Dopamine quinone chemistry: a study of the influence of amide, amidine and guanidine substituents [-NH-CX-Y] on the mode of reaction

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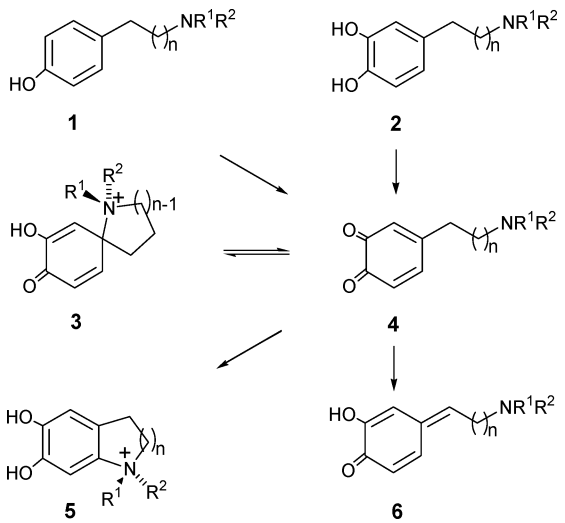
The influence of N-substituents on the mode of reaction of *ortho*-quinones generated by oxidation of N-substituted dopamine derivatives **8** has been studied. *Ortho*-quinones with amide, urea or guanidine side chains are relatively stable, with evidence of rearrangement to *para*-quinomethanes. The *N*-methylthiourea derivative **11** rapidly cyclises giving a bicyclic product **12**. The trichloromethylamidine derivative **13** also rapidly cyclises but in this case gives a spirocyclic derivative **14**. In contrast to the transient formation of spirocyclic products by other *ortho*-quinones derived from dopamine derivatives, *e.g.*, **19**, the product **14** is stable and has been isolated and fully characterised.

Introduction

We have previously described investigations of the reactions of a variety of dopamine *ortho*-quinone derivatives **4**.[1,2] In these studies we have generated the *ortho*-quinones **4** by (i) tyrosinase oxidation of phenols **1** or catechols **2**,[3–7] (ii) pulse radiolytic oxidation of catechols **2**,[3–6,8] and (iii) chemical oxidation of catechols **2**.[9–11]

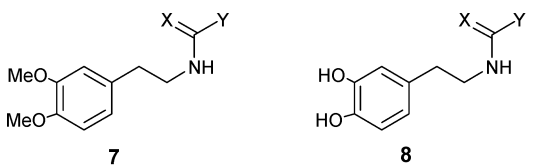
We have found that cyclisation to the 5-position to give the bicyclic products **5** (Scheme 1) is usually fast but that faster transient formation of the less stable spirocyclic compounds **3** is sometimes observed. Cyclisation to the 3-position is not observed and this aspect has been analysed using quantum mechanical calculations.[12] If cyclisation is not possible then relatively slower rearrangement to a *para*-quinomethane **6** is observed. This rearrangement occurs more easily in aqueous media (*e.g.* enzymatic oxidation) than in organic solvents[7] but we have observed examples in organic media when intramolecular deprotonation by the amine side chain is favourable.[9,11]



Scheme 1

As part of a continuing interest in the oxidation mechanisms of tyrosinase[1,13] and the suicide inhibition of tyrosinase,[14,15] we have investigated a series of N-substituted dopamine derivatives having the general structure **8**. We have previously described enzyme and pulse radiolysis studies of some of these compounds,[6] and a preliminary account of our chemical studies has also been published.[16] We now report a full account of our studies of the chemical oxidation of the catechols **8a-k** together with some new enzyme and pulse radiolysis results on previously undescribed derivatives.

Results and discussion



In formula **7** and **8**

a, X = O, Y = Me;
b, X = O, Y = NH_2;
c, X = S, Y = NHMe;
d, X = $NH_2^+Cl^-$, Y = CCl_3;
e, X = $NH_2^+Cl^-$, Y = Ph;
f, X = $NH_2^+Br^-$, Y = Ph;
g, X = $NH_2^+0.5SO_4^{2-}$, Y = NH_2;
h, X = $NH_2^+Br^-$, Y = NH_2;
i, X = $NH_2^+Cl^-$, Y = NH_2;
j, X = NAc, Y = NH_2;
k, X = NCN, Y = NH_2;
l, X = NAc, Y = NHAc;

The side chains on the catechols **8a-k** were chosen to display a range of properties, particularly nucleophilicity and pK_a, in order to optimise the possibility of observing new *ortho*-quinone reactions. Most of the catechols **8** were prepared by demethylation of the corresponding *O,O*-dimethylcatechols **7** using either 48% aqueous HBr or 1M BCl_3 in CH_2Cl_2, and, unless otherwise stated, are new compounds. The ethers **7** were prepared from commercial 2-(3,4-dimethoxyphenyl)ethylamine. Relevant details and any significant features of this methodology are discussed in the appropriate section of the discussion.

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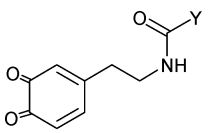
The catechol **8a** was prepared by the method of Niederstein and Peter and had spectroscopic properties identical to those previously described.[17] The urea **8b** was prepared by demethylation of the known ether **7b**.[18,19] Oxidation of $CDCl_3/CD_3OD$ solutions of the acetamide **8a** and the *N*-alkylurea **8b** by 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) was monitored by ^{1}H NMR spectroscopy. In both cases the three aromatic catechol protons (δ 6.5–6.8) were rapidly replaced by three *ortho*-quinone protons (δ 6.2–7.1). Under these conditions the *ortho*-quinones **9** (Y = Me, NH_2) were relatively stable and underwent slow decomposition over several hours. However, no specific products could be identified and after twenty four hours complex mixtures had formed. This contrasts with the behaviour of the same *ortho*-quinones **9** (Y = Me, NH_2) in aqueous phosphate buffer (pH 7.4) in which conversion to the 2-hydroxy-*para*-quinomethanes **10** (Y = Me, NH_2), with well-defined isosbestic points, occurs over several minutes.[6] We have described other examples of 4-alkyl-*ortho*-quinones that readily rearrange in aqueous buffer but are relatively stable in organic solvents, with slow formation of complex mixtures.[7] Clearly, the aqueous environment favours proton transfer and *para*-quinomethane formation. These results for *ortho*-quinones **9** (Y = Me, NH_2) therefore show a consistent pattern of behaviour for derivatives in which the catechol side chain is neither sufficiently nucleophilic to cyclise onto the *ortho*-quinone ring (Scheme 1) nor sufficiently basic to catalyse rearrangement.



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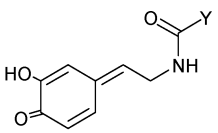

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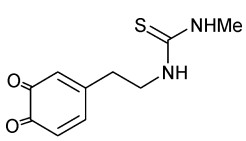
In contrast to the urea **8b**, DDQ oxidation of the thiourea **8c** rapidly gave a single product that showed only two aromatic protons in the ^{1}H NMR spectrum. This was identified as a 5-thia-7-aza-benzocycloheptene and isolated as its hydrochloride salt **12**. Cyclisation was so rapid that the *ortho*-quinone precursor **11** was not observed by ^{1}H NMR spectroscopy. This rapid cyclisation is consistent with the results of a pulse radiolysis study in which the cyclisation **11** → **12** was complete after approximately three seconds in phosphate buffer.[6] This ring formation is in accord with the side chain being a powerful S-nucleophile. The chloride **12** was isolated as a crystalline solid (mp 227–228 °C) (59%) and the bicyclic structure is fully supported by its spectroscopic properties. The ^{1}H NMR spectrum shows two uncoupled aromatic protons at δ 6.59 and 6.66, corresponding to a 4,5-disubstituted catechol, and this is confirmed by the ^{13}C NMR spectrum, which shows two aromatic CH signals (δ 118.6 and 119.8), and also a C=N signal (δ 167.3). The UV spectrum is consistent with a catechol structure [λ_{max} 287 (ε 2800)] and the mass spectrum confirmed the constitution and shows a strong fragment ion at *m*/*z* 168 corresponding to elimination of MeNH.C≡N.



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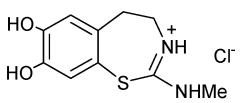

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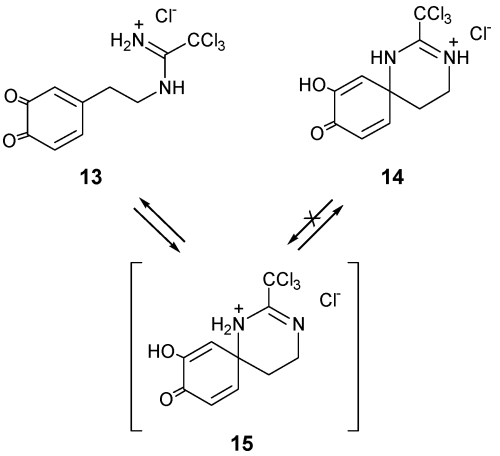
We next turned our attention to amidines (**8**; X = NH, Y = alkyl, aryl), which are much stronger bases than ureas and thioureas. The trichloromethyl hydrochloride salt **8d** was prepared by reaction of trichloroacetonitrile with dopamine.[20] The trichloromethyl derivative was chosen simply because these are the easiest amidines to prepare directly from primary amines and a nitrile, due to the enhanced reactivity of trichloroacetonitrile.

When the amidine **8d** was oxidised using one equivalent of DDQ an unexpected result was obtained. ^{1}H NMR examination of the reaction solution revealed the quantitative formation of a single product that on superficial examination appeared to be the *ortho*-quinone **13**. However, in contrast to the *ortho*-quinones **9** (Y = Me, NH_2), this product remained stable in solution over several days with no evidence of decomposition. The reaction was repeated on a preparative scale, and the product isolated as colourless needles, mp 144 °C (decomp.), and identified as the spirocyclic compound **14**. The ^{1}H NMR spectrum shows two methylene groups (δ 2.20 and 3.85). The signal at δ 2.20 is at too high a field to be a benzylic type signal (~δ 2.65 in **9** and ~δ 2.75 in **7** or **8**) but is entirely consistent with a spirocyclic CH_2 attached to a quaternary carbon atom. In addition, this signal (δ 2.20) appears as a pseudo-quartet, which can be attributed to the non-equivalence of the protons. The second methylene signal (δ 3.85) is entirely consistent with the NCH_2 group. The CH protons of the dienone fragment in compound **14** appear at δ 5.95, 6.35 and 7.00 with mutual coupling constants of 3 and 10 Hz. The signals resemble those recorded for the *ortho*-quinones **9** (Y = Me, NH_2) except that the 'enolic' CH (δ 5.95) is at significantly higher field than the corresponding protons in the *ortho*-quinones **9** (~δ 6.25). The ^{13}C NMR spectrum also fully supports the spirocyclic structure **14** with notable signals at δ 55.3 (spiro C), 159.0 (N=C-N) and 182.0 (C=O).

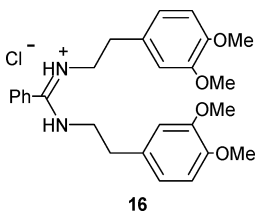
We have not previously isolated a spirocyclic product in our *ortho*-quinone studies. However, we have detected the transient formation of the very short-lived species **3** ($t_{1/2}$ ~0.1 s) using pulse radiolysis.[9] The UV spectrum of the product **14** [λ_{max} 244 (ε 7415) and 320sh (ε 1440) nm] is comparable to those of the transient species **3** [λ_{max} 250 and 310 nm],[9] and is attributable to the dienone fragment.

The mechanism of irreversible formation of the derivative **14** merits some discussion. We propose that the initially formed *ortho*-quinone **13** undergoes spirocyclisation to give the kinetic product **15**. In contrast to other spirocyclic species **3** that we have generated, the 1-proto cation **15** can rapidly tautomerise to the resonance stabilised 3-proto cation **14**, which is much more stable. Since the tautomerisation **15** → **14** is irreversible, the amidinium chloride **14** can no longer equilibrate with the *ortho*-quinone precursor **13**.

Because the trichloromethyl substituent is atypical, we decided to investigate the benzamidinium derivative **8e**. Attempts to prepare the precursor **7e** by reaction of benzonitrile with 2-(3,4-dimethoxyphenyl)ethylamine in the presence of aluminium trichloride, using the method of Brodrick and Short,[21] were unsuccessful. Using this procedure we obtained a product that was identified as the N^1,N^2-bis-alkylamidinium chloride **16**, mp 138 °C (26%). This product had insufficient aromatic protons relative to the methylene and methyl protons for it to be the amidine **7e**. The mass spectrum showed a strong ion at *m*/*z* 450 corresponding to the cation and this product (**16**) is presumably formed by further reaction of the desired amidine **7e** with excess amine. We therefore prepared the derivative **7e** by an alternative

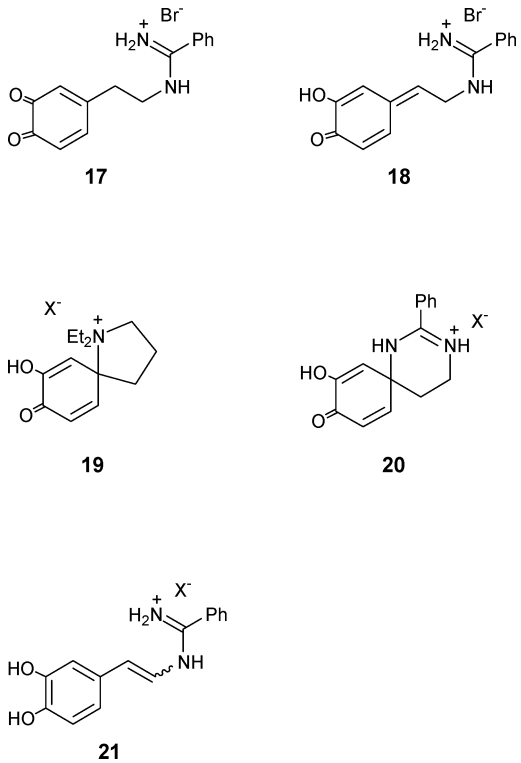


route involving reaction of 2-(3,4-dimethoxyphenyl)ethylamine with ethyl benzamidate in methanol. Recrystallisation of the crude product gave a 73% yield of the amidinium chloride **7e**. Demethylation of this product using 48% aqueous hydrobromic acid gave the bromide **8f** and demethylation using boron trichloride in methylene chloride gave the chloride **8e**. The bromide **8f** was then oxidised using pulse radiolysis, tyrosinase and DDQ.



In a pulse radiolysis study, $Br_2^{\bullet-}$ oxidation of the bromide **8f** gave the semiquinone (k 1.7×10^8 M^{-1} s^{-1}) (λ_{max} 310 and 350sh nm). This disproportionated to the *ortho*-quinone **17** (λ_{max} 400 nm; ε 1000 M^{-1} cm^{-1}), which was stable for at least ten seconds. Under the same conditions the trichloromethylamidine **8d** gave the short-lived *ortho*-quinone **13** which rapidly cyclised ($t_{1/2}$ ~0.003 s) to the spiro-product **14**.[6]

Tyrosinase oxidation of the benzamidinium bromide **8f** showed no spectral evidence of *ortho*-quinone formation (λ_{max} 400 nm), despite clear oximetric evidence of tyrosinase-catalysed oxidation. This suggests that a rapid reaction of the initially formed *ortho*-quinone **17** takes place. The spectral scans showed generation of a product(s) with a well-defined absorption at 305 nm, which is not consistent with formation of the *para*-quinomethane **18**. An HPLC study, using mass spectral and UV-vis analysis, showed one major product (rt 2.89 min) and two minor products (rt 1.82 and 2.07 min), together with the precursor **8f** (rt 2.68 min). The major product showed a strong molecular ion (*m/z* 255, 100%) and UV absorption at λ_{max} 234 and 305 nm. This UV spectrum is comparable to that of the trichloromethyl spiro-derivative **14** and similar short-lived species, *e.g.* **19**, λ_{max} 250 and 305 nm.[9] Since the spectrum of this product corresponds closely to that of the original spectral scan (λ_{max} 305 nm), we conclude that this is the major product of tyrosinase oxidation and propose that this product has the spirocyclic structure **20**. The mass spectrum shows two weak fragment ions at *m/z* 226 (loss of CH_2NH or COH) and *m/z* 151 (loss of $PhCNH_2$). The minor products show virtually identical UV spectra (λ_{max} 232 and 300 nm) and mass spectra (*m/z* 255 and 135 (100%)) indicating that they are closely related, and isomeric with the major product and *ortho*-quinone precursor **17**. The UV spectra suggest that they are catechol derivatives and the most likely structures are the (*E*)- and (*Z*)-isomers **21**.

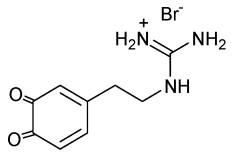


In a ^1H NMR study, DDQ oxidation of the catechol **8f** in CD_3OD gave the *ortho*-quinone **17** [δ 7.17 (d, *J* 10 Hz), 6.41 (d, *J* 10 Hz) and 6.33 (s)] which was stable in solution (>1 h). In contrast to the derivative **13**, which under the same conditions very rapidly formed the spirocyclic product **14**, there was no evidence of cyclisation. In particular, there were no signals in the region δ 1.5–2.5 which would be characteristic of a spirocyclic CCH_2. Similar NMR studies using D_2O as solvent, with and without the presence of base, also failed to show any evidence of spirocyclisation.

The difference in behaviour of the amidinium *ortho*-quinones **13** (CCl_3) and **17** (Ph) is of some interest. Assuming that the spirocyclic product **20** is the main product of tyrosinase oxidation in phosphate buffer, it forms significantly more slowly than product **14** based on the relative stabilities of the *ortho*-quinones **13** and **17** in phosphate buffer measured by pulse radiolysis. This difference in behaviour may be related to base strength: the weaker base **13** may be a more effective N-nucleophile.

We next turned our attention to guanidine derivatives. Because guanidines are strong bases, we investigated the unsubstituted salts **8h**,**i** and two N-substituted derivatives **8j**,**k** (X = $NCOCH_3$, NCN) selected to be weaker bases. The parent guanidinium bromide **8h** was prepared from the known sulfate **7g** by demethylation using aqueous HBr. DDQ oxidation of the catechol **8h** (pK_a ~13) in CD_3OD gave a ^1H NMR spectrum [d 7.30 (d, *J* 7 Hz), 6.57 (d, *J* 7 Hz) and 6.50 (s)] consistent with formation of the *ortho*-quinone **22**. Over a period of four hours the *ortho*-quinone **22** equilibrates to an equimolar mixture of the quinone **22** and the catechol precursor **8h**. The partial regeneration of the catechol **8h** can be attributed to bromide reduction ($2Br^- \rightarrow Br_2^{\bullet-} \rightarrow Br_2$)

of the quinone to the semi-quinone ($Q \rightarrow Q^{\bullet -}$) which rapidly disproportionates ($2Q^{\bullet -} \rightarrow Q + Q^{2-}$). Similar behaviour was not observed using the more electronegative chloride salt **8i**.



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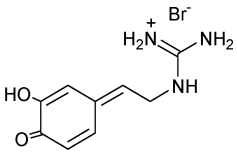

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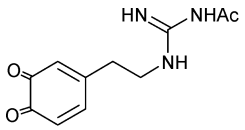
Pulse radiolytic oxidation of the catechol **8h** gave the semi-quinone (λ_{max} 310 and 350sh nm); this disproportionates to the *ortho*-quinone **22** (λ_{max} 400 nm; ε 1400 M^{-1} cm^{-1}) which is stable for at least ten seconds. Tyrosinase oxidation of the catechol **8h** showed evidence of *ortho*-quinone formation (λ_{max} ~400 nm) after 30 and 60 seconds but in later scans the main product absorbs at ~480 nm, which can be attributed to rapid rearrangement to the *para*-quinomethane **23**. This is comparable to the behaviour of the *ortho*-quinones **9** (Y = Me, NH_2),[6] and is consistent with our previous observations of rapid conversion to a quinomethane in aqueous buffer.[6,7]

The *N*-acetyl derivative **8j** can be expected to be a significantly weaker base (pK_a ~8) than the parent guanidine (pK_a ~13).[22] Prolonged heating of the sulfate **7g** with acetic anhydride and triethylamine gave the *N,N′*-diacetylguanidine **7l** in poor yield. This was demethylated using BCl_3 to give the catechol **8j**. The ^{13}C NMR spectrum of compound **7l** shows two carbonyl signals at δ 172.8 and 186.5 which we assign to the groups –NH.C=O and =N.C=O, respectively. An alternative route to catechol **8j** involved a shorter reaction time with acetic anhydride and triethylamine to give the *N*-acetylguanidine **7j** which was demethylated (BCl_3) to give the desired guanidine **8j**. The product **8j** was isolated as the free base and all spectral details were in accord with the proposed structure. The ^{13}C NMR spectrum showed a carbonyl signal at δ 173.7 and, on the basis of the ^{13}C NMR spectrum of compound **7l**, we conclude that this product is the –NH.C=O tautomer, as shown in structures **24** and **25**.

DDQ oxidation of the catechol **8j** in CD_3OD gave the *ortho*-quinone **24** [δ 6.30 (s), 6.50 (d) and 7.15 (d)] which slowly decayed over a period of twenty four hours. A complex set of signals develops as the *ortho*-quinone decays. There is some evidence to suggest that the *para*-quinomethane **25** is an initial product, and formation is possibly assisted by intramolecular deprotonation by the basic guanidine function. After two hours new signals appeared in the catechol region (δ 6.6–6.9) suggesting intramolecular cyclisation by the *para*-quinomethane side chain. However, the ^1H NMR was complex and all attempts on a preparative scale to isolate products were unsuccessful. In a similar manner to compound **8h**, pulse radiolytic oxidation of the catechol **8j** gave the *ortho*-quinone **24** (λ_{max} 400 nm; ε 1600 M^{-1} cm^{-1}) which was stable for more than ten seconds. Tyrosinase oxidation showed a clear 400 nm absorbance indicating initial formation of the *ortho*-quinone **24**. This converts, with isosbestic points at 372 and 428 nm, to a product absorbing at longer wavelengths (~500 nm) which is presumed to be the *para*-quinomethane **25**.



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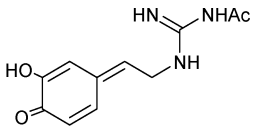

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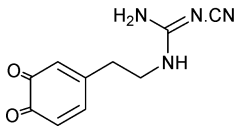

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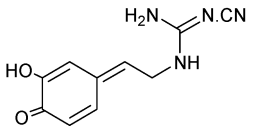

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Finally, we investigated the *N*-cyanoguanidine **8k** on the basis that it should be an even weaker base (pK_a ~0) than the *N*-acetyl derivative. The 3,4-dimethoxy derivative **7k** was prepared from 2-(3,4-dimethoxyphenyl)ethylamine and dimethyl *N*-cyanothioiminocarbonate [$(MeS)_2C$=N.CN] using standard procedures,[23] and demethylated (aq. HBr) to give the catechol **8k**. In a pulse radiolysis study of the oxidation of the catechol **8k** the cyano substituent appeared to interfere with the one-electron oxidation by $Br_2^{\bullet -}$ and gave a radical of unknown structure that did not decay to the *ortho*-quinone. This study therefore provided no useful information on the stability of the *ortho*-quinone **26**. Tyrosinase oxidation of catechol **8k** did not show evidence (λ_{max} 400 nm) of the *ortho*-quinone **26** but the final spectrum was very similar to that shown by the *N*-acetyl derivative **8j** with a very broad peak at λ_{max} ~470 nm. This suggests formation of the *para*-quinomethane **27** that is too fast for the *ortho*-quinone to be observed. When the catechol **8k** was oxidised using one equivalent of DDQ in CD_3OD, the ^1H NMR spectrum showed immediate formation of the *ortho*-quinone [δ 6.27 (s), 6.40 (d) and 7.15 (d)]. The *ortho*-quinone decayed over five hours: after three hours catecholic protons (δ 6.5–6.8) dominated the spectrum but the product was clearly a mixture. Attempts to identify or isolate products on a preparative scale were unsuccessful.

Conclusions

The purpose of this investigation was to explore the diversity of 4-substituted *ortho*-quinone chemistry by oxidation of a series of catechols **8** in which the side chains were chosen to have a wide variety of properties (*e.g.*, pK_a, nucleophilicity). In addition to extending our knowledge of *ortho*-quinone chemistry, new reactions of synthetic interest or of use for pro-drug activation by tyrosinase are a potential outcome. The formation of bicyclic products, *e.g.*, **12**, from thioureas provides access to novel heterocyclic derivatives and a wider variety of thiourea derivatives merits further investigation. In addition, the formation of the spirocyclic product **14** and the difference in behaviour of the corresponding phenyl precursor **17** is unexplained, and a more extensive investigation of amidine derivatives would be of interest.

Experimental

^1H and ^{13}C NMR spectra were recorded using a Bruker Advance DPX300 NMR spectrometer. IR spectra were recorded on a

Perkin-Elmer Paragon 1000 FT-IR spectrometer or a Thermo Nicolet Avatar 320 FT-IR spectrometer. IR spectra were measured as thin films (liquids) or potassium bromide discs (solids). NMR spectra were measured in $CDCl_3$ with tetramethylsilane as internal standard unless otherwise stated. Only significant bands for the IR spectra are quoted. Mass spectrometry was carried out by the EPSRC National Mass Spectrometry Service, Swansea. Melting points were determined on a Kofler block or a Bibby Stuart Scientific SMP3 melting point apparatus and are uncorrected. Column chromatography was carried out using BDH silica gel (particle size 33–70 μm) and chromatotron chromatography was carried out using a LDC Analytical Consta Metric 3200 solvent delivery system and plates made using Merck silica gel 60 PF_{254} containing gypsum. New compounds were shown to be pure by both tlc and NMR spectroscopy.

Preparation of *O,O*-dimethylcatechols 7

Compounds **7a**,[24] **7b**,[18,19] **7g**,[25] and **7k**[23] were prepared according to literature procedures.

***N*^{1}-[2-(3,4-Dimethoxyphenyl)ethyl]-*N*^{2}-methylthiourea 7c.** A mixture of 2-(3,4-dimethoxyphenyl)ethylamine (1.81 g, 10.0 mmol) and methyl isothiocyanate (0.88 g, 12.0 mmol) in EtOH (25 mL) was heated under reflux (1 h). The solution was concentrated to give compound **7c** (2.4 g, 93%), colourless needles, mp 95 °C; ν_{max}/cm^{-1} 3345, 3221, 3019, 2996, 2961, 2936, 1563, 1514, 1466, 1259, 1234, 1193, 1158, 1138 and 1026; δ_H ($CDCl_3$) 2.80 (5H, m), 3.65 (2H, m), 3.79 (6H, s, 2 × OC*H*$_3$), 5.70 (1H, br s, N*H*), 6.00 (1H, br s, N*H*) and 6.70 (3H, m, arom. *H*); δ_C ($CDCl_3$) 31.0 (Ar*C*H$_2$), 35.2 (*C*H$_3$N), 46.1 (*C*H$_2$N), 56.3 (2 × O*C*H$_3$), 111.8 (*C*H), 112.3 (*C*H), 121.1 (*C*H), 131.5 (*C*), 148.0 (*C*), 149.3 (*C*) and 182.6 (*C*=S). This product was converted to the catechol **8c** without further purification.

***N*-[2-(3,4-Dimethoxyphenyl)ethyl]benzamidinium chloride 7e.** 2-(3,4-Dimethoxyphenyl)ethylamine (2.83 g, 15.6 mmol) and ethyl benzimidate hydrochloride (2.90 g, 15.6 mmol) in MeOH (50 mL) were stirred overnight under an N_2 atmosphere. After evaporation, the crude product was recrystallised from MeOH–Et_2O to give compound **7e** (3.8 g, 73%), colourless plates, mp 201–202 °C (lit.[21] 200.5–201.5 °C); ν_{max}/cm^{-1} 3176, 3018, 1672, 1614, 1581, 1516, 1467, 1259, 1240, 1159, 1143, 1026 and 748; δ_H (CD_3OD) 2.99 (2H, t, *J* 7.0 Hz, ArC*H*$_2$), 3.70 (2H, t, *J* 7.0 Hz, NHC*H*$_2$), 3.80 (6H, s, 2 × OC*H*$_3$), 6.9 (3H, m, aromatic *H*), and 7.6 (5H, m, C_6H_5); δ_C ($CDCl_3$) 33.3 (Ar*C*H$_2$), 44.5 (N*C*H$_2$), 55.5 (2 × O*C*H$_3$), 112.3 (*C*H), 112.9 (*C*H), 121.4 (*C*H), 127.8 (*C*H), 129.4 (*C*H), 129.7 (*C*H), 130.8 (*C*.C=N), 133.7 (*C*CH$_2$), 148.6 (*C*.OMe), 149.7 (*C*.OMe) and 164.9 (*C*=N); *m/z* (Electrospray) 285 (M^+, cation)(25%), 165 (40), 150 (50), 135 (75), 119 (55), 107 (77), 103 (80), 91 (100); Found: M^+ (cation), *m/z* 285.1599; Calc. for $C_{17}H_{21}N_2O_2$; 285.1598.

***N*-Acetyl-*N*′-[2-(3,4-dimethoxyphenyl)ethyl]guanidine 7j.** *N*-[2-(3,4-Dimethoxyphenyl)ethyl]guanidinium sulfate **7g** (2.0 g, 7.4 mmol), triethylamine (0.74 g, 7.4 mmol) and acetic anhydride (0.76 g, 7.4 mmol) in CH_2Cl_2 (25 mL) were heated under reflux (5 h) and then stirred at room temperature (48 h). The solution was filtered and concentrated under vacuum to give a solid product that was identified as compound **7j** (0.7 g, 36%), cream prisms, mp 101–103 °C; ν_{max}/cm^{-1} 3253, 3083, 2992, 2928, 2840, 1634, 1566, 1517, 1473, 1263, 1235, 1157, 1140, 1020, 850, 815, 766 and 611; δ_H ($CDCl_3$) 2.07 (3H, s, C*H*$_3$.CO), 2.10 (1H, br s, N*H*), 2.77 (2H, t, *J* 6.9 Hz, ArC*H*$_2$), 3.60 (2H, m, NC*H*$_2$), 3.80 (3H, s, OC*H*$_3$), 3.82 (3H, s, OC*H*$_3$), 6.71 (3H, m, aromatic *H*), 8.99 (1H, br s, C=N*H*) and 13.07 (1H, br s, N*H*.Ac); δ_C ($CDCl_3$) 25.2 (C*H*$_3$), 28.8 (Ar*C*H$_2$), 35.0 (N*C*H$_2$), 56.0 (2 × O*C*H$_3$), 111.4 (*C*H), 112.0 (*C*H), 120.8 (*C*H), 131.1 (*C*), 147.9 (*C*OH), 149.1 (*C*OH), 155.6 (*C*=N) and 172.6 (NH.*C*=O). This material was converted into compound **8j** without further characterisation.

***N*,*N*-Diacetyl-*N*′-[2-(3,4-dimethoxyphenyl)ethyl]guanidine 7l.** A suspension of *N*-[2-(3,4-dimethoxyphenyl)ethyl]-guanidinium sulfate **7g** (10.0 g, 37.0 mmol) in CH_2Cl_2 (100 mL) was stirred with triethylamine (3.74 g, 37.0 mmol) (12 h). Acetic anhydride (3.77 g, 37.0 mmol) was then added and the mixture was heated under reflux (7 d). After cooling, the mixture was filtered and washed (× 3) with H_2O. Concentration gave a hygroscopic solid that was recrystallised from CH_2Cl_2–hexane and identified as compound **7l** (1.14 g, 10%), colourless prisms, mp 92–93 °C; δ_H ($CDCl_3$) 2.13 (3H, s, CO.C*H*$_3$), 2.14 (3H, s, CO.C*H*$_3$), 2.83 (2H, t, *J* 7.0 Hz, ArC*H*$_2$), 3.65 (2H, m, C*H*$_2$N), 3.86 (3H, s, OC*H*$_3$), 3.88 (3H, s, OC*H*$_3$), 6.80 (3H, m, aromatic *H*), 9.05 (1H, br s, N*H*) and 13.14 (1H, br s, N*H*); δ_C ($CDCl_3$) 25.4 (CO.*C*H$_3$), 29.1 (CO.*C*H$_3$), 35.2 (Ar*C*H$_2$), 42.9 (*C*H$_2$N), 56.2 (O*C*H$_3$), 56.3 (O*C*H$_3$), 111.7 (*C*H), 112.3 (*C*H), 121.1 (*C*H), 131.4 (*C*), 148.2 (*C*), 149.4 (*C*), 155.8 (*C*=N), 172.8 (NH.*C*=O) and 186.5 (=N.*C*=O). This material was converted to compound **8j** without further characterisation.

***N*^{1},*N*^{2}-Bis-[2-(3,4-dimethoxyphenyl)ethyl]benzamidinium chloride 16.** Powdered anhydrous $AlCl_3$ (10.4 g, 80 mmol) was added (over 20–30 s) to a stirred mixture of benzonitrile (8.8 g, 85 mmol) and 2-(3,4-dimethoxyphenyl)ethylamine (14.3 g, 80 mmol) and heated at (200 °C)(1 h). After cooling, the thick orange oil was stirred with water (150 mL). The aqueous component was filtered and extracted with ether (3 × 50 mL) to remove unreacted benzonitrile. The aqueous solution was then extracted with CH_2Cl_2 (3 × 50 mL) and the combined extracts were dried (Na_2SO_4). Concentration under vacuum gave a light brown solid that was identified as compound **16** (5.0 g, 26%). A small sample was recrystallised from CH_2Cl_2–Et_2O to give colourless crystals, mp 138 °C; ν_{max}/cm^{-1} 3173, 2936, 1638, 1516, 1448, 1420, 1262, 1237, 1157, 1141, 1025, 764 and 703; δ_H ($CDCl_3$) 2.70 (4H, t, *J* 6.5 Hz, ArC*H*$_2$), 3.08 (4H, m, NHC*H*$_2$), 3.78 (6H, s, OC*H*$_3$), 3.82 (6H, s, OC*H*$_3$), 6.3–7.4 (11H, m, aromatic *H*) and 10.54 (2H, t, *J* 2.0 Hz, NH); δ_C ($CDCl_3$) 35.7 (Ar*C*H$_2$), 46.7 (N*C*H$_2$), 56.0 (2 × O*C*H$_3$), 111.2 (*C*H), 112.4 (*C*H), 121.2 (*C*H), 125.0 (*C*H), 127.0 (*C*H), 129.2 (*C*H), 129.6 (*C*.C=N), 131.7 (*C*CH$_2$), 147.8 (*C*.OMe), 149.0 (*C*.OMe) and 168.0 (*C*=N); *m/z* (Electrospray) 450 [M – Cl^+](50%), 449 (100), 299 (5), 285 (5), 165 (75) and 150 (5).

Preparation of *N*-substituted dopamine derivatives 8. Compounds **8a**[17] and **8d**[20] were prepared according to literature procedures.

***N*-[2-(3,4-Dihydroxyphenyl)ethyl]guanidinium bromide 8h.** The guanidinium sulfate **7g** (2.0 g, 7.4 mmol) was dissolved in 48% aq HBr (50 mL) and under an N_2 atmosphere was heated at 90 °C (16 h). Most of the HBr was removed by evaporation under reduced pressure to afford a brown solid. This residue was extracted with EtOH and the solution filtered. Concentration

under reduced pressure gave a hygroscopic solid, which rapidly became an oil, that was identified as the bromide **8h** (0.6 g, 27%), brown oil; ν_{max}/cm^{-1} 3346, 1653, 1527, 1446, 1358, 1197, 1116, 1059, 1002, 870, 815, 781; δ_H ($CDCl_3$) 2.73 (2H, d, *J* 7.0 Hz, ArCH_2), 3.52 (2H, m, CH_2N), 6.57 (1H, dd, *J* 2.1 and 8.0 Hz, aromatic 6*H*), 6.68 (1H, d, *J* 2.1 Hz, aromatic 2*H*), 6.71 (1H, d, *J* 8.0 Hz, aromatic 5*H*); δ_C ($CDCl_3$) 35.4 (Ar*C*H_2), 44.1 (*C*H_2N), 116.6 (*C*H), 117.0 (*C*H), 121.2 (*C*H), 130.8 (*C*), 145.2 (*C*.OH), 146.5 (*C*.OH), 158.6 (*C*=N); *m/z* (Electrospray) 196 [M – Br$^+$](27%), 137(100), 119 (33), 91(53), 60 (92); Found: M – Br$^+$, *m/z* 196.1081; Calc. for $C_9H_{14}N_3O_2$; 196.1081.

In a similar manner, the following derivatives were prepared from the *O,O*-dimethylcatechols **7b**, **7e** and **7k**.

N-[2-(3,4-dihydroxyphenyl)ethyl]urea **8b** (0.46 g, 89%), mp 133–135 °C (with softening at 78 °C); ν_{max}/cm^{-1} 3317, 1687, 1639, 1570, 1521, 1468, 1332, 1257, 1185, 1115, and 786; δ_H ($CDCl_3$/CD_3OD) 2.69 (2H, t, *J* 7.0 Hz, ArCH_2) 3.37 (2H, t, *J* 7.0 Hz, CH_2N), 6.55 (1H, dd, *J* 8.0 and 1.7 Hz, aromatic 6*H*), 6.68 (1H, d, *J* 1.7 Hz, aromatic 2*H*) and 6.72 (1H, d, *J* 8.0 Hz, aromatic 5*H*); δ_C (CD_3OD) 38.9 (Ar*C*H_2), 46.5 (N*C*H_2), 119.5 (*C*H), 120.0 (*C*H), 124.2 (*C*H), 134.4 (*C*), 147.8 (*C*OH), 149.2 (*C*OH) and 165.5 (*C*=O); *m/z* (EI) 196 [M$^{\bullet+}$](5%), 136 (100), 124 (20), 123 (50), 77 (20), Found (Electrospray): MH$^+$, *m/z* 197.0921; Calc. for $C_9H_{13}N_2O_3$; 197.0921.

N-[2-(3,4-Dihydroxyphenyl)ethyl]benzamidinium bromide **8f** (0.65 g, 72%), colourless powder, mp 111–112 °C; ν_{max}/cm^{-1} 3141, 1666, 1622, 1581, 1526, 1446, 1354, 1284, 1256, 1196, 1115 and 781; δ_H (CD_3OD) 2.89 (2H, t, *J* 7.0 Hz, ArCH_2), 3.66 (2H, m, CH_2NH), 6.62 (1H, dd, *J* 2.0 and 8.0 Hz, aromatic 6*H*), 6.73 (2H, m, aromatic 2*H* and 5*H*), 7.62 (5H, m, C_6H_5), 8.76 (1H, br s, N*H*), 9.29 (1H, br s, N*H*) and 9.53 (1H, br s, N*H*); δ_C (CD_3OD) 33.2 (Ar*C*H_2), 44.8 (*C*H_2N), 115.6 (*C*H), 116.0 (*C*H), 120.2 (*C*H), 127.9 (*C*H), 129.4 (*C*H), 129.5 (*C*H), 129.9 (*C*), 133.6 (*C*), 144.3 (*C*), 145.6 (*C*) and 165.1 (*C*=N); *m/z* (Electrospray) 257 (M$^+$, cation)(100%), 121 (5); Found: M$^+$ (cation), *m/z* 257.1282; Calc. for $C_{15}H_{17}O_2N_2$; 257.1285.

N^1-Cyano-N^2-[2-(3,4-dihydroxyphenyl)ethyl]guanidine **8k** (0.26 g, 45%), dark oil, after column chromatography [silica gel: eluent CH_2Cl_2–CH_3OH (4:1)]; ν_{max}/cm^{-1} 3333, 2476, 1626, 1524, 1440, 1348, 1283, 1203, 1112 and 973; δ_H (CD_3OD) 2.72 (2H, t, *J* 7.0 Hz, ArCH_2), 3.37 (2H, t, *J* 7.0 Hz, CH_2N), 6.55 (1H, d, *J* 8.0 Hz, aromatic 6*H*), 6.66 (1H, s, aromatic 2*H*), 6.69 (1H, d, *J* 8.0 Hz, aromatic 5*H*); δ_C (CD_3OD) 34.4 (Ar*C*H_2), 43.4 (*C*H_2N), 115.9 (*C*H), 116.3 (*C*H), 121.0 (*C*H), 130.4 (*C*), 143.5 (*C*OH), 144.7 (*C*OH), 157.4 and 157.5 (*C*=N and *C*≡N).

N-Acetyl-*N*′-[2-(3,4-dihydroxyphenyl)ethyl]guanidine 8j.

Method 1. Under an N_2 atmosphere, 1M BCl_3 in CH_2Cl_2 (56.0 mL, 56 mmol)) was slowly added to a solution of compound **7l** (1.74 g, 5.65 mmol) in CH_2Cl_2 (25.0 mL) and the mixture was stirred at room temperature (72 h). The reaction mixture was then quenched with H_2O (50 mL) and the aqueous fraction extracted with CH_2Cl_2 (3 × 10 mL). The combined CH_2Cl_2 solutions were dried (Na_2SO_4) and concentrated to give a solid that was identified as compound **8j** (1.30 g, 95%), identical with a sample prepared by Method 2.

Method 2. Under an N_2 atmosphere, 1M BCl_3 in CH_2Cl_2 (4.0 mL, 4 mmol) was slowly added to a solution of compound **7j** (0.5 g, 2 mmol) in CH_2Cl_2 (5.0 mL) and the mixture was stirred at room temperature (56 h). The reaction mixture was then quenched with H_2O (10.0 mL) and the aqueous fraction extracted with CH_2Cl_2 (3 × 10 mL). The combined CH_2Cl_2 solutions were dried (Na_2SO_4) and concentrated to give a solid that was identified as compound **8j** (0.16 g, 34%), hygroscopic solid, mp ill-defined; ν_{max}/cm^{-1} 3157, 1721, 1688, 1624, 1594, 1517, 1443, 1371, 1247, 1199, 1153, 1116 and 1039; δ_H (CD_3OD) 2.16 (3H, s, CH_3), 2.80 (2H, t, *J* 7.0 Hz, ArCH_2), 3.52 (2H, t, *J* 7.0 Hz, NCH_2), 6.60 (1H, dd, *J* 2.0 and 7.0 Hz, aromatic 6*H*), 6.71 (1H, d, *J* 2.0 Hz, aromatic 2*H*) and 6.72 (1H, d, *J* 7.0 Hz, aromatic 5*H*); δ_C (CD_3OD) 23.5 (*C*H_3), 33.6 (Ar*C*H_2), 43.1 (N*C*H_2), 115.6 (*C*H), 115.9 (*C*H), 120.2 (*C*H), 129.1 (*C*), 144.4 (*C*OH), 145.6 (*C*OH), 154.4 (*C*=N) and 173.7 (NH.*C*=O); *m/z* (Electrospray) 238 [MH$^+$](30%), 196 (100), 137 (20), 105 (25), 74 (25), Found: MH$^+$, *m/z* 238.1187; Calc. for $C_{11}H_{16}N_3O_3$; 238.1186.

In a similar manner to Method 2, the following derivatives was prepared from the *O,O*-dimethylcatechols **7c** and **7e**.

N^1-[2-(3,4-Dihydroxyphenyl)ethyl]-N^2-methylthiourea **8c** (0.35 g, 76%), tiny crystals, mp 103 °C; ν_{max}/cm^{-1} 3290, 1576, 1521, 1452, 1355, 1282, 1224, 1200, 1111, 951 and 790; δ_H (CD_3OD) 2.69 (2H, t, *J* 7.0 Hz, ArCH_2), 2.91 (3H, br s, NCH_3), 3.55 (2H, m, NCH_2), 6.55 (1H, dd, *J* 8.0 and 1.7 Hz, aromatic 6*H*), 6.68 (1H, d, *J* 1.7 Hz, aromatic 2*H*) and 6.72 (1H, d, *J* 8.0 Hz, aromatic 5*H*); δ_C (CD_3OD) 29.6 (Ar*C*H_2), 33.7 (*C*H_3N), 45.6 (*C*H_2N), 114.7 (*C*H), 115.3 (*C*H), 119.6 (*C*H), 129.6 (*C*), 143.0 (*C*), 144.4 (*C*) and 174.9 (*C*=S); *m/z* (EI) 226 [M$^{\bullet+}$](80%), 195 (40), 136 (90), 123 (90), 107 (40) and 91 (100), Found: MH$^+$, *m/z* (Electrospray) 227.0847; Calc. for $C_{10}H_{14}N_2SO_2$; 227.0849.

N-[2-(3,4-dihydroxyphenyl)ethyl]benzamidinium chloride **8e** (0.11 g, 40%), hygroscopic solid; δ_H (CD_3OD) 2.92 (2H, t, *J* 7.0 Hz, ArCH_2), 3.68 (2H, m, CH_2N), 6.76 (3H, m, aromatic *H*), 7.63 (5H, m, C_6H_5), 8.80 (1H, br s, N*H*), 9.31 (1H, br s, N*H*) and 9.56 (1H, br s, N*H*); δ_C (CD_3OD) 33.3 (Ar*C*H_2), 44.7 (*C*H_2N), 115.6 (*C*H), 116.0 (*C*H), 120.2 (*C*H), 127.8 (*C*H), 129.4 (2 × *C*H), 129.9 (*C*), 133.6 (*C*), 145.6 (*C*), 148.2 (*C*) and 165.1 (*C*=N): the sample was spectroscopically almost identical to the bromide **8f** and was used without further analysis.

Oxidation of catechols 8

6-Methylamino-8,9-dihydro-5-thia-7-azabenzocycloheptene-2,3-diol hydrochloride 12. The thiourea **8c** (0.25 g, 1.1 mmol) in MeOH–$CHCl_3$ (1:9) (10 mL) was treated with 2,3-dichloro-5,6-dicyanobenzoquinone (0.25 g, 1.1 mmol). The precipitate that immediately formed was collected and dissolved in ethanolic HCl (5.0 mL). The solution was concentrated to give a solid that was recrystallised from ethanolic HCl and identified as the hydrochloride **12** (0.17 g, 59%), colourless prisms, mp 227–228 °C; ν_{max}/cm^{-1} 3132, 1637, 1511, 1441, 1367, 1245, 1173, 875, 812 and 724; λ_{max} (0.1 M phosphate buffer): pH 7.4, 287 (ε 2800) nm; δ_H (CD_3OD) 2.78 (3H, s, NCH_3), 3.07 (2H, t, *J* 6.0 Hz, ArCH_2), 3.69 (2H, t, *J* 6.0 Hz, CH_2N), 6.59 (1H, s, aromatic *H*) and 6.66 (1H, s, aromatic *H*); δ_C (CD_3OD) 31.3 (Ar*C*H_2), 32.4 (*C*H_3N), 46.8 (*C*H_2N), 115.1 (*C*), 118.6 (*C*H), 119.8 (*C*H), 133.4 (*C*), 146.2 (*C*), 149.3 (*C*) and 167.3 (*C*=N); *m/z* (EI) 224 [M$^{\bullet+}$](25%), 168 (100), 167 (80), 149 (30), 121 (30), Found: MH$^+$, *m/z* (Electrospray) 225.0691; Calc. for $C_{10}H_{13}N_2O_2S$; 225.0692.

8-Hydroxy-2-trichloromethyl-1-aza-spiro[5.5]undeca-2,7,10-trien-9-one hydrochloride 14. The amidine **8d** (0.37 g, 1.1 mmol)

in MeOH–$CHCl_3$ (1:9) (10 mL) was treated with 2,3-dichloro-5,6-dicyanobenzoquinone (0.25 g, 1.1 mmol). The solution was concentrated and the mixture separated by chromatotron chromatography (silica gel: ethyl acetate). The crude product was recrystallised from ethanolic HCl and identified as the hydrochloride **14** (0.28 g, 76%), colourless rods, mp 144 °C (decomp.); ν_{max}/cm^{-1} 2940, 1670, 1637, 1570, 1437, 1351, 1259, 1185, 1087, 1044, 858, 831 and 653; λ_{max} (0.1 M phosphate buffer): pH 7.4, 220 (ε 7000), 244 (ε 7415) and 320sh (ε 1440); δ_H (CD_3OD) 2.20 (2H, m, CCH_2), 3.85 (2H, t, *J* 6.0 Hz, CH_2N), 5.95 (1H, d, *J* 3.0 Hz, C7-*H*), 6.35 (1H, d, *J* 10.0 Hz, C10-*H*) and 7.00 (1H, dd, *J* 3.0 and 10.0 Hz, C11-*H*); δ_C (CD_3OD) 29.1 (CCH_2), 38.5 (CH_2N), 55.3 (spiro-*C*), 87.9 (CCl_3), 115.1 (*C*7), 128.8 (*C*10), 147.5 (*C*11), 149.4 (*C*8), 159.0 (*C*=N) and 182.0 (*C*=O); *m/z* (Electrospray) 395 $[M - Cl]^+$(10%), 161 (45%) and 135 (100%), Found: $[M - Cl]^+$, *m/z* (Electrospray) 294.9802 (^{35}Cl); Calc. for $C_{10}H_{10}O_2N_2Cl_3$; 294.9802.

Oxidative pulse radiolysis studies

The pulse radiolysis experiments were carried out on the 12 MeV linear accelerator at the Daresbury Laboratory, using the Free Radical Research Facility.[26–28] This accelerator provides pulse lengths between 0.2 and 2 μsec with doses up to 30 Gy. Absorbed doses were determined from the transient $(SCN)_2^{\bullet-}$ formation in air-saturated KCNS solutions (10 mM) as described by Adams and co-workers,[29] but using the updated Gε value of 2.59×10^{-4} m^2J^{-1} obtained by Buxton and Stuart,[30] G being the radiation chemical yield of $(SCN)_2^{\bullet-}$ and ε its molar absorption coefficient at 475 nm. Generation of the single-electron oxidising species $Br_2^{\bullet-}$ was carried out by irradiating nitrous oxide-saturated buffered aqueous solutions of 0.1 M KBr.[5]

Enzymatic oxidation studies using oximetry and spectrophotometry

These investigations were carried out using the apparatus previously described,[3] consisting of a 1 cm light path quartz spectrophotometer cuvette of 3.65 mL capacity adapted to hold a Clark-type polarimetric electrode (Yellow Springs Instruments, Yellow Springs, Ohio, Model 5300) with the tip located orthogonal to the light path of a Hewlett-Packard diode-array spectrophotometer (Model 8452A). The reaction mixture was stirred by a vertically mounted magnetic stirrer and the cell closed with a capillary stopper through which additions were made. The experiments were made in 0.1 M phosphate buffer (pH 7.4) at 24 °C to which the dopamine substrates were added. Stock solutions of the catechols were made up freshly in glass distilled water. A standard solution of tyrosinase, consisting of 300 Sigma units mL^{-1} made up in 0.1 M phosphate buffer (pH 7.4), and stored at –20 °C, was used in the experiments. Following enzyme addition the spectral changes were followed at 30 second scanning intervals using the kinetic mode of a software program (UV-Vis Chemstation A0801(66) from Agilent Technologies, Hannover, Germany). The oximetric readings were continuously recorded on a chart recorder and converted to nanomoles of oxygen utilised. The oximeter was calibrated using dithionite as previously described.[3] Unless otherwise stated, the amount of enzyme used was 30 Sigma units (100 μl), giving an enzyme concentration in the reaction mixture of approximately 8.2 units mL^{-1}.

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