

STUDIES ON THE STRUCTURE OF THE BLACK POLYMERS OBTAINED FROM 4,7-DIMETHOXYINDOLE

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Abstract—Studies of the transformations undergone by 4,7-dimethoxyindole during heating in acid suggest a mechanism for the formation of the resulting linear polymers and a possible structure.

The 4,7-dimethoxyindoles¹⁻⁹ display some interesting properties, which can be summarized as follows:

(a) In an acidic aqueous medium they easily undergo conversion into black compounds, polymeric in nature, much less soluble in water and in common organic solvents, and apparently similar to the natural melanins.^{10,11}

(b) They can be completely demethylated without the formation of black polymers by heating with anhydrous aluminium chloride in benzene; the 4,7-dihydroxyindole derivatives **2** thus formed are in tautomeric equilibrium with the corresponding 4,7-dioxo-4,5,6,7-tetrahydro derivatives **3**.^{9,12}

Some years ago we began to study the structure of the black compounds obtained by heating 4,7-dimethoxyindole **1** in an acidic aqueous alcoholic solution achieving some results which, however, were not sufficient for a complete formulation of their structures.¹⁰

In this paper we describe further experiments which allow us to suggest a structure of these black compounds and a mechanism for their formation.

RESULTS AND DISCUSSION

In a previous paper¹⁰ we described some results obtained by studying the black compounds formed by heating 4,7-dimethoxyindole in an aqueous alcoholic solution containing hydrochloric acid. Although not conclusive, these results gave some indications about the molecular structure of the black compounds and the pathway of their formation. The first step appeared to be the demethylation of the 4,7-dimethoxyindole **1**; one of the two methoxys was hydrolyzed very rapidly, while the second one was hydrolyzed much more slowly, perhaps after the formation of the black compound. The black compounds appeared to be polymers, formed by a number of partially demethylated indole units; the

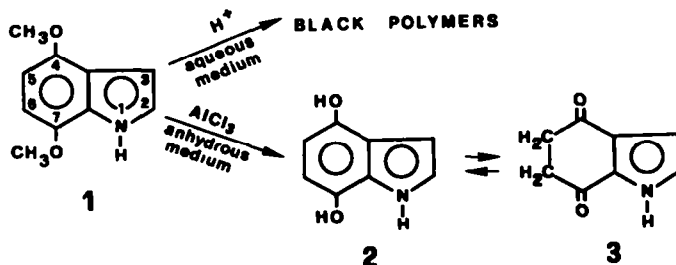
molecular weight, and therefore the number of indole units, increased by increasing the time of heating. Therefore, the polymers should be linear. The hydroxyls originating from the easily hydrolyzable methoxyl groups were not free in the black polymers; they were probably involved as links between indole units. By contrast, the hydroxyl groups originating by the slow hydrolysis of the second methoxyl group remained free. No C-C linkages between two units involving the C-2 and C-3 positions of the indole nucleus were present in the black polymers.

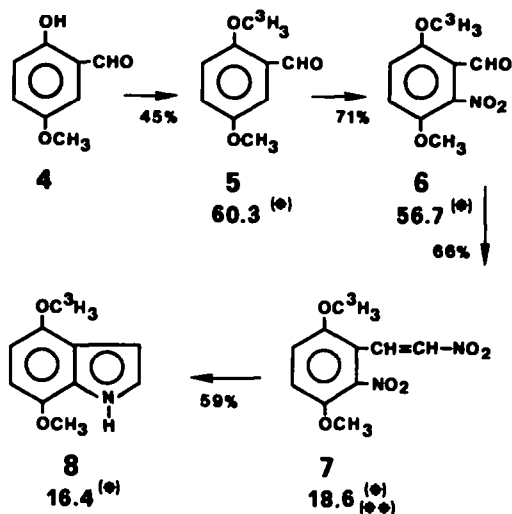
Moreover, the above-mentioned paper¹⁰ described a compound with constant properties and composition obtained by long reflux heating (18 h) of an aqueous alcoholic solution of 4,7-dimethoxyindole containing hydrochloric acid; on the basis of its molecular weight, this compound was considered an heptamer in which three methoxyl groups were still present; moreover, it contained four free hydroxyl groups.

After a relatively short time of heating (3 h) of a same aqueous alcoholic solution of 4,7-dimethoxyindole containing hydrochloric acid, we have now been able to isolate, among the various products formed, a simpler black compound, with a molecular weight corresponding to that of a dimer and in which two of the initial four methoxyl groups were still present.

The composition of this dimer confirms our previous suggestion, that is, for the formation of the polymer the hydrolysis of one of the two methoxys is necessary and sufficient; the hydrolysis of the second methoxyl group takes place slowly after polymerisation. Therefore, it was of interest to establish which of the two methoxys is hydrolyzed first, because the resulting hydroxyl group is involved in the linkage with a second indole molecule.

In order to clarify this problem, we prepared a 4,7-dimethoxyindole having a ³H-labelled methoxyl group at the C-4 position. This compound was prepared following the same synthetic method used for obtaining the 4,7-





Scheme 1.

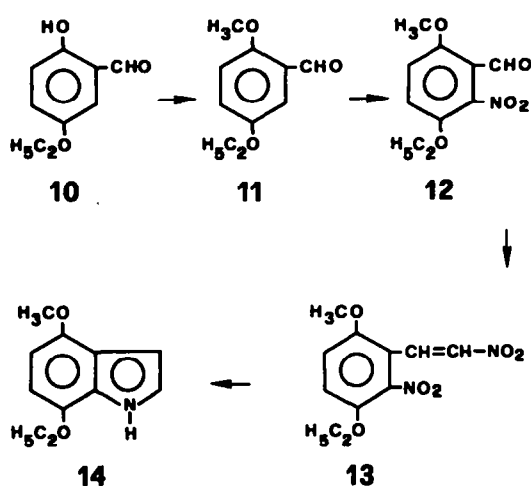
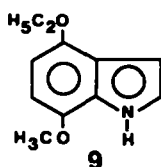
*Specific radioactivity in $\text{DPM}/\mu\text{mol} \times 10^3$.

**This low value of radioactivity is due to a dilution (1:2) with cold 2,5-dimethoxy-6-nitrobenzaldehyde before condensation to styryl derivative.

dimethoxyindole 1: labelling was performed by methylation of 2-hydroxy-5-methoxybenzaldehyde 4 by means of $\text{di}[\text{H}]$ methyl sulphate; the subsequent steps are indicated in Scheme 1, in which the chemical yields and the specific radioactivities of the various intermediates are reported.

By reflux heating for 3 h the 4-[^3H]methoxy-7-methoxyindole 8 in an aqueous alcoholic solution containing hydrochloric acid, a black compound was isolated, corresponding in all properties to the dimer previously indicated. In this dimer, containing two methoxyl groups, the whole radioactivity initially present in two molecules of indole derivative was present. The preparation of the heptamer starting from the same labelled 4,7-dimethoxyindole 8 by 18 hr reflux, confirmed that all the methoxyl groups still present were radioactive. These results demonstrate that the first methoxyl group which undergoes hydrolysis is that present at the C-7 position.

In previous experiments using both 4-methoxy-7-ethoxyindole 14, now prepared following a method analogous to Nenitzescu's synthesis¹³ (see Scheme 2), and 4-ethoxy-7-methoxyindole 9, which preparation has been already described,⁶ this problem failed to be clarified.



Scheme 2.

In fact, by reflux heating of both these compounds in an acidic medium, black polymers analogous to those formed from 4,7-dimethoxyindole were obtained; however, in both cases only ethoxyl groups were still present in the black polymers. The reason for the anomalous behaviour of the 4-methoxy-7-ethoxyindole, which underwent dealkylation at the C-4 position, is at present unknown.

As a result of the present and previously described¹⁰ investigations and on the basis of the properties showed by various 4,7-dimethoxyindole derivatives,¹⁻⁹ it is possible to suggest a scheme for the mechanism of formation and for the structures of these linear black polymers.

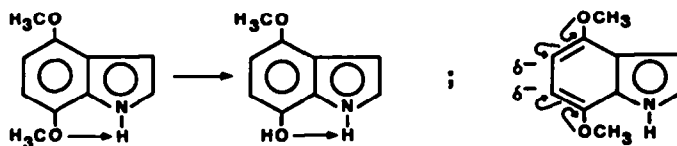
The first event of the polymerisation may be the hydrolysis of the methoxyl present at C-7 position, which is facilitated by the engagement of one of the two lone pairs of oxygen in hydrogen bonding with the imino group.

In order to interpret the following transformations it must be supposed that in the 4,7-dimethoxyindole and perhaps to a much greater extent in its demethylated derivatives an increased electron density on the C-5 and C-6 positions may take place, conferring a partial negative charge on them.

This hypothesis seems to explain the isomerisation of the 4,7-dihydroxyindole derivatives, in which a migration of the two hydrogens of the hydroxyls on the C-5 and C-6 positions occurs, thus yielding the 4,7-dioxo-4,5,6,7-tetrahydro forms; this isomerisation is totally or partially prevented by the presence of an electron-withdrawing group at the C-2 or C-3 positions of the indole nucleus.^{4,6,9}

Considering therefore the character of the bond between the C-5 and C-6 positions, it is likely to think out an acid-catalyzed addition, in which H^+ is the attacking species.

The resulting carbonium ion of the first indole unit

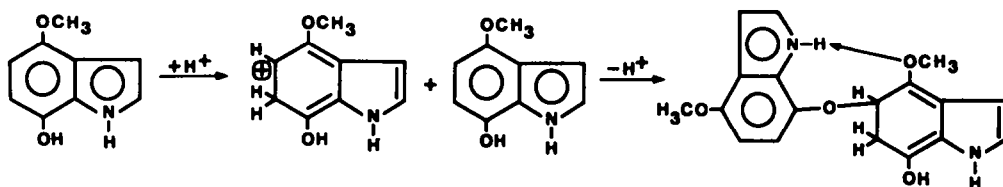


combines with the free hydroxyl of a second molecule, thus forming a dimer.

The proposed structure of this dimer is presumably stabilized and made more compact by the possibility of a hydrogen bond between the imino group of the second indole unit and the methoxyl group on the C-4 position of the first molecule.

With a similar mechanism the polymerisation may continue linearly, binding an increasing number of indole units.

2-[³H]methoxy-5-methoxybenzaldehyde 5. To a suspension of 3.75 g (0.025 mol) of 2-hydroxy-5-methoxybenzaldehyde 4 in 24 ml of water, was added dropwise and under continuous stirring a solution of 10.26 g (0.18 mol) of KOH in 21 ml of water and 4.8 ml (6.35 g, 0.05 mol) of tritiated (CH₃)₂SO₄ (specific radioactivity: 120 DPM/μmol × 10³) obtained from the addition of 6 mg of di[³H]methyl sulphate to 6.344 g of cold product. After 12 h of stirring and heating at 62°, the brown mixture was cooled and extracted with Et₂O (3 × 300 ml). The collected ethereal layers were stirred with aqueous KOH, washed with water, dried over anhydrous Na₂SO₄ and evaporated. The residual oil (1.859 g) on



EXPERIMENTAL

M.ps (uncorrected) were determined using a "Büchi-Tottoli" SMP-20 capillary m.p. apparatus. Infrared spectra were recorded on a "Perkin-Elmer" 457 spectrophotometer as KBr discs or between NaCl plates. Nuclear magnetic resonance spectra were obtained on a "Hitachi-Perkin-Elmer" R-24 A spectrometer using (CD₃)₂CO or DMSO-*d*₆ as solvents. Chemical shifts are given in parts per million (δ), downfield from tetramethylsilane, used as internal standard. Integrals correspond satisfactorily to the chemical formula. Molecular weight determinations were carried out with a "Hewlett-Packard" 302-B vapor pressure osmometer. Elemental analyses were performed in the Micro-analytical Laboratory of this Institute. The analytical determinations of the sole ethoxyl groups¹⁴ were carried out using the sulphochromic oxidative method proposed by Kuhn and L'Orsa¹⁵ and readapted on a microscale by Kuhn and Roth.¹⁶ The analyses of both acetoxyl groups and ethoxyl groups were performed by the hydrolytic oxidative method described by Wiesenberger.¹⁷ Radiochemical determinations were carried out by means of a "Packard Tri-Carb" 3375 liquid scintillation spectrometer. Measured volumes of each solution at established concentration were added to 10 ml of dioxan base liquid scintillator, containing 120 g of naphthalene, 4 g of 2,5-diphenyloxazole, 0.075 g of 2,2'-p-phenyl-bis-(5-phenyloxazole) in dioxane up to a solution of 1000 ml. In these conditions the counting efficiency for the tritium was in the range of 25–35 per cent.

The 2-hydroxy-5-methoxybenzaldehyde 4 used as starting material for the synthesis of the labelled compounds outlined in Scheme 1, was purchased from Riedel-de Haën AG.

The di[³H]methyl sulphate (50 mCi/mmol), used for labelling of the compound 4, was purchased from Radiochemical Centre, Amersham.

All substances cited in Scheme 1 were prepared and purified according to the procedures described in our previous publication¹ and will not be reviewed here.

Compound 9, employed as a starting material in the polymerisation, was synthesized as described in our previous paper⁸ and compound 10 was prepared by literature procedures.^{18,19}

Preparation of a dimer from 4,7-dimethoxyindole. 0.4 g of 4,7-dimethoxyindole 1 dissolved in a mixture of 40 ml of EtOH and 40 ml of conc HCl was refluxed on a water bath for 3 h. The crystalline black precipitate which separated on refrigeration, was collected, washed several times with water, dried and dissolved in absolute boiling EtOH (40 ml). Concentration to a ca. 2 ml by rotary evaporator produced the separation, upon refrigeration, of a black crystalline substance, infusible up to 320°C. Found: C, 65.83; H, 5.71; OCH₃, 19.48; m.w. 326.6–319.1. Calc. for C₁₈H₁₄N₂O₄ (dimer formed from two molecules of methoxy-hydroxyindole): C, 66.24; H, 5.56; OCH₃, 19.02%; m.w. 326.34.

standing overnight at room temperature solidified into a dense mass (m.p. 51–52°). Specific activity: 60.3 DPM/μmol × 10³.

2-[³H]methoxy-5-methoxy-6-nitrobenzaldehyde 6. This compound, m.p. 166°, was obtained in a very good yield by mild nitration of 2-[³H]methoxy-5-methoxybenzaldehyde, operating as it was already described.¹ Specific activity: 56.7 DPM/μmol × 10³.

2-[³H]methoxy-5-methoxy-6-β-dinitrostyrene 7. A mixture of 1 g of 2-[³H]methoxy-5-methoxy-6-nitrobenzaldehyde and 2 g of cold 2,5-dimethoxy-6-nitrobenzaldehyde by condensation with nitromethane in the conditions previously described¹ yielded 2.393 g of tritiated styryl derivative, m.p. 192°. Specific activity: 18.6 DPM/μmol × 10³.

4-[³H]methoxy-7-methoxyindole 8. The reduction of an ethanol-acetic acid solution of 2.3 g of 2-[³H]methoxy-5-methoxy-6-β-dinitrostyrene with iron powder, according to the conditions already reported,¹ produced 0.947 g of 4-[³H]methoxy-7-methoxyindole, m.p. 129–130°. Specific activity: 16.4 DPM/μmol × 10³.

Polymerisation of 4-[³H]methoxy-7-methoxyindole. (a) 3 h of reflux. A mixture of 200 mg of 4-[³H]methoxy-7-methoxyindole in 20 ml of concentrated HCl and 20 ml of EtOH was heated at reflux on a water bath for 3 h. The brown reaction mass was cooled, filtered and washed several times with water and finally dried to yield 22.1 mg of a brown solid, which was dissolved in absolute boiling EtOH (20 ml); the resulting solution was concentrated by rotary evaporator to ca. 1.5 ml. Upon refrigeration, a crystalline brown polymer was collected, infusible up to 320°.

The m.w. found (320.3–339.2) and the analytical data for methoxyl groups (found 19.01–18.73%) support the structure of a dimethoxyindole dimer. Calcd. for C₁₈H₁₄N₂O₄ (dimer corresponding to two molecules of hydroxy-methoxyindole) m.w. 326.34; methoxyl groups 19.02%.

The specific radioactivity of this polymer (32.81 DPM/μmol × 10³) indicated that polymerisation in this case left the tritiated methoxyl groups unchanged.

(b) 18 h of reflux. Following a previously described procedure,¹⁰ the solution of 200 mg of 4-[³H]methoxy-7-methoxyindole in a mixture of 20 ml of EtOH and 20 ml of concentrated HCl was refluxed for 18 h. Filtering, washing with water and drying left 42.5 mg of a black solid, which could be dissolved in 40 ml of boiling EtOH. Concentration of the solution with a rotary evaporator (ca. 2 ml) produced a polymer infusible up to 320°.

Analytical data: m.w. 1119; found OCH₃, 9.17%; Specific radioactivity: 46.75 DPM/μmol × 10³. Calcd. for C₃₀H₂₂N₂O₁₄ (heptamer corresponding to three molecules of 4-[³H]methoxy-7-hydroxyindole and four molecules of 4,7-dihydroxyindole) m.w. 1086.1; OCH₃, 8.57; calcd. specific activity: 49.08 DPM/μmol × 10³.

Preparation of the black polymer from 4-ethoxy-7-methoxyindole. A solution of 4-ethoxy-7-methoxyindole **9** (0.5 g) in a mixture of absolute EtOH (50 ml) and concentrated HCl (50 ml) was refluxed on a water bath for 18 h. The dark suspension obtained was cooled and the solid collected by suction filtration, washed repeatedly with water and dried. This crude polymer was dissolved in absolute EtOH (100 ml) and, after filtration, the solution was concentrated with a rotary evaporator to ca. 10 ml. After standing at 0°C overnight, the black solid was collected and crystallized again from absolute EtOH, repeating the procedure previously described. Crystalline black substance, infusible up to 320°C. Found: C, 65.87, 66.02, 65.86 (65.92)†; H, 5.22, 5.48, 5.03 (5.24)†; N, 8.24, 8.43, 8.21 (8.29)†; O, 19.76, 19.34, (19.55)†; OC₂H₅, 11.66, 12.65, 11.90 (12.07)†. This polymer contained no methoxyl groups. Calcd. for C₆₂H₈₁N₇O₁₄ (heptamer corresponding to three molecules of 4-ethoxy-7-hydroxyindole and four molecules of 4,7-dihydroxyindole): C, 66.00; H, 5.45; N, 8.69; O, 19.86; OC₂H₅, 11.98%.

Ethylation of the black polymer obtained from 4-ethoxy-7-methoxyindole. To a stirred suspension of 0.12 g of black heptamer in 8 ml of water in a three-necked flask, 4.2 ml of 10% NaOH solution and 1.2 ml of Et₂SO₄ were added dropwise. When addition was complete, the mixture was heated at 50° under continuous stirring and N₂ for 5 h. The resulting mixture was then cooled, the black crude product collected, washed repeatedly with water and dried in vacuum. This compound was dissolved in absolute boiling EtOH (300 ml); the solution was filtered, concentrated to ca. 8 ml with a rotary evaporator and leaved to crystallize at 0°. For analysis it was recrystallized from EtOH; it was infusible up to 320°. Found: C, 68.14, 67.65, 68.05 (67.94)†; H, 6.17, 5.80, 6.30 (6.09)†; OC₂H₅, 25.38, 25.46 (25.42)†. Calc. for C₇₀H₇₇N₇O₁₄ (heptamer) C, 67.78; H, 6.26; OC₂H₅, 25.42%.

Acetylation of the black polymer obtained from 4-ethoxy-7-methoxyindole. A mixture of 0.10 g of black heptamer, 1.2 g of powdered anhydrous sodium acetate and 6 ml of acetic anhydride was refluxed for 6 h. Upon cooling, sufficient water to hydrolyse the acetic anhydride was added to the reaction mixture. After ca. 1 h stirring, the solvents were evaporated in vacuum and the residue washed with water. After drying, the crude product was dissolved in 250 ml of absolute boiling EtOH; the solution, concentrated with rotary evaporator at ca. 10 ml and allowed to stand overnight at 0°, yielded a crystalline precipitate, which was filtered and recrystallized from absolute EtOH. Crystalline black polymer, infusible up to 320°. Found: C, 64.99, 64.97 (64.98)†; H, 5.26, 5.13 (5.20)†; CH₃⁽¹⁷⁾ 8.32, 8.38 (8.35)†. Calc. for C₇₀H₆₉N₇O₁₈ (heptamer corresponding to three molecules of 4-ethoxy-7-hydroxyindole and four molecules of 4-acetoxy-7-hydroxyindole): C, 64.85; H, 5.37; CH₃⁽¹⁷⁾, 8.11%.

2-Methoxy-5-ethoxybenzaldehyde 11. To a suspension of 13.34 g (0.08 mol) of 2-hydroxy-5-ethoxybenzaldehyde **10** in 80 ml of water, a solution of 33.2 g (0.59 mol) of KOH in 70 ml of water and 15.3 ml (20.38 g; 0.15 mol) of Me₂SO₄ was slowly added under stirring and heating at 60°. After 6 h, the brown mixture was cooled and extracted with Et₂O (3 × 500 ml). The organic layer was washed with water, dried over anhydrous Na₂SO₄ and stripped of solvent under reduced pressure; the crude yellowish oily residue (13.78 g) was distilled then at 40° (10⁻² Torr), to give a colourless fluid oil.

Found (Calc. for C₁₀H₁₂O₃): C, 66.40 (66.65); H, 6.52 (6.71)%. IR: 2864–2976 (ν CH₃, ν C₂H₅), 1675 (ν C=O) cm⁻¹. NMR [(CD₃)₂CO]: 10.25 (1H, s, CHO), 7.07 (3H, s, 2d, aromatic protons), 3.99 (2H, q, CH₂ of ethyl), 3.86 (3H, s, OCH₃) and 1.33 (3H, t, CH₃ of ethyl).

2-Methoxy-5-ethoxy-6-nitrobenzaldehyde 12. The nitration was carried out with 65% HNO₃ at 0° on slightly stirred aliquots of compound **11**. Each reaction flask containing 3 g of 2-methoxy-5-ethoxybenzaldehyde was cooled in an ice water bath while 5 ml of HNO₃ was added dropwise under stirring. Immediately afterwards, 25 g of crushed ice were added to the mixture. After 10 min the crude yellow product was collected, thoroughly washed with water, dried and crystallized from EtOH. Two recrystal-

izations from the same solvent afforded the title compound, m.p. 183°. Found (Calc. for C₁₀H₁₁NO₅): C, 53.39 (53.33), H, 5.09 (4.92), N, 6.26 (6.22)%. IR: 1547 and 1384 (ν NO₂) cm⁻¹. NMR [(CD₃)₂CO]: 10.31 (1H, s, CHO), 7.61–7.35 (2H, 2d, aromatic protons), 4.19 (2H, q, CH₂ of ethyl), 4.01 (3H, s, OCH₃) and 1.32 (3H, t, CH₃ of ethyl).

2-Methoxy-5-ethoxy-6,β-dinitrostyrene 13. A cooled mixture of 2.95 g of 2-methoxy-5-ethoxy-6-nitrobenzaldehyde (0.013 mol) and 1.7 g of CH₃NO₂ (1.53 ml; 0.027 mol) in 60 ml of EtOH was treated with a solution of 1.64 g of KOH in 4 ml of water (0.029 mol) added dropwise, temperature being maintained at 6–8°. After standing 19 h at the same temperature, the mixture was poured into a large quantity of water, acidified with conc. HCl, and then extracted with Et₂O. The organic phase was washed with water, dried (Na₂SO₄) and concentrated under vacuum to give 3.18 g of a solid, which was then dehydrated under reflux for 15 min with Ac₂O (10 ml) and AcONa (2 g). After cooling, the mixture was diluted to 50 ml with water, stirred to obtain a homogeneous suspension, and the precipitate collected and washed several times with water. The crude product was crystallized from dioxane: bright yellow crystals (1.99 g), m.p. 175°. Found (Calc. for C₁₁H₁₂N₂O₆): C, 48.98 (49.25); H, 4.55 (4.51); N, 10.68 (10.45)%. NMR (DMSO-d₆): 7.97–7.49 (2H, 2d, HCα and HCβ), 7.52–7.32 (2H, 2d, HC₂ and HC₄), 4.17 (2H, q, CH₂ of ethyl), 3.97 (3H, s, OCH₃), 1.29 (3H, t, CH₃ of ethyl).

4-Methoxy-7-ethoxyindole 14. To a solution of 1.95 g of 2-methoxy-5-ethoxy-6,β-dinitrostyrene **13** in 45 ml of EtOH, 8.6 g of reduced iron powder and 32 ml of AcOH were added. After heating at reflux for 0.5 h, the mixture was cooled and filtered, washing carefully the solid part with warm EtOH. The combined filtrates, diluted with water and adjusted to ca. pH 8 by addition of NaHCO₃, were extracted with Et₂O (4 × 400 ml). The extracts were dried (Na₂SO₄) and concentrated under reduced pressure, leaving a viscous oil, which solidified upon standing. Sublimation at 65–70° (2 × 10⁻² Torr) yielded a compound which was crystallized from benzene: white needles, m.p. 72–73°. Found (Calc. for C₁₁H₁₃NO₂): C, 69.28 (69.09); H, 6.79 (6.85); N, 7.01 (7.33)%; OC₂H₅, 23.71 (23.56)%; OCH₃, 16.26 (16.22)%. IR: 3414 (ν NH) cm⁻¹. NMR [(CD₃)₂CO]: 10.15 (broad band, NH), 7.09–6.46 (2H, 2a, HC₂, HC₃), 6.40–6.31 (2H, 2d, HC₄ and HC₆), 4.06 (2H, q, CH₂ of ethyl), 3.81 (3H, s, OCH₃) and 1.37 (3H, t, CH₃ of ethyl).

Polymerisation of 4-methoxy-7-ethoxyindole. Following the procedure described above for preparing the black polymer from the 4-ethoxy-7-methoxyindole **4**, the solution of 4-methoxy-7-ethoxyindole **9** (0.5 g) in a mixture of 50 ml of absolute EtOH and 50 ml of concentrated HCl was refluxed for 18 h. After cooling, the precipitate was collected, washed several times with water and dissolved in 60 ml of boiling absolute EtOH. The filtered solution, concentrated with rotary evaporator to ca. 7 ml, yielded, after standing overnight at 0°, the black polymer, which was collected and recrystallized from absolute EtOH. The compound (0.07 g) was infusible up to 320°. Found: C, 65.64; H, 5.18; OC₂H₅, 11.64%. Calc. for C₆₀H₆₁N₇O₁₄ (heptamer corresponding to three molecules of 4-hydroxy-7-ethoxyindole and four molecules of 4,7-dihydroxyindole): C, 66.00; H, 5.45; OC₂H₅, 11.98%.

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