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Oxidation Chemistry of 5,6-Dihydroxy-2-methylindole

Alessandra Napolitano, Orlando Crescenzi, Kostantino Tsiakas and Giuseppe Prota*

Department of Organic and Biological Chemistry, University of Naples, Via Mezzocannone 16, I-80134 Naples, Italy.

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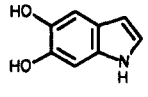
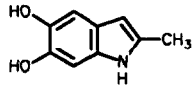
Abstract: In aqueous buffer at neutral pHs, 5,6-dihydroxy-2-methylindole (**2**) is rapidly oxidised by the peroxidase/H_2O_2 system to give in the early phases a complex mixture of oligomers, the major of which were isolated as the *O*-acetyl derivatives. Five of these were formulated as the 3,3'-biindolyl **3**, the isomeric dimers **4-6** and trimer **9**, arising by coupling of the indole ring at the nuclear positions, whereas the remaining ones were assigned structures **7-8**, marked by methylene bridges between the indole moieties, and notably **10**, featuring a tetrahydrocyclopentadiindole ring system.

The 5,6-dihydroxyindole system has attracted considerable attention over the years in view of the peculiarity of its chemistry, which combines the features associated to the oxidisability of *o*-diphenols with those characteristic of the electron rich indole ring.[1] Moreover, a major stimulus for research in the field stems from the critical role of 5,6-dihydroxyindoles in the biosynthesis of melanins, the pigments of human hair, skin and brain *substantia nigra*.[2,3]

In previous studies we found that the oxidative polymerisation of the major melanogenetic precursor, 5,6-dihydroxyindole (**1**), to the pigment proceeds by coupling of the 2- position of one indole unit with the 4- or 7- positions of another,[4] rather than at the 3- and 7- positions, as previously suggested.[5] A similar oxidative behaviour was exhibited by the 1-methyl derivative,[6] whereas 5,6-dihydroxyindole-2-carboxylic acid was found to polymerise invariably through the 4- and 7- positions.[7]



1



2

As an extension of this investigation, we have now turned our attention to the effect of substitution on the mode of polymerisation of the 5,6-dihydroxyindole system. Early studies carried out in the fifties[8,9] showed that, in the case of 3-, 4-, and 7-methyl-5,6-dihydroxyindole, as well as of some dimethyl derivatives, the normal course of the aerial or enzyme-promoted polymerisation of the 5,6-dihydroxyindole system was deflected, as judged from the color changes observed during the reaction and the solubility of the resulting pigments. On the other hand, oxidation of 5,6-dihydroxy-2-methylindole (**2**) was found to lead to the rapid formation of a dark brown pigment closely resembling a true melanin. While the oxidative behaviour of the

former indoles is consistent with the critical involvement of the 4- and 7- positions in the polymerisation process, the reported reactivity of **2** was intriguing and a re-examination of the its oxidation chemistry under the conditions of melanin biosynthesis appeared desirable.

As the oxidising system, the peroxidase/hydrogen peroxide couple was chosen in view of its effectiveness in converting 5,6-dihydroxyindoles to melanin pigments, as shown in our previous investigations.[4,10] Periodical HPLC analysis of the reaction mixture showed that, under these oxidation conditions, consumption of **2** was complete within a few minutes. When the course of the enzymic oxidation of **2** was followed spectrophotometrically, no transient chromophoric species reminiscent of the purple intermediate (melanochrome) described in the polymerisation of **1** [8] could be detected. Analysis of the pigment formed at complete consumption of the starting indole proved difficult because of its insolubility in all solvents, even after acetylation treatment. However, if the oxidation was stopped in the early stages, a cromatographically well-defined mixture could be obtained, from which eight oligomeric products were isolated in the acetylated form by HPLC fractionation.

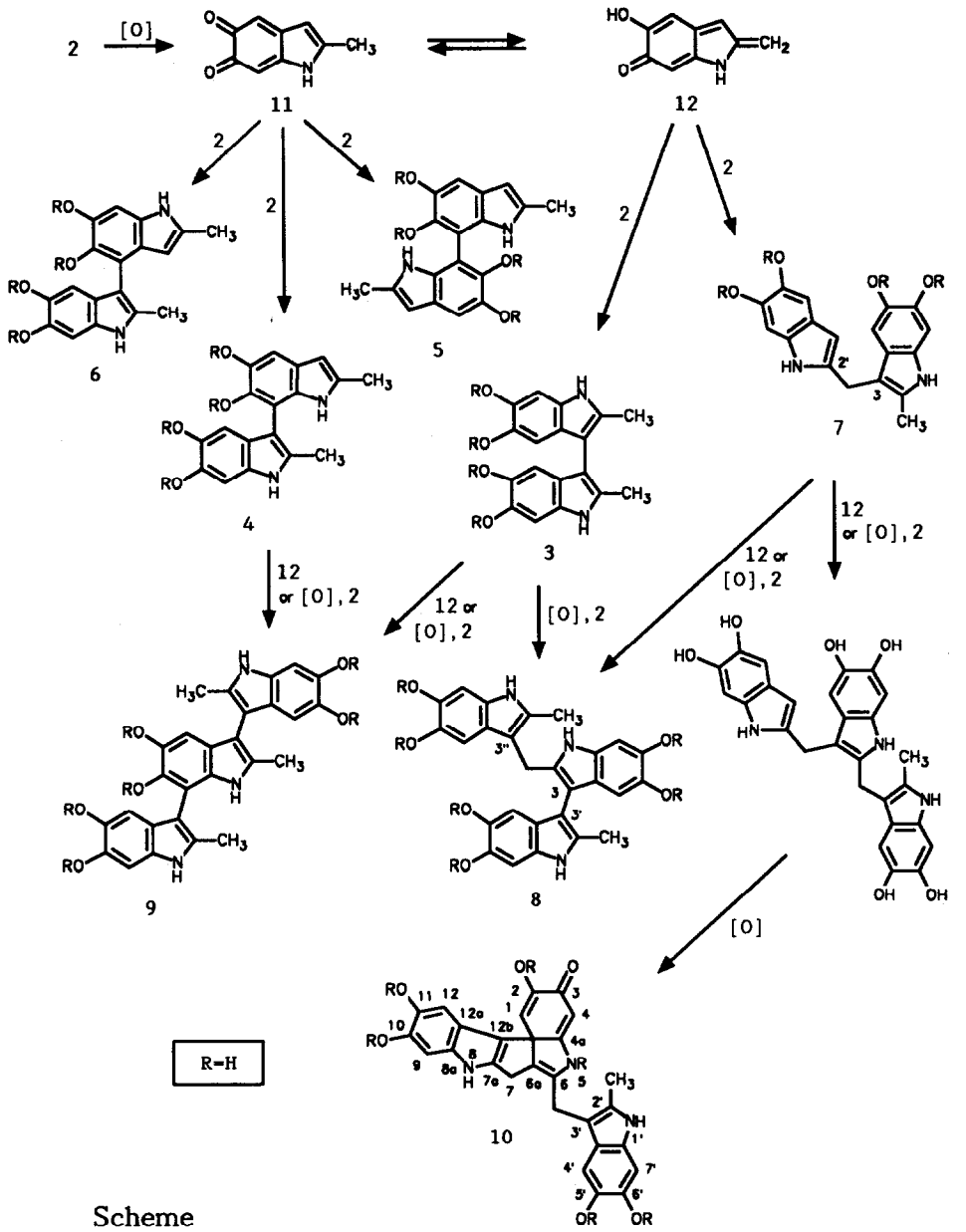
The most abundant of these was characterised as the symmetrical dimer **3** (R=Ac) by straightforward spectral analysis, whereas other three components of the mixture proved to be the isomeric dimers **4-6** (R=Ac).

The ^{1}H NMR spectrum of the fifth oligomer displayed five signals in the aromatic region, attributable to a C-3 substituted 2-methyl indole unit and to the H-4, H-7 and H-3 protons of another indole moiety, whereas in the sp^3 region a 2H singlet at δ 4.17 ppm was clearly distinguishable. These features pointed to a dimeric structure in which the indole moieties are linked through a methylene bridge, as in **7** (R=Ac). Consistent with this structural assignement was the presence in the ^{13}C NMR spectrum of a C-3 carbon singlet at δ 108.40 ppm and a methylene resonance at δ 23.62 ppm which, as evidenced by 2D heteronuclear correlation experiments, was coupled with the CH_2 protons at δ 4.17 ppm.

The other three oligomers were assigned structures **8**, **9** and **10** (R=Ac) on the following grounds. Compounds **8** and **9** (R=Ac), accounting for about 30% of the total oligomeric fraction, exhibited virtually identical FAB mass spectra, with pseudomolecular ion peaks at *m/z* 738, corresponding to trimeric structures.

The ^{1}H NMR spectrum of **8** (R=Ac) exhibited six aromatic 1H singlet, arising from H-4 and H-7 protons, and a deshielded methylene resonance at δ 4.10 ppm. These data, coupled with analysis of the ^{13}C NMR spectrum, showing six doublets for the C-4 and C-7 carbons, three C-3 carbon singlets and an sp^3 triplet at δ 22.67 ppm, suggested the trimeric structure **8** (R=Ac), which comprises those of both dimers **3** and **7** (R=Ac).

Formulation of the terindolyl **9** (R=Ac) followed from careful analysis of the pattern of resonances in the carbon NMR spectrum, providing evidence for a central indole unit linked through the 3- and 7- positions to the 3-positions of the outer indole rings. An intriguing feature of the proton was the presence of a number of signals doubled with respect to that required for structure **9** (R=Ac). All of these, however, could be clearly divided into two closely related, but distinct, sets, with areas approximately in a 1:1 ratio. 2D-Heteronuclear correlation experiments showed that six out of the ten sp^2 protons between δ 6.95 and 7.07 were coupled with the three C-4 carbons, whereas the remaining four, falling in the δ 7.23-7.25 ppm region, were directly attached to the two C-7 carbons. When considered also in the light of the chromatographic behaviour of the product, which moved as a single band under a variety of TLC and HPLC conditions, these data led us to interpret the unusual features exhibited by the proton spectrum in terms of a rotational isomerism of the trimer, two conformations being preferentially adopted. The energy barrier for the interconversion between the two rotamers should be considerably high, as judged from the failure to observe coalescence of the signals even


2
[O]
11
12
2
2
2
2
2
2
6
5
4
3
7
12 or [O], 2
12 or [O], 2
12 or [O], 2
[O], 2
12 or [O], 2
9
8
[O]
10
R=H

Scheme

when the proton spectrum was taken in DMSO at temperatures up to 140 °C.

The remaining oligomer, which was obtained in rather low yields, displayed in the positive FAB mass spectrum the pseudomolecular ion peak at *m/z* 736, two mass units lower than that of trimers **8** and **9** (R=Ac). The ^{1}H NMR spectrum showed in the aliphatic region seven -CH_3 signals and two geminal AB systems, apparently due to methylene groups adjacent to an asymmetric center; six sp^2-singlets and two indolic NH proton resonances were also present. The most salient features of the ^{13}C NMR spectrum were the signals of an aliphatic quaternary carbon (δ 61.61 ppm), a conjugated carbonyl group at δ 189.10 ppm, and a deshielded sp^2 carbon at δ 152.63 ppm, the latter two suggestive of a β-aminoenone system. All together, these data could be accommodated by structure **10** (R=Ac), marked by a tetrahydrocyclopenta[1,2-*b*:4,3-*c*']diindole ring system. Support to the proposed structure was provided by the presence in the mass spectrum of a major peak at *m/z* 260, apparently generated from fragmentation of the compound with loss of the tetrahydrocyclopentadiindole moiety.

From a mechanistic view point, formation of the oligomers **3-10** (R=H) can be envisaged as the result of an ionic-type process in which the 3- and to a much lesser extent the 7- position of the indole **2** brings about nucleophilic attack to the electron deficient sites of an oxidised counterpart, seemingly the indole-*o*-quinone **11** or its tautomeric quinone methide **12**. As depicted in the Scheme, the major dimeric product **3** (R=H) can undergo oxidative coupling with the starting indole **2** to give trimers **8** and **9** (R=H); alternatively, these can be derived from dimers **7** and **4** (R=H), respectively, through an analogous series of reactions. Dimer **7** (R=H) is also involved in the formation of **10** (R=H), through the intermediacy of a bis(indolylmethyl)indole, which by intramolecular oxidative cyclisation generates eventually the cyclopentadiindole ring system. Interestingly, evidence supporting the tautomerisation of indol-5,6-quinones to the corresponding quinone-methides has been recently provided by pulse radiolysis kinetic experiments.[11,12] Moreover, the formation of methylene bridges during oxidative polymerisation of 2-methyl substituted indoles is not unprecedented in the literature.[13]

Apart from the chemical interest, elucidation of the reactivity of **2** toward the peroxidase/H_2O_2 couple, a major enzymic system in brain,[14] may have some bearing to the metabolism in the central nervous system of α-methyldopa, a potent antihypertensive agent[15] from which the indole is readily generated by oxidation.[16]

EXPERIMENTAL SECTION

M.ps. were determined with a Kofler hot-stage apparatus and are uncorrected. UV spectra were performed with a Perkin-Elmer Lambda 7 spectrophotometer. EIMS and high resolution mass spectra were determined with a Kratos MS 50 spectrometer. Samples were ionized with a 70 eV beam. Main fragmentation peaks (above *m/z* 100) are reported with their relative intensities (percent values are in brackets). Fast atom bombardment (FABMS) mass spectra, positive ion mode (matrix: glycerol) were run on a Kratos MS 50 spectrometer. ^{1}H NMR (270 MHz) and ^{13}C NMR (67.9 MHz) spectra were recorded on a Bruker AC 270 spectrometer, using TMS as an internal standard. HPLC was performed using a Gilson model 305 pump with a Gilson 316 UV detector. A 21.4 x 250 mm C18 Rainin Dynamax column (20 mL/min), a 10 x 250 mm Alltech Econosil C18 column (6 mL/min) and a Spherisorb S5 ODS2 column (1 mL/min) were used for preparative, semipreparative and analytical runs, respectively. Detection was carried out at 280 nm. α-Methyldopa and horseradish peroxidase (donor: H_2O_2 oxidoreductase, EC 1.11.1.7.) type II (175 U/mg, RZ E_{430}/E_{275}= 2.0) were from Sigma.

Synthesis of 2

Preparation of **2** was carried out by a modication of a previously reported procedure[16]. In a typical run, a solution of α-methyldopa sesquihydrate (7.20 g) in water (3 L), extensively deareated by purging with nitrogen, was treated with a solution of potassium ferricyanide (39.6 g) and sodium bicarbonate (15.0 g) in water (360 mL). The reaction mixture was allowed to stand under nitrogen until the red-orange tint, which developed on addition of the oxidant, had turned to pale brown. At this stage, the mixture was treated with sodium dithionite and extracted repeatedly with ethyl acetate. The combined organic layers were washed with a saturated solution of sodium chloride, dried over sodium sulphate and taken to dryness. Chromatographic fractionation of the resulting residue (2.40 g) over a polyamide column (3 x 40 cm) using a 5-20% methanol-ethyl acetate gradient as the eluent afforded pure **2** [17] as a pale-yellow solid (2.10 g).

Oxidation of 2

To a solution of **2** (2.00 g) and horseradish peroxidase (6300 pyrogallol units) in 0.1 *M* phosphate buffer, pH 7.0 (920 mL), 1.5% hydrogen peroxide (27 mL) was added under vigorous stirring. The reaction mixture immediately turned to dark-brown and a precipitate began to form. After 3 minutes the oxidation was stopped by addition of sodium dithionite.The pale-brown mixture was repeatedly extracted with ethyl acetate and the combined organic layers were washed with saturated sodium chloride solution, dried over sodium sulphate and taken to dryness. The residue thus obtained (1.80 g) was acetylated with acetic anhydride-pyridine at room temperature overnight. Preparative HPLC fractionation of the resulting brown oil (2.40 g), using water-acetonitrile 60:40 as the mobile phase, afforded six main bands with Rt 11.6, 18.1, 21.4, 25.0, 31.1 and 45.0 min. The first (680 mg) and the third (200 mg) of these were found to consist of the acetylated starting indole and the symmetrical dimer **3** (R=Ac), respectively, in pure form. Crystallisation of the Rt 31.1 band from ethanol afforded product **4** (R=Ac, 60 mg) as colourless needles. The mother liquors (80 mg), consisting mainly of two components, yielded, after purification by preparative HPLC (methanol-water 55:45), dimers **5** (R=Ac, 10 mg) and **7** (R=Ac, 50 mg). The Rt 18.1 (30 mg) and Rt 25.0 (25 mg) bands were purified by semipreparative HPLC using methanol-water 50:50 as the mobile phase to give dimer **6** (R=Ac, 20 mg) and trimer **10** (R=Ac, 5 mg), respectively. Trimers **8** (R=Ac, 70 mg) and **9** (R=Ac, 65 mg) were obtained in pure form by HPLC fractionaction of the slowest moving band (150 mg) on the semipreparative column using water-acetonitrile 60:40 as the eluent.

5,6-Diacetoxy-2-methylindole. M.p. 114-116 °C. UV: λ_{max} 288 nm (log ε 3.87); EIMS, *m/z*: 247 (M^+, 34), 205 (28), 163 (100); ^{1}H NMR (acetone-d_6), δ (ppm): 2.22 (3Hx2, -$COCH_3$), 2.40 (3H, d, *J*= 0.7 Hz, -CH_3), 6.14 (1H, m, H-3), 7.11 (1H, d, *J*= 0.7 Hz, H-7), 7.18 (1H, s, H-4), 10.15 (1H, bs, NH); ^{13}C NMR (acetone-d_6), δ (ppm): 13.48 (q, -CH_3), 20.52 (q, -$COCH_3$), 100.45 (d, C-3), 105.48 (d, C-7), 113.09 (d, C-4), 127.40 (s, C-9), 134.34 (s, C-2), 137.00 (s, C-5), 137.96 (s, C-8), 138.07 (s, C-6), 169.32, 169.45 (s, -$COCH_3$).

5,5',6,6'-Tetracetoxy-2,2'-dimethyl-3,3'-biindolyl (3, R=Ac). Prisms from ethanol, m.p. 191-193 °C (dec); UV: λ_{max} (EtOH) 295 nm (log ε 4.10); HRMS, *m/z* 492.1517 (M^+) (calc. for $C_{26}H_{24}N_2O_8$: 492.1533). EIMS, *m/z*: 492 (48), 450 (33), 408 (88), 366 (100), 324 (100), 163 (48); ^{1}H NMR (DMSO-d_6), δ (ppm): 2.18 (6H, s, -CH_3, -CH_3'), 2.26 (6Hx2, s, -$COCH_3$), 6.82 (2H, s, H-7, H-7'), 7.18 (2H, s, H-4, H-4'), 11.28

(2H, bs, -NH, NH'); ^{13}C NMR (DMSO-d_6), δ (ppm): 12.21 (q, -CH_3, -CH_3'), 20.09, 20.18 (q, -$COCH_3$), 104.69 (s, C-3, C-3'), 104.80 (d, C-7, C-7'), 111.30 (d, C-4, C-4'), 125.83 (s, C-9, C-9'), 132.36 (s, C-2, C-2'), 134.68 (s, C-5, C-5'), 135.27 (s, C-8, C-8'), 136.54 (s, C-6, C-6'), 168.61 (s, -$COCH_3$).

5,5',6,6'-Tetracetoxy-2,2'-dimethyl-3,7'-biindolyl (4, R=Ac). Needles from ethanol, m.p. 177-179 °C; UV: λ_{max} (EtOH) 292 nm (log ε 4.29); HRMS, *m/z* 492.1545 (M^+) (calc. for $C_{26}H_{24}N_2O_8$: 492.1533); EIMS, *m/z*: 492 (7), 450 (36), 408 (54), 366 (61), 324 (100); ^{1}H NMR (DMSO-d_6), δ (ppm): 1.83 (3H, s, -CH_3), 2.19 (3H, s, -$COCH_3$), 2.21 (3H, s, -$COCH_3$), 2.25 (3H, s, -$COCH_3$), 2.27 (3H, s, -$COCH_3$), 2.31 (3H, d, *J*= 0.7 Hz, -CH_3'), 6.18 (1H, dq, *J*= 1.9, 0.7 Hz, H-3'), 6.73 (1H, s, H-7), 7.22 (1H, s, H-4 or H-4'), 7.23 (1H, s, H-4 or H-4'), 10.51 (1H, bs, NH'), 11.46 (1H, bs, NH); ^{13}C NMR (DMSO-d_6), δ (ppm): 12.57, 13.40 (q, -CH_3, -CH_3'), 19.92, 20.34, 20.47, 20.51 (q, -$COCH_3$), 99.58 (d, C-3'), 104.00 (s, C-3), 105.00 (d, C-7), 111.05, 111.46 (d, C-4, C-4'), 111.28 (s, C-7'), 125.05, 125.37 (s, C-9, C-9'), 132.56, 133.07 (s, C-2, C-2'), 134.98, 135.55 (s, C-5, C-5'), 135.96, 136.38 (s, C-6, C-6'), 136.77, 137.75 (s, C-8, C-8'), 168.33, 168.85, 168.92, 169.02 (s, -$COCH_3$).

5,5',6,6'-Tetracetoxy-2,2'-dimethyl-7,7'-biindolyl (5, R=Ac). Colourless oil; UV: λ_{max} (EtOH) 295 nm; HRMS, *m/z* 492.1514 (M^+) (calc. for $C_{26}H_{24}N_2O_8$: 492.1533); EIMS, *m/z*: 492 (10), 450 (85), 408 (100), 366 (99), 324 (92); ^{1}H NMR (acetone-d_6), δ (ppm): 1.91 (6H, s, -$COCH_3$), 2.22 (6H, s, -$COCH_3$), 2.33 (6H, d, *J*= 0.8 Hz, -CH_3, -CH_3'), 6.19 (2H, dq, *J*=2.0, 0.8 Hz, H-3, H-3'), 7.27 (2H, s, H-4, H-4'), 9.71 (2H, bs, NH, NH'); ^{13}C NMR, DEPT (acetone-d_6), δ (ppm): 13.51 (q, -CH_3, -CH_3'), 20.17, 20.65 (q, -$COCH_3$), 100.77 (d, C-3, C-3'), 113.30 (d, C-4, C-4').

5,5',6,6'-Tetracetoxy-2,2'-dimethyl-3,4'-biindolyl (6, R=Ac). Colourless oil; UV: λ_{max} (EtOH) 295 nm; HRMS, *m/z* 492.1517 (M^+) (calc. for $C_{26}H_{24}N_2O_8$: 492.1533); EIMS, *m/z*: 492 (17), 450 (25), 408 (54), 366 (66), 324 (100); ^{1}H NMR (acetone-d_6), δ (ppm): 1.84 (3H, s, -CH_3), 2.20 (3H, s, -$COCH_3$), 2.24 (3Hx2, s, -$COCH_3$), 2.29 (3H, s, -$COCH_3$), 2.37 (3H, d, *J*=0.7 Hz, -CH_3'), 5.86 (1H, ddq, *J*= 1.5, 0.8, 0.7 Hz, H-3'), 6.93 (1H, s, H-7), 7.17 (1H, d, *J*= 0.8 Hz, H-7'), 7.20 (1H, s, H-4), 10.15 (1H, bs, NH or NH'), 10.35 (1H, bs, NH or NH'); ^{13}C NMR (acetone-d_6), δ ppm): 13.00, 13.64 (q, -CH_3, -CH_3'), 20.20, 20.50, 20.62, 20.70 (q, -$COCH_3$), 101.18 (d, C-3'), 104.59, 105. 65 (d, C-7, C-7'), 108.62 (s, C-3), 113.37 (d, C-4), 119.58 (s, C-4'), 126.47, 128.07 (s, C-9, C-9'), 133.72, 133.90 (s, C-2, C-2'), 135.47, 136.19 (s, C-5, C-5'), 137.10, 137.51 (s, C-6, C-6'), 138.48, 138.82 (s, C-8, C-8'), 168.86, 169.27, 169.32, 169.39 (s, -$COCH_3$).

5,6-Diacetoxy-3-[(5,6-diacetoxyindol-2-yl)methyl]-2-methylindole (7, R=Ac). Prisms from ethanol, m.p. 145-147 °C; UV: λ_{max} (EtOH) 291 nm; HRMS, *m/z* 492.1532 (M^+) (calc. for $C_{26}H_{24}N_2O_8$: 492.1533). EIMS, *m/z*: 492 (44), 450 (87), 408 (92), 366 (100), 324 (10), 162 (67); ^{1}H NMR (acetone-d_6), δ (ppm): 2.18 (3H, s, -$COCH_3$), 2.21 (3H, s, -$COCH_3$), 2.22 (3Hx2, s, -$COCH_3$), 2.38 (3H, s, -CH_3), 4.17 (2H, s, -CH_2-), 6.18 (1H, m, H-3'), 7.07 (1H, d, *J*=0.7 Hz, H-7'), 7.12 (1H, s, H-7), 7.13 (1H, s, H-4 or H-4'), 7.18 (1H, s, H-4 or H-4'), 10.05 (1H, bs, NH'), 10.12 (1H, bs, NH); ^{13}C NMR (acetone-d_6), δ (ppm): 11.59 (q, -CH_3), 20.47, 20.53, 20.54, 20.55 (q, -$COCH_3$), 23.62 (t, -CH_2-), 100.14 (d, C-3'), 105.60, 105.72 (d, C-7, C-7'), 108.40 (s, C-3), 111.83, 113.37 (d, C-4, C-4'), 127.10, 127.23 (s, C-9, C-9'), 133.53, 134.48 (s, C-2, C-2'), 135.32, 136.95 (s, C-5, C-5'), 137.10, 138.12 (s, C-6, C-6'), 138.31, 141.93 (s, C-8, C-8'), 169.24, 169.30, 169.40 (s, -

$COCH_3$).

2-[(5,6-Diacetoxy-2-methylindol-3-yl)methyl]-2'-methyl-5,5',6,6'-tetraacetoxy-3,3'-biindolyl (8, R=Ac). Colourless oil; UV: λ_{max} (EtOH) 294 nm; FABMS, *m/z*: 738 $(M+H)^+$, 696, 654, 612; ^1H NMR (acetone-d_6), δ (ppm): 2.17 (3Hx2, s, $-CH_3$ or $-COCH_3$), 2.19 (3H, s, $-CH_3$ or $-COCH_3$), 2.20 (3H, s, $-CH_3$ or $-COCH_3$), 2.21 (3H, s, $-CH_3$ or $-COCH_3$), 2.25 (3Hx2, s, $-CH_3$ or $-COCH_3$), 2.39 (3H, s, $-CH_3$), 4.10 (2H, s, $-CH_2-$), 6.91 (1H, s, H-7, H-7' or H-7"), 6.97 (1H, s, H-7, H-7' or H-7"), 7.05 (1H, s, H-7, H-7' or H-7"), 7.06 (1H, s, H-4, H-4' or H-4"), 7.07 (1H, s, H-4, H-4' or H-4"), 7.22 (1H, s, H-4, H-4' or H-4"), 10.05 (1H, bs, NH, NH' or NH"), 10.10 (1H, bs, NH, NH' or NH"), 10.40 (1H, bs, NH, NH' or NH"); ^{13}C NMR (acetone-d_6), δ (ppm): 11.54, 12.83 (q, $-CH_3$), 20.43, 20.52, 20.57, 20.58, 20.62 (q, $-COCH_3$), 22.67 (t, $-CH_2-$), 105.50, 105.80, 105.98 (d, C-7, C-7', C-7"), 106.27, 106.66 (s, C-3, C-3'), 108.53 (s, C-3"), 111.78, 112.88, 112.94 (d, C-4, C-4', C-4"), 127.06, 127.48, 127.67 (s, C-9, C-9', C-9"), 133.47, 133.97, 134.00 (s, C-2, C-2', C-2"), 135.33, 136.07, 136.86 (s, C-5, C-5', C-5"), 137.13, 137.20, 138.27 (s, C-6, C-6', C-6"), 138.49, 138.50, 139.10 (s, C-8, C-8', C-8"), 169.27, 169.33, 169.37 (s, $-COCH_3$).

5,5',5",6, 6', 6"-Hexaacetoxy-3,3':7',3"-terindolyl (9, R=Ac). Prisms from ethanol, m.p. 183-186 °C (dec); UV: λ_{max} (EtOH) 296 nm (log ε 4.30); FABMS, *m/z*: 738 $(M+H)^+$, 696, 654, 612, 570, 528, 486; ^1H NMR (acetone-d_6), δ (ppm): 1.86 (3H, s, $-CH_3$), 1.87 (3H, s, $-CH_3$), 2.21 (3Hx2, s, $-COCH_3$), 2.22 (3Hx2, s, $-COCH_3$), 2.23 (3H, s, $-COCH_3$), 2.24 (3H, s, $-COCH_3$), 2.26 (3Hx2, s, $-COCH_3$), 2.27 (3Hx3, s, $-COCH_3$), 2.28 (3H, s, $-COCH_3$), 2.32 (3H, s, $-CH_3$), 2.33 (3H, s, $-CH_3$), 2.34 (3H, s, $-CH_3$), 2.36 (3H, s, $-CH_3$), 6.95 (1H, s, H-4, H-4' or H-4"), 6.97 (1H, s, H-4, H-4' or H-4"), 7.05 (1Hx 2, s, H-4, H-4' or H-4"), 7.06 (1H, s, H-4, H-4' or H-4"), 7.07 (1H, s, H-4, H-4' or H-4"), 7.23 (1H, s, H-7 or H-7"), 7.24 (1H, s, H-7 or H-7"), 7.25 (1Hx2, s, H-7 or H-7"), 10.02 (1H, bs, NH, NH' or NH"), 10.04 (1H, bs, NH, NH' or NH"), 10.30 (1Hx2, bs, NH, NH' or NH"), 10.45 (1H, bs, NH, NH' or NH"), 10.47 (1H, bs, NH, NH' or NH"); ^{13}C NMR (acetone-d_6), δ (ppm): 12.69, 12.80, 12.81 (q, $-CH_3$), 20.17, 20.50, 20.54, 20.61 (q, $-COCH_3$), 105.73, 105.75 (d, C-7, C-7"), 105.81 (s, C-7'), 106.78, 106.94, 112.48 (s, C-3, C-3', C-3"), 112.03, 112.96, 113.02 (d, C-4, C-4', C-4"), 126.47, 126.74, 127.48 (s, C-9, C-9', C-9"), 133.80, 133.88, 133.98 (s, C-2, C-2', C-2"), 135.56, 135.69, 137.04 (s, C-5, C-5', C-5"), 137.13, 137.20, 137.26 (s, C-6, C-6', C-6"), 137.67, 138.43, 138.50 (s, C-8, C-8', C-8"), 168.95, 168.98, 169.40, 169.49 (s, $-COCH_3$).

5-Acetyl-6-[(5',6'-diacetoxy-2'-methylindol-3'-yl)methyl]-3-oxo-2,10,11-triacetoxy-3,5,7,8-tetrahydrocyclopenta[1,2-b:4,3-c']diindole (10, R=Ac). Colourless oil; UV: λ_{max} (EtOH) 290 nm; FABMS, *m/z*: 736 $(M+H)^+$, 694, 652, 610, 260; ^1H NMR (acetone-d_6), δ (ppm): 2.01 (3H, s, $-COCH_3$ or $-CH_3$), 2.09 (3H, s, $-COCH_3$ or $-CH_3$), 2.13 (3H, s, $-COCH_3$ or $-CH_3$), 2.17 (3H, s, $-COCH_3$ or $-CH_3$), 2.23 (3H, s, $-COCH_3$ or $-CH_3$), 2.25 (3H, s, $-COCH_3$ or $-CH_3$), 2.26 (3H, s, $-COCH_3$ or $-CH_3$), 2.85 (1H, d, *J*=14.3 Hz, H-7-a), 3.87 (1H, d, J= 14.7 Hz, H-7-b), 4.48 (1H, d, *J*=18.5 Hz, -CH(H)-), 4.56 (1H, d, *J*=18.5 Hz, -CH(H)-), 6.00 (1H, s, H-4), 6.23 (1H, s, H-1), 7.13 (1H, s, H-9 or H-7'), 7.17 (1H, s, H-9 or H-7'), 7.35 (1H, s, H-12 or H-4'), 7.40 (1H, s, H-12 or H-4'), 10.35 (1H, bs, N-8-H or N-1'-H), 10.55 (1H, bs, N-8-H or N-1'-H); ^{13}C NMR (acetone-d_6), δ (ppm): 17.11 (q, $-CH_3$), 20.18, 20.35, 20.41, 20.54 (q, $-COCH_3$), 23.75 (t, $-CH_2-$), 35.31 (t, C-7), 61.61 (s, C-12c), 105.87, 106.52 (d, C-9, C-7'), 107.67, 110.46 (s, C-12b, C-3'), 111.44, 111.61 (d, C-12, C-4'), 115.70, 118.70 (d, C-1, C-4), 125.10, 125.75 (s, C-12a, C-9'), 132.71, 132.92, 132.53

(s, C-7a, C-6, C-2'), 137.09, 137.50, 137.78 (s, C-11, C-2, C-5'), 138.57, 138.72, 140.87 (s, C-10, C-2, C-6'), 143.14, 143.18 (s, C-8a, C-8'), 152.63 (s, C-4a), 168.56, 168.75, 169.01, 169.12, 169.30, 169.45 (s, -$COCH_3$), 189.09 (s, C-3).

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REFERENCES

1. Jones R.A. Pyrroles and their Benzo Derivatives: Reactivity. In *Comprehensive Heterocyclic Chemistry*; Bird, C.W; Cheeseman, G.W. Eds.; Pergamon Press: Oxford, 1984; pp. 201-312. Sundberg, R.J. *The Chemistry of Indoles*; Academic Press: New York, 1970; pp. 341-392.
2. Prota, G. *Melanins and Melanogenesis*; Academic Press: New York, 1992.
3. Prota, G. *Fortsch. Chem. Org. Naturst.*, **1993**, in press.
4. d' Ischia, M.; Napolitano, A.; Tsiakas, K.; Prota, G. *Tetrahedron*, **1990**, *46*, 5789-5796.
5. Bu'Lock, J.D. *J. Chem. Soc.*, **1960**, 52-59.
6. Corradini, M.G.; Napolitano, A.; Prota, G. *Tetrahedron*, **1986**, *42*, 2083-2088.
7. Palumbo, P; d' Ischia, M.; Prota, G. *Tetrahedron* **1987**, *43*, 4203-4206.
8. Beer, R.J.S.; Broadhurst, T.; Robertson, A. *J. Chem. Soc.* **1954**, 1947-1953.
9. Cromartie, R.I.T.; Harley-Mason, J. *Biochem. J.* **1957**, *66*, 713-720.
10. d' Ischia, M; Napolitano, A.; Prota, G. *Biochim. Biophys. Acta* **1991**, *1073*, 423-430.
11. Lambert, C.; Chacon, J.N.; Chedekel, M.R.; Land, E.J.; Riley, P.A.; Thompson, A.; Truscott, T.G. *Biochim. Biophys. Acta* **1989**, *993*, 12-20.
12. Al-Kazwini, A.T.; O'Neill, P; Adams, G.E.; Cundall, R.B.; Lang, G.; Junino, A. *J. Chem. Soc. Perkin Trans. 2*, **1991**, 1941-1945.
13. Remers, W.H. Properties and Reactions of Indoles. In *The Chemistry of Heterocyclic Compounds*; Houlian, W.J., Ed.; John Wiley and Sons, Inc.: New York, 1972; p. 155.
14. Ambani, L.M.; Van Woert, M.H.; Murphy, S. *Arch. Neurol.* **1975**, *32*, 114-118.
15. *Martindale: The Extra Pharmacopoeia*; Reynolds, J.E.F. Ed.; The Pharmaceutical Press: London, 1989; pp. 487-490.
16. Bu'Lock, J.D.; Harley-Mason, J. *J. Chem. Soc.* **1951**, 2248-2252.
17. Beer, R.J.S.; Clarke, K.; Khorana, H.G.; Robertson, A. *J. Chem. Soc.* **1948**, 2223-2226.