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On the Structure of Eumelanins: Identification of Constitutional Patterns by Solid-State NMR Spectroscopy

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known as dimerization products of phosphaalkenes.[11] The intermediacy of **4** was confirmed by trapping experiments with 2,3-dimethylbutadiene; the product **6**, formed by [2 + 4] cycloaddition to the P=C bond, was obtained in nearly quantitative yield.[12] Similarly, the reaction of **7a, b** with $BF_3 \cdot OEt_2$ in the presence of 2,3-dimethylbutadiene in excess leads to the heterocycles **8a** and **8b**. However, the reaction of **7b** is not uniform. Loss of F_2BPh, instead of F_2BNiPr_2, results in the formation of **2b** as a side product. Compounds **6, 8a**, and **8b**[13] are formed as racemates with no observed formation of diastereomers. This finding supports a concerted [2 + 4] cycloaddition. Compounds **2a** and **2b** do not react with 2,3-dimethylbutadiene under the conditions described here. Moreover, products of a [1 + 4] cycloaddition were not observed.[14]

The bonding parameters obtained from a structure investigation and the data derived from the UV/VIS spectra of **2a** and **2b**, as well as the reactivity of compounds **4, 7a**, and **7b**, are similar to those of previously reported phosphaalkenes. The syntheses described here offer attractive variants for the synthesis of phosphaalkenes, since the phosphinomethylenetriphenylphosphoranes required as starting materials are readily accessible and widely variable.

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[6] **2a**: $Pbn2_1$; $a = 9.225(5)$, $b = 15.097(9)$, $c = 19.419(12)$ Å, $V = 2704$ Å^3, $Z = 4$. 1035 observed reflections with $I > 2\sigma(I)$ (four-circle diffractometer, $Mo_{K\alpha}$ radiation, ω scan). $R = 0.089$, $R_w = 0.048$ (P refined anisotropically, F, N, C, B isotropically; the disordered anion refined with two rigid BF_4 tetrahedrons, the methyl groups and phenyl rings as rigid groups with a common temperature factor for the H atoms). Further details of the crystal structure investigation may be obtained from the Fachinformationszentrum Karlsruhe, Gesellschaft für wissenschaftlich-technische Information, D-7514 Eggenstein-Leopoldshafen 2 (FRG), on quoting the depository number CSD-53721, the names of the authors, and the journal citation.
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[13] General experimental procedure: The respective phosphinomethylenetriphenylphosphorane (**1a,b, 3, 7a,b**; 5 mmol), was dissolved in 30 mL of dry CH_2Cl_2 and treated at −78 °C with 1.13 mL of freshly distilled $BF_3 \cdot OEt_2$ (10 mmol) in ether. In the cycloaddition reactions, 2,3-dimethylbutadiene (2.1 g, 25 mmol) was then added. The reaction solution was warmed to room temperature, the volatiles were removed at 0.01 torr, and the residue was washed with ether and recrystallized from CH_2Cl_2/ether. **2a**: m.p. 146 °C. $^{31}P\{^1H\}$ NMR (36.19 MHz, $CDCl_3$, 27 °C): $\delta = 19.5$ (d, PPh_3); 303.5 (d, PN, $^2J = 124.6$ Hz). ^1H NMR (89.99 MHz, $CDCl_3$, 27 °C): $\delta = 1.25$ (d, 12H, CH_3, $^3J = 6.6$ Hz); 3.75–4.46 (m, 2H, CH); 6.41 (dd, 1H, CH, $^2J(P^{III}H) = 10.7$, $^2J(P^VH) = 1.7$ Hz); 7.49–7.60 (m, 15H, arene-CH). $^{13}C\{^1H\}$ NMR (22.49 MHz, $CDCl_3$, 25 °C): $\delta = 20.96$ (s, CH_3); 25.87 (s, CH_3); 51.25 (s, CH); 52.76 (s, CH); 88.40 (dd, CH, $^1J(P^{III}C) = 92.5$, $^1J(P^VC) = 34.3$ Hz); 121.58 (d, *ipso*-C, $^1J(P^VC) = 89.4$ Hz); 129.72 (d, *m*-C, $^3J(P^VC) = 12.5$ Hz); 133.2 (d, *o*-C, $^2J(P^VC) = 10.1$ Hz), 134.2 (s, *p*-C). **2b**: m.p. 143 °C. $^{31}P\{^1H\}$ NMR (36.19 MHz, $CDCl_3$, 27 °C): $\delta = 28.5$ (d, PPh_3), 302.5 (d, PN, $^2J = 168.6$ Hz). ^1H NMR (89.99 MHz, $CDCl_3$, 27 °C): $\delta = 1.11$ (d, 12H, CH_3, $^3J = 6.9$ Hz); 2.15 (dd, 3H, CH_3, $^3J(P^VH) = 17$, $^3J(P^{III}H) = 7.2$ Hz); 4.03 (sept., 2H, CH, $^3J = 6.9$ Hz); 7.46–7.55 (m, 15H, arene-CH). $^{13}C\{^1H\}$ NMR (22.49 MHz, $CDCl_3$, 27 °C: $\delta = 19.02$ (dd, CH_3, $^2J(P^VC) = 8.8$, $^2J(P^{III}C) = 3.0$ Hz); 24.16 (d, CH, $^3J(P^{III}C) = 7.4$ Hz); 51.37 (d, CH, $^2J(P^{III}C) = 6.1$ Hz); 104.29 (dd, CCH_3, $^1J(P^{III}C) = 76.2$, $^1J(P^VC) = 68.9$ Hz) 119.79 (dd, *ipso*-C, $^1J(P^VC) = 89.4$, $^3J(P^{III}C) = 7.3$ Hz); 129.45 (d, *m*-C, $^3J(P^VC) = 13.2$ Hz); 133.64 (d, *o*-C, $^2J(P^VC) = 13.2$ Hz); 133.83 (s, *p*-C). **5**: m.p. 249 °C. $^{31}P\{^1H\}$ NMR (36.19 MHz, CD_3CN 27 °C): $\delta = 21.42$ (m, AA'XX' system). ^1H NMR (89.99 MHz, CD_3CN, 27 °C): $\delta = 0.43–0.57$ (m, 18H, CH_3); 3.5 (m, 2H, CH); 7.34–7.75 (m, 30H, arene CH). **6**: m.p. 166–167 °C. $^{31}P\{^1H\}$ NMR (36.19 MHz, $CDCl_3$, 27 °C): $\delta = -25.2$ (d, P*t*Bu); 24.76 (d, PPh_3, $^2J = 80.6$ Hz). **8a**: m.p. 193 °C. $^{31}P\{^1H\}$ NMR (36.19 MHz, CD_3CN, 27 °C): $\delta = -24.8$ (d, PCH_3); 36.3 (d, PPh_3, $J = 95.1$ Hz). **8b**: $^{31}P\{^1H\}$ NMR (36.19 MHz, $CDCl_3$, 0 °C): $\delta = -20.97$ (d, PPh); 31.2 (d, PPh_3, $^2J = 85.3$ Hz).
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On the Structure of Eumelanins: Identification of Constitutional Patterns by Solid-State NMR Spectroscopy**

By *Martin G. Peter** and *Hans Förster*

Melanins are complex polyphenolic polymers. They are usually formed in nature by enzyme-catalyzed oxidative polymerization of *o*-diphenols (for reviews, see Refs. [1–3]). The deep black eumelanins, derived from Dopa **1** or dopamine **3**, are distinguished from the yellow to brown phaeomelanins obtained from Dopa in the presence of cysteine. Characteristic of eumelanins are the indole units, which are formed from catecholamines by intramolecular addition of the amino groups to the oxidatively generated *o*-quinones (Fig. 1). During polymerization of these indoles, C–C and C–O bonds are formed by nucleophilic additions and by oxidative radical phenol couplings. Allomelanins are polyarenes obtained from nitrogen-free catechols.

In contrast to Dopa-melanin, dopamine-melanin is basic in character and is capable of being benzoylated to a greater extent than Dopa-melanin.[1] According to *Swan*,[2] Dopamelanin has the following statistical composition (Fig. 1): ca. 10% **1** and **2**, ca. 10% **7** and **9**, ca. 65% **11** and **12**, and ca. 15% pyrrolecarboxylic acids. Dopamine-melanin contains ca. 35% **3** and **4**, ca. 55% **11, 12, 8,** and **10**, and ca. 10% pyrrolecarboxylic acid units. The latter are not shown in Figure 1 (cf. Refs. [1, 2, 5]). The relative amounts of noncyclic units in Dopa- and dopamine-melanin correspond roughly to the ratio of the rate constants k_c of the cyclization (Dopa, $k_c = 72$ s^{-1};[6] dopamine, $k_c = 25.6$ s^{-1}[7]). The rates of rearrangement of dopachrome **9** and dopaminochrome **10** to 5,6-dihydroxyindole **11** have not yet

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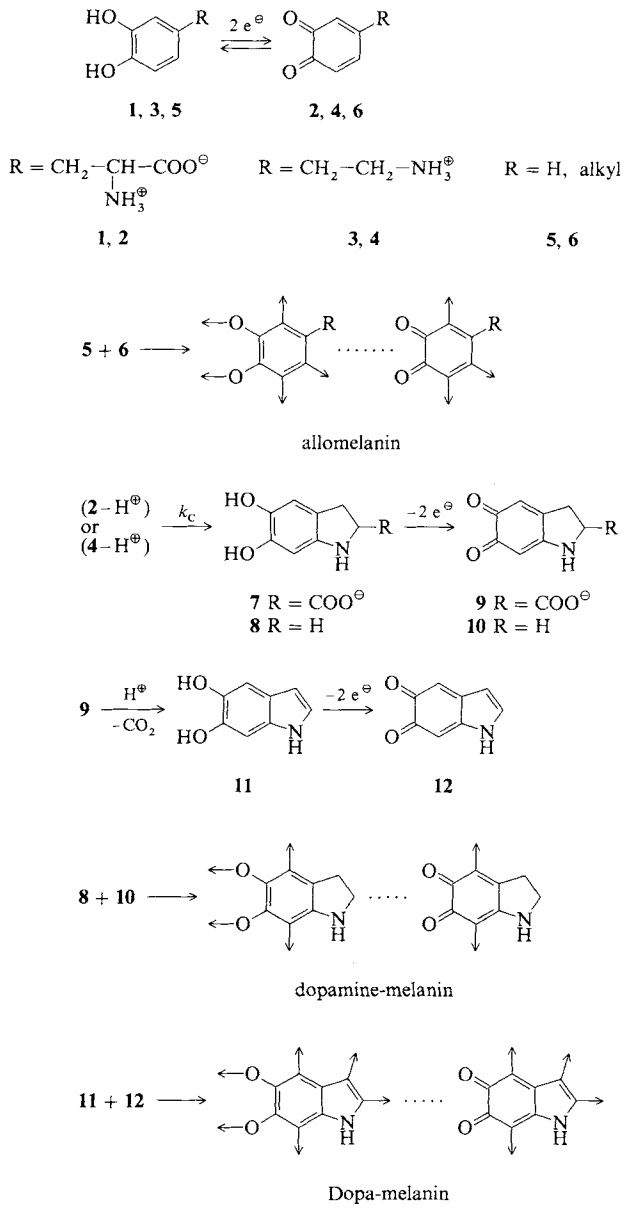

Fig. 1. Schematic drawing of the formation of melanins from catechols.

been determined to the best of our knowledge. In basic to neutral media, however, the decarboxylation of the dihydroindole should proceed faster than the deprotonation. The dopamine-melanin generated at pH 7 should therefore consist to a larger extent of dopamine and dihydroindole units than of indole units.

These conclusions are confirmed by solid-state ^{13}C NMR spectra. We prepared Dopa- and dopamine-melanin by oxidation of Dopa and dopamine, respectively, with excess $K_3[Fe(CN)_6]$ in water. The elemental analysis (double determination) gave the following results for Dopa-melanin: 49.02 ± 0.03% C, 3.71 ± 0.01% H, and 8.29 ± 0.03% N ($C_9H_{8.15}N_{1.30}$). For dopamine-melanin, the values were 50.83 ± 0.11% C, 4.20 ± 0.03% H, and 9.52 ± 0.01% N ($C_8H_{7.92}N_{1.28}$).

The CP/MAS ^{13}C NMR spectrum of Dopa-melanin is shown in Figure 2b. The signal assignment given in Table 1 was made by comparison with the solid-state ^{13}C NMR spectrum of Dopa (Fig. 2a).

In the aliphatic region, the side-chain C atoms of the Dopa units and C2 of the dihydroindole units can also be identified upon improvement of the resolution by Gauss modification.

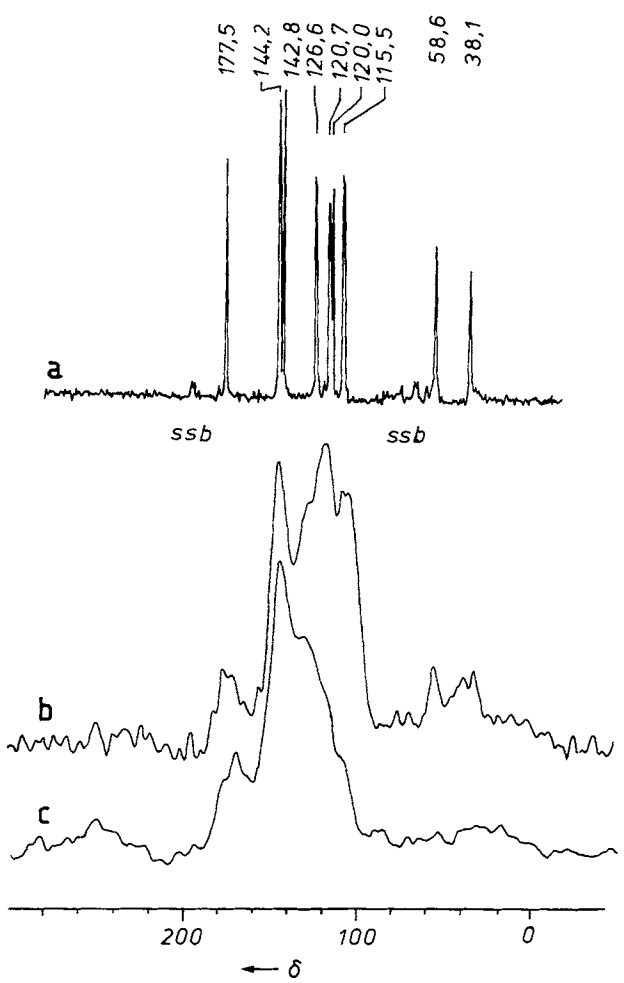

Fig. 2. CP/MAS ^{13}C NMR spectra of a) Dopa, b) Dopa-melanin, and c) Dopa-melanin recorded with an NQS sequence using 60-μs delay times; $^1H-^{13}C$ cross-polarization with sample rotation around the magic angle; spin frequency 5.19 kHz; decoupling field strength 20 G; contact time 500 μs; 1606 accumulations at 297 K; rotation side bands (SSB) were largely suppressed.

Table 1. Assignment of the signal groups in the CP/MAS ^{13}C NMR spectrum of Dopa-melanin (Fig. 2b).

δ	Assignment
175	Carboxyl and quinoid carbonyl C atoms
144	Diphenolic phenoxy C atoms
127–116	Arene C-H and C-C; C2, C3a, C4, and C6a of indole units
107–104	C3 of indole and pyrrole units
54, 36, and 31	Side-chain C atoms of noncyclized Dopa and C2 of dihyroindole units

If the resonances of non-quaternary C atoms are suppressed with an NQS sequence, the signals in the aliphatic region as well as those of the respective indole and pyrrole C atoms at $\delta = 107-104$ disappear (Fig. 2c). In the region of the arene C atoms, a decrease in intensity is observed owing to the suppression of the resonances of the unsubstituted phenyl and indole C atoms. Thus, the partial structures contained in the constitutional scheme of Dopa-melanin are unambiguously confirmed by the solid-state ^{13}C NMR spectrum.

The CP/MAS ^{13}C NMR spectrum of dopamine-melanin, shown in Figure 3b, differs markedly from that of Dopa-melanin (cf. Fig. 2b). Owing to the absence of carboxyl groups in the starting material, the carbonyl resonances ($\delta \approx 170$) are weaker than in Dopa-melanin. The C-3 signals of the unsubstituted indole and pyrrole units can hardly be

identified for dopamine-melanin. The intense aliphatic signals correlate unambiguously with the side-chain C atoms of dopamine (Fig. 3a); the signal of dihydroindole C2, expected at $\delta \approx 46$, is not resolved. Suppression of non-quaternary C atoms affords a spectrum very similar to that of the NQS spectrum of dopa-melanin (Fig. 3c).

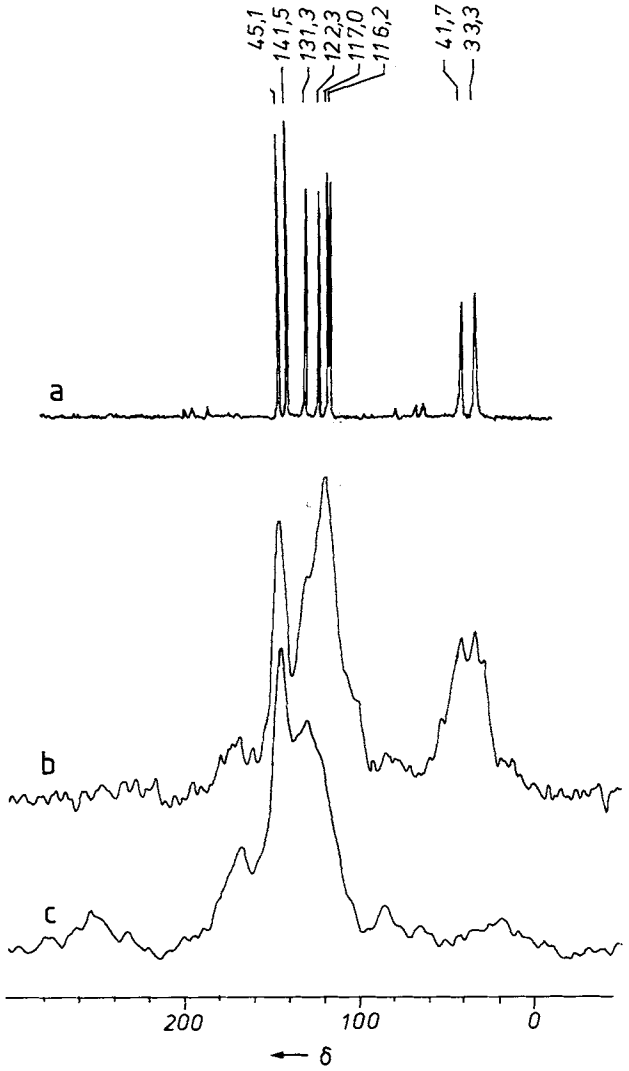

Fig. 3. CP/MAS ^{13}C NMR spectra of a) dopamine (hydrochloride), b) dopamine-melanin, and c) dopamine-melanin recorded with an NQS sequence using 60-μs delay times. For measuring parameters, see Figure 2.

Comparison of the relative intensities of individual groups of signals in the spectra of the monomeric precursor and the polymer reveal that dopamine-melanin contains a much larger proportion of unmodified side chains compared with Dopa-melanin. Accordingly, dopamine-melanin is formed to a correspondingly greater extent by polymerization of the open-chain precursors **3** and **4** (Fig. 1) and contains substructures of the allomelanin type (cf. the polymerization of **5** and **6** in Fig. 1). In addition, constitutional patterns resulting from copolymerization of **3** and **4** with **8, 10, 11,** and **12** are present to a lesser extent. In Dopa-melanin, on the other hand, the polymerization of **11** with **12** predominates.

The solid-state ^{13}C NMR spectra also allow an estimate of the oxidation state of the polymer. Comparison of the intensities of the carbonyl and the diphenolic phenoxy C signals reveals a high proportion of catechol units; this indicates that the polymerization occurs by oxidative phenol couplings.

Duff et al.[8] recently described CP/MAS ^{13}C and ^{15}N NMR spectra of autooxidatively generated Dopa-melanin as well as of melanoma- and sepia-melanin, isolated from biological material. The signal assignments largely agree with those in Figure 2b discussed here. However, the indole, pyrrole, and dihydroindole units in the spectrum published in Ref. [8] are not unambiguously identifiable owing to the comparatively low resolution. In the CP/MAS ^{15}N NMR spectrum, on the other hand, signals for aliphatic amino groups are present.

The CP/MAS ^{13}C NMR spectrum of melanin generated enzymatically from tyrosine with tyrosinase (monophenol monooxygenase, EC 1.14.18.1) agrees qualitatively with Figure 2b, although the carboxyl/carbonyl signals in tyrosine-melanin are more intense. The signals of the phenoxy C atoms at $\delta \approx 145$ are less intense than those of the other arene C atoms. However, indole C3 atoms and pyrrole C atoms can be unambiguously identified. The CP/MAS ^{13}C NMR spectrum of a phaeomelanin, prepared by oxidation of a mixture of Dopa and cysteine with $K_3[Fe(CN)_6]$, is characterized above all by the prominent carboxyl signal and the signals of the aliphatic amino-acid C atoms.

The results show that solid-state NMR spectroscopy is very suitable for nondestructive investigation of complex biopolymers. The analysis of the constitutional pattern not only leads to new insights into structural relationships, but also is essential for the assignment of unknown polyphenolic natural products to specific melanin classes.

Experimental Procedure

Preparation of Dopa-melanin: A solution of L-Dopa (1.0 g, 5.07 mmol) and $K_3[Fe(CN)_6]$ (8 g, 23.4 mmol) in 1 L of H_2O was adjusted to pH 8 with ca. 2 N KOH and then stirred vigorously in an open Erlenmeyer flask at 22 °C. After 7 h, the reaction mixture was acidified with 10 mL of conc. HCl. The black melanin that settled out was centrifuged off and washed thoroughly with 10 × 50 mL of H_2O. It was then suspended in H_2O and lyophilized. Yield: 897 mg Dopa-melanin. Dopamine-melanin was synthesized similarly.

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$[Et_4N]_6[Na_{14}Mo_{24}P_{17}O_{97}(OH)_{31}] \cdot xH_2O$: A Hollow Cluster Filled with 12 $Na^{\oplus}$ Ions and a H_3PO_4 Molecule

By *Robert C. Haushalter** and *Frank W. Lai*

Dedicated to Professor Hans Bock on the occasion of his 60th birthday

Compared to the solid-state layer and tunnel structures composed of either all octahedral or all tetrahedral building blocks, relatively little attention has been given to solids built up from octahedral-tetrahedral frameworks.[1] During our

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