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Oxidation of the Neurotoxin 6-Nitrodopamine and Related 4-Nitrocatechols Under Biomimetic Conditions

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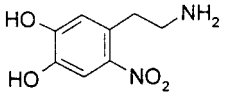
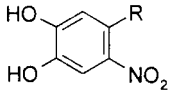
Abstract—Oxidation of 6-nitrodopamine (**1**) with horseradish peroxidase (HRP)/H_2O_2 in 0.05 M phosphate buffer, pH 7.4 afforded as main product the novel 5-[4-(2-aminoethyl)-2-hydroxy-5-nitrophenoxy]-6,7-dihydroxy-4-nitro-2,3-dihydroindole (**4**). Similar oxidation of the 4-nitrocatechols **2** and **3** with HRP/H_2O_2 or $K_3Fe(CN)_6$ gave chiefly the dimers **5/6** and **7/8**. These products arise probably via 4-nitro-*o*-quinone or 4-nitro-2,3-dihydroindole-6,7-dione intermediates which are trapped by the starting nitrocatechols.

6-Nitrodopamine[†] (**1**) and its congeners, e.g. 6-nitronorepinephrine, have recently emerged as a novel class of catecholamine metabolites putatively implicated in a variety of pathophysiological conditions associated with oxidative stress, impaired catecholaminergic neurotransmission and enhanced synthesis of nitric oxide (nitrogen monoxide, NO), a powerful physiological mediator.[1–5] Although the mechanisms of formation of nitrocatecholamines have been the focus of particular interest, and several NO- and nitrite-dependent routes have been elucidated,[2,4,5] the oxidative chemistry of these metabolites has remained virtually unexplored. Preliminary observations indicated that **1**, though less oxidisable than dopamine, was susceptible to oxidation under mild, biologically relevant conditions, a property which may account at least in part for its moderate neurotoxic effects.[5]

Prompted by the biological relevance of nitrocatecholamines as well as by the apparent lack of a systematic picture of the oxidative behaviour and mode of polymerisation of nitrocatechols, we undertook a detailed investigation of the reactivity of **1**, as well as of the model compound 4-methyl-5-nitrocatechol (**2**) and 4-nitrocatechol (**3**) under oxidative conditions supposed to mimic those accounting for nitrocatechol formation and degradation in vivo.[5]



1



2: R= CH_3
3: R= H

Oxidation of 1 by the Horseradish Peroxidase/Hydrogen Peroxide System

The horseradish peroxidase (HRP)/H_2O_2 system, which can mediate formation of **1** from dopamine and nitrite ions,[5] proved effective in inducing oxidation of **1** and was accordingly chosen as a convenient biologically relevant oxidant. Reaction of **1** with HRP/H_2O_2 in 0.05 M phosphate buffer, pH 7.4, and at 37°C proceeded smoothly to give complex mixtures of chromatographically ill-defined oligomeric species with a broad yellow chromophore centred at about 410 nm. Careful HPLC inspection of the reaction mixture at various stages of oxidation failed to reveal detectable amounts of intermediary products. However, when the HRP/H_2O_2 reaction was carried out under conditions causing only a limited substrate conversion and the oxidation was halted by the addition of $NaHSO_3$, chromatographic analysis revealed the formation of, besides large amounts of ill defined oligomeric material, a main product positive to the $FeCl_3$ test for phenolic compounds, which was eventually obtained as a yellow oil by preparative HPLC.

The product exhibited a pH-dependent chromophore resembling that of **1**, with an apparent pK_a=5.8 versus a pK_a of 6.2 for **1**,[5] and resisted reductive treatment with $NaBH_4$ but not with $Na_2S_2O_4$, a potent reducing agent for

Keywords: coupling reactions; nitro compounds; dimerisation; biomimetic reactions.

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† For the sake of simplicity we adopted throughout this paper the common system for catecholamine nomenclature which assigns numbers 3 and 4 to the OH-bearing carbons of the aromatic ring. Such a system is clearly not applicable to other catechol derivatives, for which the correct numbering of the ring was used.

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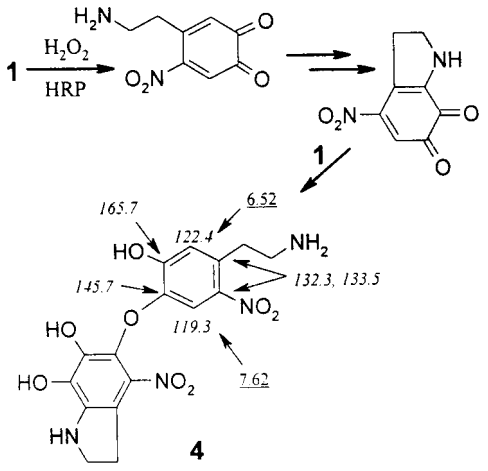


Figure 1. Proposed origin and structure of the main oxidation product of **1**. Salient ^{1}H (underlined) and ^{13}C (italicised) NMR resonances and their assignments as deduced on the basis of 2D ^{1}H, ^{13}C correlation experiments are highlighted.

nitro groups.[6] It proved moderately stable to autoxidation but was readily oxidised to chromophoric species absorbing at ca. 430–440 nm in the presence of sodium periodate or potassium ferricyanide.

The ^{1}H NMR spectrum displayed two 1H singlets at δ 7.66 and 6.52 and a complex 8H multiplet at δ around 3.1, whereas the ^{13}C NMR spectrum showed twelve carbon resonances in the aromatic region and four resonances for the methylene carbons. ^{1}H, ^{13}C HMBC experiments showed that the signal at δ 7.66 correlated with carbons resonances at δ 133.5, 145.7 and 165.7, whereas the proton signal at δ 6.52 displayed cross-peaks with carbon resonances at δ 132.3 and, notably, 145.7. The FAB-MS spectrum showed two distinct groups of peaks at *m/z* 391–393 and 413–415 ($+Na^{+}$). Taken together, these data pointed to a dimeric structure in which one of the catechol units retained both aromatic protons and the other was fully substituted. These structural constraints suggested that the product originated from attack of a phenoxyl group of **1** to an oxidised quinonoid counterpart, forming a C–O–C ether linkage.

To distinguish among the various structures compatible with this formulation, the 1D and 2D spectra of the product were carefully analysed in comparison with those of **1**.[‡] As a result, several crucial differences were recognised, which were revealing of the modifications suffered by the 4-nitrocatechol system of **1** following dimerisation. In particular, within the unsubstituted nitrocatechol moiety the hydroxyl-bearing carbon *para* to the nitro group (C-3) appeared at δ 165.7, the adjacent oxygenated carbon (C-4) resonated at δ 145.7, the C-2 and C-5 carbons gave CH resonances at δ 119.3 and 122.4, respectively, whereas the quaternary carbons linked to the nitro group and the aminoethyl chain (C-6 and C-1) resonated at δ133.5 and 132.3. This analysis highlighted a pronounced downfield shift (5–10 ppm) of the signals for the C-1, C-3 and C-5 carbons, i.e. *ortho* and *para* to the C-4 hydroxylated carbon, indicating that the shielding influence of the germane hydroxyl group was partly decreased by substitution, e.g. linkage to the nitrocatechol ring. Conversely, an appreciable upfield shift of some of the carbon signals belonging to the fully substituted ring was apparent, all resonances appearing at δ values lower than 150. Based on literature data on polyoxygenated benzene systems[7–9] and with the aid of additivity rules,[10] four of these resonances (δ 137.0, 140.0, 142.1, 147.7) were assigned to the carbons bearing the hydroxyl and nitro groups, whereas the signal at δ 114.3 (HMBC cross-peak with protons at δ 3.12) was ascribed to the carbon bearing the aminoethyl chain. Based on mechanistic considerations, and keeping into account the molecular weight restrictions indicated by the FAB-MS spectrum, it was argued that the quaternary carbon responsible for the remaining resonance (δ 125.4) was linked to a nitrogen atom derived from cyclisation of the aminoethyl functionality. Accordingly, the product was formulated as having the unusual structure of 5-[4-(2-aminoethyl)-2-hydroxy-5-nitrophenoxy]-6,7-dihydroxy-4-nitro-2,3-dihydroindole (**4**) (Fig. 1).

This structure would conceivably arise from the intramolecular cyclisation of the aminoethyl chain of **1**-quinone followed by oxidation to afford 4-nitro-2,3-dihydroindole-6,7-dione susceptible to nucleophilic attack by another unit of **1** via a hydroxyl functionality. It may be noted, in this connection, that C–O–C linkages are not unprecedented in dimerisation products of catechols, *o*-aminophenols and pyrogallols.[11–13] The proposed structure would be consistent with the spectroscopic (chromophoric) and chemical features of the product: in particular, the 1,2-dihydroxy-3-amino substitution pattern pertaining to one of the aromatic rings would reasonably account for the apparently higher facility to oxidation compared to **1**, whereas the substituted 4-nitrophenol moiety might be responsible for the slightly lower pKa value.

Oxidation of **1** with other reagents commonly used for catechol oxidation, e.g. potassium ferricyanide, proceeded sluggishly leading to chromatographically ill-defined mixtures of oligomeric products but no detectable amount of **4** or other isolable products, in spite of carefully controlled conditions and reductive treatments with $NaHSO_3$. Considering the greater facility to oxidation of **4** compared to **1**, it seems conceivable that, when generated at slow rate with poor oxidants, **4** and other dimeric products hardly accumulate because of rapid conversion to higher oligomeric species, engaging the oxidant in processes other than substrate conversion.

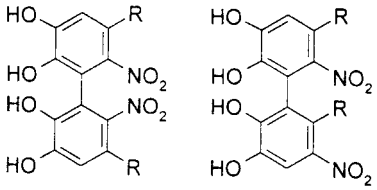
Oxidation of 4-Nitrocatechols 2 and 3

To gain a deeper insight into the oxidative chemistry of the 4-nitrocatechol system featured by **1**, in another series of experiments we focused to compounds **2** and **3** which could lead expectedly to products more amenable to structural characterisation.

Oxidation of **2** and **3** with the HRP/H_2O_2 system under the above conditions afforded mixtures of relatively polar

‡ $^{1}H/^{13}C$ NMR of **1** (DMSO-d_6); δ 2.81/30.86 ($CH_2CH_2NH_2$), 2.81/40.41 ($CH_2CH_2NH_2$), 7.22/111.42 (C5), 6.72/118.32 (C2), 127.02 (C1), 136.53 (C6), 145.58 (C4), 156.40 (C3).

materials, including in both cases two main dimeric products with chromophoric features similar to those of the parent nitrocatechols. These products could be conveniently obtained in 10–25% isolated yields by oxidation of **2** and **3** with potassium ferricyanide at pH 7.0 and were formulated as **5** and **6** (from **2**), and **7** and **8** (from **3**), by ^{1}H and ^{13}C NMR analysis as integrated by 2D ^{1}H, ^{13}C HETCOR and ^{1}H, ^{13}C HMBC connectivity experiments.



5: R= CH_3 **6**: R= CH_3
7: R= H **8**: R= H

Whereas the structure of the asymmetric dimer **6** was unambiguously assigned because of the restricted positional reactivity of **2**, that of **8** was not so straightforward and was deduced from careful scrutiny of the ^{1}H NMR spectrum, in which the resonance for the H-6 proton in one of the units was lacking, and a *meta* coupling constant between the H-3 and H-5 protons was well detectable. Attempts to obtain satisfactory molecular ion peaks in the EI-MS spectra of **5**–**8** were frustrated by the marked tendency of the products to suffer extensive fragmentation under a variety of conditions. In an attempt to overcome these problems, products **5**–**8** were converted to their tetraacetyl derivatives which gave small, yet detectable molecular ion peaks.

Since HRP/H_2O_2 and ferricyanide promote one-electron oxidation of catechols to yield semiquinone radicals, which are susceptible to further oxidation or disproportionation to give *o*-quinones,[14] two possible mechanisms of dimerisation can be envisaged, via either semiquinone radical coupling or catechol–quinone ionic interaction. Available evidence would argue strongly in favour of a catechol–quinone reaction rather than of a free radical coupling of *o*-semiquinones which, to the best of our knowledge, does not seem to be adequately supported in the literature as a general mechanism. In principle, the possible modes of coupling of semiquinone radicals and the range of resulting products would be less restricted than indicated by the formation of only two dimers, nor is there any evidence that the missing dimers have eluded detection. An additional argument against a semiquinone radical coupling is that such a process should be primarily controlled by the spin density distribution which, in most semiquinones, is higher on the positions *para* to the hydroxyl groups,[15] implying in the case of **3** a significant involvement of the 5-position, at variance with experimental evidence. In fact, the preferential formation of the asymmetric over the symmetric dimers would be more in line with a catechol–quinone coupling mechanism. The similar positional reactivity of the 4-nitrocatechol system in **3** and **2** would entail that in both cases the mode of dimerisation is mainly a reflection of electronic effects, being little affected by the methyl substituent.

Taking the catechol–quinone coupling mechanism for granted, we briefly investigated whether the prevalent mode of coupling of **2** via the 6-position near the nitro group and, to a lesser extent, the 3-position (corresponding in the case of **3** to the 3- and 6-positions, respectively) was more compatible with charge- or frontier orbital-controlled processes. Inspection of the general properties of **2** by a semiempirical approach[16] (AM1 and PM3 methods) revealed a higher charge density on the position adjacent to the methyl group, but a larger HOMO coefficient on the alternate position, whereas for the corresponding quinone similar analysis of the unsubstituted positions predicted the highest ionic-type reactivity near the nitro group but comparable LUMO-controlled reactivity. In the case of **3**, most of the charge density was localised on the 6-position, whereas the largest HOMO coefficients were on the 5- and 3-positions. Calculations for **3**-quinone predicted a comparable LUMO-controlled reactivity on the 3- and 6-positions, but provided slightly different results for total charge densities: whereas AM1 calculations favoured nucleophilic attack to the 5-position, PM3 data also argued so but to a much lesser degree, the 3-position displaying comparable charge density.

Within the limitations of the preliminary approach used, it appears that neither a simple frontier orbital-controlled reactivity nor merely charge density–oriented interactions would provide an entirely satisfactory rationalisation of the regiochemistry of the dimerisation reactions, although the ionic mechanism would overall seem more consistent with the experimental evidence. This conclusion, in fact, was not entirely unanticipated, since canonical resonance structures for 4-nitrocatechols and 4-nitro-*o*-quinones would also identify the 3- and 6-positions as the most reactive in a charge-controlled coupling. In any case, a more sophisticated theoretical analysis would be desirable before drawing any definitive conclusion.

As an additional remark, it was noted that in the geometry-optimised stereostructure of **2** the nitro group was twisted out of the plane of the aromatic ring, probably because of the steric hindrance caused by the adjacent methyl group,[17] whereas the expected coplanarity of the ring and the nitro group was confirmed for the energy-minimised structure of **3**. However, the changing nature of the orbital interaction between the nitro group and the aromatic ring was not reflected in the general properties and reactivity of the nitrocatechols **2** and **3** as indicated by the virtually similar NMR spectra and product distributions.

Conclusions

The results of this study provide, to the best of our knowledge, the first insight into the oxidative behaviour and mode of polymerisation of nitrocatechols, filling an important gap in the relevant literature. The products isolated, though far from providing an exhaustive picture, can be taken as being very representative of the oxidative behaviour of nitrocatechols at physiologically relevant pH. Of particular interest was the different mode of dimerisation of the nitrocatecholamine **1** and its model **2** via C–O–C and C–C bonds, respectively. This would apparently reflect the influence of the aminoethyl side chain which, in the case of **1**, would account for the formation of a transient

nitroindoline-6,7-quinone with a reaction behaviour expectedly different from that of the 4-nitro-*o*-quinones supposedly involved in the oxidation of **2** and **3**. Alternatively, steric effects due to the aminoethyl side chain may be considered. In any case, it can not be ruled out that C–C coupled dimers similar to **5** and **6** are also formed in the oxidation of **1**, and that they evolve further, e.g. through intramolecular cyclisation of the side chain, to afford elusive species.

The relevance of the reported chemistry to the mechanism of toxicity of **1** and related nitrocatechols is the focus of current work in our laboratories.

Experimental

General methods

For EI-MS spectra samples were ionised with a 70 eV beam, and the source was taken at 230°C. Main fragmentation peaks are reported with their relative intensities (percent values are in brackets). (HR) FAB-MS spectra were obtained using NBA as the matrix. ^{1}H (^{13}C) NMR spectra were recorded at 400 (100) MHz using a Bruker WM 400 spectrometer. ^{1}H, ^{1}H COSY, ^{1}H, ^{13}C HETCOR and ^{1}H, ^{13}C HMBC NMR experiments were run at 400 MHz using standard pulse programs from the Bruker library. UV spectra were performed with a Beckmann DU 640 spectrophotometer. Analytical and preparative HPLC was carried out on a Gilson apparatus equipped with a UV detector set at 254. Sphereclone ODS (5 μ, 4.6×250 mm) or Econosil (10 μ, 22×250 mm) columns were used for analytical or preparative runs, at a flow rate of 1 or 15 mL/min, respectively. Elution conditions: 0.1 M formic acid containing 10% acetonitrile (solution A), 0.1 M formic acid containing 40% acetonitrile (solution B) from 0 to 70% solution B gradient, 40 min; (eluant A); 0.05 M formic acid/acetonitrile 90:10 (eluant B); 0.1 M formic acid/acetonitrile 75:25 (eluant C). TLC was carried out on silica gel plates (0.25 mm) from Merck. 4-Nitrocatechol, non-stabilised hydrogen peroxide (35% solution in water), potassium ferricyanide were from Aldrich Chemie; horseradish peroxidase (HRP) (donor H_2O_2 oxidoreductase, E.C. 1.11.1.7, 200 purpurogallin units/mg E_{430}/E_{275}=2.0) type II was from Sigma Chemicals. 6-Nitrodopamine (**1**) and 4-methyl-5-nitrocatechol (**2**) were prepared by previously reported procedures.[18]

Oxidation of 1

A solution of **1** hydrogensulphate in 0.05 M phosphate buffer pH 7.4 at concentrations varying in the range 0.5–10 mM was treated with HRP (2–6 U/mL) and 3% hydrogen peroxide (0.4–2 molar equivalents). In other experiments, the oxidation of **1** was performed by potassium ferricyanide (0.8–2 molar equivalents) in 0.1 M Na_2CO_3, pH 9. The reaction course was followed by periodical HPLC analysis (eluant A) of aliquots of the reaction mixture treated with $NaHSO_3$.

5-[4-(2-Aminoethyl)-2-hydroxy-5-nitrophenoxy]-6,7-dihydroxy-4-nitro-2,3-dihydroindole (4). A solution of **1** hydrogensulphate (500 mg, 1.7 mmol) in 0.05 M phosphate buffer, pH 7.4 (1 L) was treated with HRP (11 U/mL) and 3% hydrogen peroxide (2.6 mmol) and taken under stirring at r.t. After 1 h the mixture was treated with excess $NaHSO_3$, concentrated to a small volume, centrifuged to remove solid material and fractionated by preparative HPLC (eluant B) to give pure 4 (R_t 20 min, 25 mg, 8% yield) as yellow amorphous solid: λ_{max} 251, 299, 411 nm (pH 7.0) and 289, 338 nm (pH 3.0); λ_{max} ($CHCl_3$/DMSO) 3673, 3392, 1603, 1515, 1417, 1378 cm^{-1} δ_H (DMSO-d_6) 3.05–3.12 (8H, m), 6.52 (1H, s), 7.66 (1H, s); δ_C (DMSO-d_6) 26.0 (CH_2), 32.2 (CH_2), 39.5 (CH_2), 40.1 (CH_2), 114.3 (C), 119.3 (CH), 122.4 (CH), 125.4 (C), 132.3 (C), 133.5 (C), 137.0 (C), 140.0 (C), 142.1 (C), 145.7 (C), 147.7 (C), 165.7 (C); FAB-MS: 393 $[M+H]^+$, 415 $[M+Na]^+$; HRFABMS calcd for $C_{16}H_{17}N_4O_8$ $[M+H]^+$ 393.1046, found *m/z* 393.1065.

Oxidation of 2 or 3

A solution of the appropriate catechol (10–50 mM) in 0.1 M Na_2CO_3, pH 9 (500 mL) or 0.1 M phosphate buffer, pH 7.4, was treated with potassium ferricyanide (1–2 molar equivalents) or HRP (1.5 U/ml final concentration) and 3% hydrogen peroxide (1 molar equivalent). The reaction course is followed by periodical HPLC analysis (eluant A) of aliquots of the reaction mixture treated with $NaHSO_3$.

For preparative purposes the reaction was carried on with the substrate at 50 mM using potassium ferricyanide as the oxidant. After 15 min, the reaction mixture was acidified to pH 3, worked up as in the case of **1**, and purified by preparative HPLC using eluant C. Under these conditions, fractionation of the mixture obtained by oxidation of **2** or **3** (300 mg) afforded compounds **5** (R_t 5.4, eluant C, 25 mg, 8% yield), and **6** (R_t 19, eluant C, 35 mg, 12% yield) or compounds **7** (R_t 10 eluant C, 29 mg, 10% yield) and **8** (R_t 15, eluant C, 42 mg, 14%) as yellow glassy oils. Tetraacetyl derivatives of compounds **5**–**8** were prepared by repeated treatment with acetic anhydride containing 2–5% pyridine.

2,2′,3,3′-Tetrahydroxy-5,5′-dimethyl-6,6′dinitrobiphenyl (5). λ_{max} 405 nm (pH 7.0); ν_{max} ($CHCl_3$) 3684, 3400, 1614, 1525, 1425, 1380 cm^{-1}; δ_H (DMSO-d_6) 2.19 (3H×2, s, CH_3), 6.62 (1H×2, s, H-4, H-4′); δ_C (DMSO-d_6) 18.6 (CH_3×2), 115.9 (CH×2, C-4, C-4′), 116.9 (C×2, C-1, C-1′), 122.2 (C×2, C-5, C-5′), 141.3 (C×2, C-2, C-2′), 142.6 (C×2, C-6, -6′), 149.8 (C×2, C-3, C-3′). [Found: C, 49.95; H, 3.68; N, 8.30 $C_{14}H_{12}N_2O_8$ requires C, 50.01; H, 3.60; N, 8.33%]. Tetraacetyl derivative: (R_f 0.28 eluant benzene–AcOEt 90:10). EI-MS: *m/z* 504 (2, M^+), 429 (30), 355 (100), 341 (20), 295 (25).

2,2′,3,3′-Tetrahydroxy-5,6′-dimethyl-6,5′-dinitrobiphenyl (6). λ_{max} 423 nm (pH 7.0); ν_{max} ($CHCl_3$) 3672, 3398, 1603, 1515, 1417, 1377 cm^{-1}; δ_H (DMSO-d_6) 2.13 (3H, s, CH_3), 2.18 (3H, s, CH_3′), 6.85 (1H, s, H-4), 7.48 (1H, s, H-4′); δ_C (DMSO-d_6) 16.6 (CH_3–C-6′), 17.6 (CH_3–C-5), 110.8 (CH, C-4′), 116.3 (CH, C-4), 117.9 (C, C-1), 121.3 (C, C-5), 122.7 (C, C-6′), 126.1 (C, C-1′), 140.3 (C, C-5′), 141.5 (C, C-6), 142.7 (C, C-3′), 142.9 (C, C-2), 147.9 (C, C-3), 149.1 (C, C-2′). [Found: C, 50.12; H, 3.55; N, 8.32 $C_{14}H_{12}N_2O_8$ requires C, 50.01; H, 3.60; N, 8.33%].

Tetraacetyl derivative: (R_f 0.24 eluant benzene–AcOEt 90:10). EI-MS: *m/z* 504 (1, M^+), 474 (5), 386 (25), 368 (20), 341 (20), 281 (20), 256 (30), 214 (100).

2,2′,3,3′-Tetrahydroxy-6,6′-dinitrobiphenyl (7). λ_{max} 422 nm (pH 7.0); ν_{max} ($CHCl_3$) 3675, 3420, 1615, 1520, 1487, 1410, 1377 cm^{-1}; δ_H (DMSO-d_6) 6.86 (1H×2, d, *J*=8.3 Hz, H-4, H-4′), 7.62 (1H×2, d, *J*=8.3 Hz, H-5, H-5′); δ_C (DMSO-d_6) 112.8 (CH×2, C-5, C-5′), 117.2 (CH×2, C-4, C-4′), 119.2 (C×2, C-1, C-1′), 139.7 (C×2, C-6, C-6′), 142.8 (C×2, C-2, C-2′), 151.7 (C×2, C-3, C-3′). [Found: C, 46.61; H, 2.60; N, 9.13; $C_{12}H_8N_2O_8$ requires C, 46.77; H, 2.62; N, 9.09%]. Tetraacetyl derivative: (R_f 0.70 eluant $CHCl_3$–MeOH 90:10). EI-MS *m/z* 476 (2, M^+), 430 (2), 279 (70), 256 (100).

2,2′,3,3′-Tetrahydroxy-5,6′-dinitrobiphenyl (8). λ_{max} 425 nm (pH 7.0); ν_{max} ($CHCl_3$) 3673, 3416, 1603, 1518, 1470, 1420, 1377 cm^{-1}; δ_H (DMSO-d_6) 6.92 (1H, d, *J*=8.3 Hz, H-4′), 7.52 (1H, d, *J*=8.3 Hz, H-5′), 7.55 (1H, d, *J*=2.4 Hz, H-6), 7.61 (1H, d, *J*=2.4 Hz, H-4); δ_C (DMSO-d_6) 108.6 (CH, C-5′), 113.1 (CH, C-6), 116.9 (CH, C-4), 118.1 (CH, C-4′), 119.6 (C, C-1′), 122.0 (C, C-1), 138.1 (C, C-5), 141.2 (C, C-6′), 144.3 (C, C-3), 145.3 (C, C-2′), 151.2 (C, C-3′), 151.7 (C, C-2). [Found: C, 46.70; H, 2.56; N, 9.14; $C_{12}H_8N_2O_8$ requires C, 46.77; H, 2.62; N, 9.09%]. Tetraacetyl derivative: (R_f 0.75 eluant $CHCl_3$–MeOH 90:10). EI-MS: *m/z* 476 (less than 1, M^+), 279 (100), 256 (30), 228 (35), 213 (50).

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