboxylic acid (ICA), (c) *N*-methyl-5-hydroxy-6-methoxyindole (MHM1), (d) MHM1 dimer and (e) 5,6-dihydroxyindole-2-carboxylic acid (DHICA). B3LYP calculated spectra are on top (thin) and experimental spectra are on bottom (thick).

by the addition of functional groups to the indole compound, is due mostly to  $C_2$ - $C_3$  stretching within the pyrrole ring, consistent with the previous work (29). The remaining peaks experience some shift and/or change in intensity. Of particular interest are the indole peaks near 1000 cm<sup>-1</sup>, attributed to pyrrole in-plane bending, which disappear almost entirely in MHMI.

The MHMI dimer spectrum exhibited more noise, but a number of peaks still stand out prominently (Fig. 1d). The peak observed at  $762 \text{ cm}^{-1}$  in the monomer has shifted to

Table 1. Vibrational character of the most intense Raman bands.\*

Indole			
Raman shift (cm <sup>-1</sup> )	Character		
536.1	Symmetric distributed ring stretch		
598.8	Antisymmetric 1-4 stretch		
753.6	Symmetric 4-7 ring stretch		
1010.9	Symmetric 5-6 face ring opening mode		
1065.6	Asymmetric C(2,3)-H in plane bend		
1117.1	Antisymmetric C(4,5,6,7)-H in plane bend		
1272.0	Symmetric(?) N1-C-C7 and C3-C-C4 bond alternation and in plane C(5.6.7)/N1-H in plane bend		
1339.6	Symmetric kekule benzene		
1419.1	Asymmetric C-H bend		
1454.0	Symmetric $C(4,7)$ -H in plane bend		
1495.6	Antisymmetric C(5,6)-H in plane bend		
1523.0	C2-C3 stretch		
1585.9	4-7 axis Dewar benzene		
1626.7	C6-axis Dewar benzene		

\*A correction factor of 0.97 has been applied to the Raman shift wavenumber values.

817 cm<sup>-1</sup> in the dimer, and calculations indicate that this incorporates several weak vibrations not listed in Table 2. In addition to the in-plane ring stretching observed in the dimer, several out-of-plane asymmetric bending vibrations are included. Although out-of-plane vibrations were generally absent from indole and MHMI Raman spectra, they play a

Table 2. Vibrational character of the most intense Raman bands.\*

larger role in the dimer because a 72° twist about the 4-4′ bond (determined from crystallographic results which will be published separately) destroys the C<sub>s</sub> symmetry of the monomer, such that it is no longer a two-dimensional molecule (data not shown). The peak at 1524 cm<sup>-1</sup> is largely unaffected by dimerization, and this is consistent with its assignment to C<sub>2</sub>-C<sub>3</sub> stretching and asymmetric methyl vibrations. Because dimerization is through the 4-4′ position, it would have only a weak effect on the vibrational structure of the pyrrole end of the molecule. The peak at 1642 cm<sup>-1</sup> resulting from benzene ring stretching is also shifted to shorter wavenumbers.

Experimental spectra for DHICA agreed reasonably well with previously published IR absorbance spectra for DHICA (20), which were also corroborated by B3LYP density functional theory calculations. Because IR absorption and Raman spectroscopies have different selection rules, it can be expected that some peaks appearing in one will be absent in the other. Peaks appearing in both spectra are found near 1250, 1540 and 1650 cm<sup>-1</sup>, among others (Fig. 1e). The DHICA spectrum also shows great similarity to that of ICA. Peaks near 950, 1140, 1540 and 1740 cm<sup>-1</sup> experience virtually no shift after addition of hydroxy groups to the 5 and 6 positions. This might be expected for the 950 and 1740 cm<sup>-1</sup> peaks, which are primarily caused by stretching within the pyrrole ring and carboxylic acid, but the other two peaks have significant contributions from benzene ring vibrations as well as those in the pyrrole ring and carboxylic acid. The peak near 1400 cm<sup>-1</sup> in DHICA has several weak vibrations contributing to the observed peak, including bending within the benzene ring and hydroxy groups.

MHMI monomer		MHMI dimer		
Raman shift (cm <sup>-1</sup> )	Character	Raman shift (cm <sup>-1</sup> )	Character	
737.3	Symmetric benzene stretch along 4-7 axis			
796.7	Symmetric 7,3 axis cross-ring stretch			
		804.0	Out-of-plane C-H wag (2,3)	
		1181.6	C(4)-H + C(7)-H in plane bend	
		1187.7	(O-) methyl in plane tilt	
		1290.3	COH bend	
1308.5	In plane C-H bend (5-ring,6-ring)-COH bend	1339.8	COH bend-C-C (ring joint) stretch	
1346.0	5-ring concerted stretch and COH bend	1352.2	COH bend and distributed imidazole stretch	
1368.5	6-ring stretch-5-ring stretch and methyl pyramidal	1364.9	Phenyl stretch-imidazole stretch	
		1373.0	Phenyl stretch-imidazole stretch	
1435.5	(N-)methyl pyramidalization	1434.6	(N-) methyl pyr(O-) methyl pyr. (-coupling)	
1463.6	(N-) methyl scissoring (asymmetric bend)	1469.8	(O-) methyl pyr. (+ coupling)	
1470.8	(O-) methyl scissoring (asymmetric bend)	1471.1	(O-) methyl out-of-plane tilt (single monomer) and (O-) methyl pyr. (other monomer)	
1482.3	(N-)methyl handclap-(O-)methyl handclap (symmetric bend)			
		1511.0	C(2)-H in plane bend, (N,O-) methyl handclap, phenyl ring distortion (+ coupling)	
		1521.5	C(2)-H in plane bend, (N-) methyl handclap	
1524.8	C3-C2 stretch and in plane C-H bend (3,2,4,7)	1523.2	C(2)-H in plane bend, $C(2)$ - $C(3)$ stretch	
		1591.0	Dewar benzene (4-7 axis, + coupling), COH bend, C(3,7)-H in plane bend	
1641.9	Dewar benzene (main axis through C6)	1627.7	Dewar benzene (6-para axis, + coupling), COH bend, C(7)-H in plane bend	

MHMI = N-methyl-5-hydroxy-6-methoxy-indole. \*A correction factor of 0.97 has been applied to the Raman shift wavenumber values.

Indole-2-CA		DHICA	
Raman shift (cm <sup>-1</sup> )	Character	Raman shift (cm <sup>-1</sup> )	Character
956.3	1-3 pyrrole ring stretch	959.1	1,3 axis stretch
1009.2	C(5)- $C(6)$ bond ring-opening mode		
1141.1	C(4)-H- $C(7)$ -H- $C(5)$ -H + $C(6)$ -H in plane bends	1137.6	C(4,3)-H+O(5)-H-(COO)-H in plane bend
1158.6	C(5)-H- $C(6)$ -H- $C(3)$ -H in plane bends		
1286.0	N1-C-C7+C3-C-C4 bond alternation + (COO)H in plane bend	1243.4	C(3)-H+N-H-O(6)-H in plane bend
1327.3	Ring joint stretch and in plane C-H bends	1365.7	Ring edge stretch-C(6)-H bend
1422.9	C2N1 stretch		
1442.6	C4-H and C7-H symmetric in plane bend	1479.1	C(6)- $C(7)$ stretch + $C(5)$ - $C(4)$ stretch and NH in plane bend
1542.3	Symmetric ring stretch and C(4,5,6,7)-H in plane bend	1542.4	C(2)-C(3) stretch + $O(6)-H$ bend + $C(4,7)-H$ bend
1582.1	4-7 Dewar benzene		
1626.7	C5 diagonal axis Dewar benzene	1649.6	Dewar benzene (5 axis) and C(4,7)-H bend
1747.9	C = O stretch	1743.3	C = O stretch- $C(2)$ - $C(OOH)$ stretch

Table 3. Vibrational character of the most intense Raman bands.\*

DHICA = 5,6-dihydroxyindole-2-carboxylic acid. \*A correction factor of 0.97 has been applied to the Raman shift wavenumber values.

# DISCUSSION

The calculated spectra using B3LYP showed good agreement with experimental Raman spectra, with frequencies of major peaks overlapping very well for all compounds. Some of the incongruencies which do occur can be ascribed to crystal packing effects which were not accounted for in the calculations. Agreement is excellent for indole and ICA which can, by comparison with previously reported data, be considered as benchmarks.

The number of molecular vibrations is equal to 3n-6, where n is the number of atoms in the molecule, so it was expected that more vibrational modes should be visible in the Raman spectrum of the dimer than that of the monomer. This was not apparent in the experimental spectra. The underlying reason for this, revealed by the calculations, is that many of the additional modes do not carry appreciable intensity. The good correspondence between theoretical and experimental spectra shows that each of the observed bands is actually the superposition of several weak vibrational modes.

There are two substantial differences between the change from ICA to DHICA and the change from indole to MHMI. First, the functional groups added to ICA are restricted to one moiety, whereas those added to indole to make MHMI are on both the benzene and pyrrole rings. Second, the hydroxy group attached to position 6 in DHICA is about half the size of the methoxy group in MHMI. For these reasons, it might be expected that the differences in vibrational spectra between indole and MHMI would be greater than those between ICA and DHICA. This was not observed. In both cases, there is reasonable agreement between the two compounds with only moderate peak shifts and changes in relative intensity. In MHMI, peaks resulting from both benzene  $(737, 1369 \text{ cm}^{-1})$ and pyrrole  $(1524 \text{ cm}^{-1})$  vibrations had shifted by less than 30 cm<sup>-1</sup> from those in indole. DHICA is similar, although some of the apparently unshifted peaks change in character from those in ICA. For example, the peak at  $1542 \text{ cm}^{-1}$ changes from a benzene ring stretch in ICA to a combination of  $C_2$ - $C_3$ ,  $C_4$ - $C_7$  and hydroxy group vibrations. The peak at 1327 cm<sup>-1</sup> resulting from ring joint stretching in ICA shifts to 1366  $\text{cm}^{-1}$  and is caused by ring edge stretching. These factors suggest that addition of functional groups to either moiety impacts the vibrational structure of the entire molecule.

In general, it can be noted that although there is some marginal shifting of peaks between the Raman spectra of the MHMI monomer and dimer, they do not differ radically. Peaks in the fingerprint region  $(1200-1600 \text{ cm}^{-1})$  and near  $760 \text{ cm}^{-1}$  appear at similar frequencies and with similar intensities in the two compounds. This is likely due to the 72° rotational offset between the two monomers, allowing the vibrations within each monomer to be relatively unperturbed by the presence of the other unit. Furthermore, there is hydrogen bonding not only between the hydroxy and methoxy groups within the dimer, but also between these groups in different dimers. This hydrogen bonding could have a stabilizing effect, resulting in benzene vibrations appearing at lower frequencies than in calculated spectra, which do not account for intermolecular hydrogen bonding (although they do account for intramolecular hydrogen bonding). Indeed, small shifts are observed for the peaks at 1350 and 1600 cm<sup>-1</sup>

The rotational offset plays a key role in defining the dimer's photochemistry. Because the p-orbitals within each monomer are offset from those in the other monomer both in position and in angle, it is highly unlikely that any cooperative vibrations occur. A planar oligomeric structure would be more susceptible to such mutual perturbations. This is relevant in the context of eumelanin synthesis. If planar models of eumelanin oligomers (6,30) prove to be correct, then the dimer spectrum shown here may not be relevant to eumelanin photochemistry. However, if the nonplanar models are correct (8,10), then the chemistry of individual monomers may be more significant, even in the bulk eumelanin.

Raman spectra of bulk eumelanin are characterized by two broad peaks near 1400 and 1600 cm<sup>-1</sup>, devoid of fine structure (15,16). Given the fine structure evident in both indolic monomers shown here, including strong narrow peaks near 1400 and 1600 cm<sup>-1</sup>, our results suggest that either each oligomer displays substantially broadened vibrational spectra (compared to those of its substituent monomers) or that there is such a diversity of oligomers present in eumelanin that it becomes impossible to resolve between the individual contributions. If eumelanin is planar, the broad peaks in the Raman spectrum of eumelanin are likely due both to broadening within each oligomer and superposition of many spectra. Otherwise, the similarity between monomer and dimer spectra observed here may suggest that the oligomers also exhibit substantial fine structure and that the broad spectrum of eumelanin is due primarily to contributions of many different oligomers.

It is also worthwhile to note that in addition to the vibrational spectrum of the dimer being similar to that of the monomer, so too is the energy gap between occupied and unoccupied orbitals. Despite these similarities, the quantum yield of the dimer is almost an order of magnitude lower (S.P. Nighswander-Rempel and P. Meredith, unpublished data), indicating increased propensity for nonradiative relaxation in the biindolyl structure. These similarities and differences may not hold true for a planar dimeric arrangement. In this light, the fact that the quantum yield of eumelanin is extremely low may lend further credence to the nonplanar model.

## CONCLUSIONS

We have created an indolic compound (MHMI) which lends itself well to studies of structure-function relationships in indolic dimers and eumelanin synthesis. Experimental Raman spectroscopy of the MHMI monomer and dimer (supported by B3LYP calculations) demonstrates that while dimerization does result in a significant increase in the total number of vibrational modes, it does not result in a corresponding increase in the number of strong modes or apparent spectral peaks. The presence of broadening in experimental spectra and the fact that modes are often clustered together result in the number of apparent peaks in dimer spectra being similar to that in monomer spectra. The similarity in peak width between those of the monomer and dimer supports the proposition that eumelanin consists of a wide variety of distinct oligomers, especially if nonplanar oligomeric models are correct. These results also demonstrate that certain benzene and pyrrole ring vibrations such as symmetric 4-7 and 2-3 stretching are very resilient to addition of functional groups and dimerization. This work provides new insight into the cause of the extremely low quantum yield of melanin by examining how the nonradiative relaxation pathways vary with the different stages of eumelanin synthesis.

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# SUPPLEMENTAL MATERIAL

The following supplemental materials are available for this article:

**Table S1.** Cartesian coordinates (Å) of 5-H, 6-MeO, N-Meindole, unscaled vibrational frequencies, infrared and Raman intensities, and normal modes calculated using B3LYP/ 6-31G(d). This material is available as part of the online article from: http://www.blackwell-synergy.com/doi/full/10.1111/j.1751-1097. 2007.00273.x

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