Oxidative cyclisation of *N*,*N*-dialkylcatechol amines to heterocyclic betaines *via* *o*-quinones: synthetic, pulse radiolytic and enzyme studies

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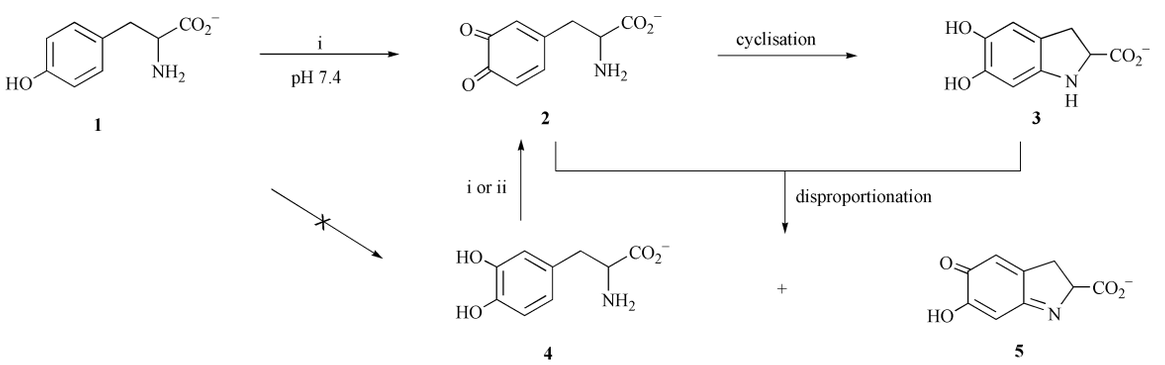
Oxidation of *N*,*N*-dialkyldopamines ‡ by either dianisyltellurium oxide or tyrosinase gives 2,3-dihydro-1*H*-indolium-5-olates which are formed by cyclisation of an intermediate *o*-quinone. The kinetics of formation and cyclisation of the *N*,*N*-dimethyl-*o*-quinone have been studied using pulse radiolysis. The indolium-5-olates do not activate met-tyrosinase and these results support a mechanism of tyrosinase oxidation of phenols to *o*-quinones in which the *o*-quinone is formed in a single step and not *via* an intermediate catechol. Similar chemical and enzymatic oxidation of a higher homologue gives an analogous 1,2,3,4-tetrahydroquinolinium-6-olate. Pulse radiolysis studies show that this product is formed *via* a spiro intermediate and not by direct cyclisation to form the six-membered quinolinium ring. The novel betaines described have been fully characterised and converted to their dimethoxy iodide salts. In a preliminary investigation of potential anti-cancer pro-drugs, amide derivatives of dopamine do not cyclise when oxidised to the *o*-quinone but cyclisation of an *N*-benzoylmethyl derivative to the corresponding betaine was observed. This betaine then appears to equilibrate with an *N*-ylide which, in contrast to the betaine, is a substrate for tyrosinase.

Introduction

Using selected phenolic and catecholic substrates we have recently demonstrated that the enzyme tyrosinase [EC 1.14.18.1] oxidises phenols to *o*-quinones in one step[1–3] and not *via* intermediate catechols as is widely claimed.[4] Thus, in the tyrosinase mediated oxidation of tyrosine **1** (Scheme 1) the dopaquinone **2** is formed directly in one step and then cyclises to cyclodopa **3**. A redox reaction subsequently occurs between the products **2** and **3** giving dopa **4** and dopachrome **5**, which then undergoes further reactions leading to melanin formation.[5] An important feature of this mechanism (Scheme 1) is that the dopa **4** is formed indirectly by non-enzymatic disproportionation and not by direct enzyme oxidation. This revised mechanism satisfactorily accounts for the induction or lag period observed during tyrosinase catalysed oxidation. In particular, the induction period arises because the enzyme occurs largely in the inactive *met* form in which the two copper atoms at the active site are in the Cu^{II} oxidation state and cannot bind dioxygen.[6] Reduction by a catechol converts the enzyme to the active *deoxy* form [Cu^{I}] together with formation of an *o*-quinone [eqn. (1)]. Deoxytyrosinase then binds dioxygen to form oxytyrosinase [eqn. (2)]. Using phenolic substrates, oxidation is initially very slow due to the small amount of enzyme in the active *deoxy* form and consequently indirect formation of activating catechol *via* redox disproportionation (Scheme 1) is also initially slow. As more catechol is formed by the indirect non-enzymatic route the rate of activation accelerates accounting for the observed kinetics. It is important

† E. J. L. is an Honorary Senior Research Fellow at Keele University.
‡ The IUPAC name for dopamine is 4-(2-aminoethyl)pyrocatechol.



Scheme 1 *Reagents*: i, O_2 + deoxytyrosinase; ii, met-tyrosinase.

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to note that although catechols are not formed directly by tyrosinase (**1**↛**4**) they are substrates for the enzyme [eqns. (1) and (3)] and like phenols [eqn. (4)] are effectively oxidised to *o*-quinones by the *oxy* form of the enzyme (*e.g.* **4**→**2**).

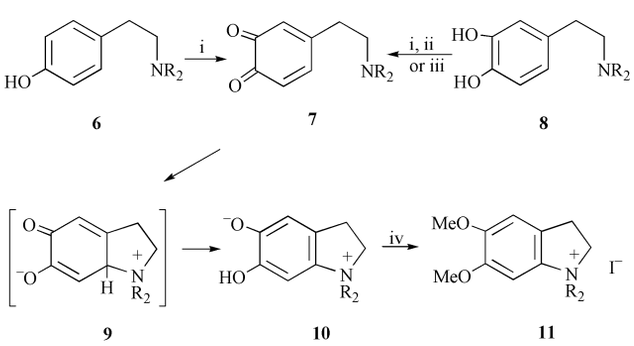
$$\text{Met}[Cu^{II}]\text{tyrosinase} + \text{catechol} \longrightarrow \text{deoxy}[Cu^{I}]\text{tyrosinase} + o\text{-quinone} + 2H^{+} \quad (1)$$

$$\text{Deoxy}[Cu^{I}]\text{tyrosinase} + O_2 \longrightarrow \text{oxy}[Cu^{II}]\text{tyrosinase} \quad (2)$$

$$\text{Oxy}[Cu^{II}]\text{tyrosinase} + \text{catechol} + 2H^{+} \longrightarrow \text{met}[Cu^{II}]\text{tyrosinase} + o\text{-quinone} + 2H_2O \quad (3)$$

$$\text{Oxy}[Cu^{II}]\text{tyrosinase} + \text{phenol} \longrightarrow \text{deoxy}[Cu^{I}]\text{tyrosinase} + o\text{-quinone} + H_2O \quad (4)$$

As part of our mechanistic study of tyrosinase we have shown that *N*,*N*-di-*n*-propyltyramine§ **6b** is not oxidised by unactivated tyrosinase.[1] This provides strong evidence that direct enzymatic catechol formation does not occur (*i.e.* **6**↛**8**). Using pre-activated tyrosinase, oxidation of the same substrate **6b** resulted in rapid formation of a product that we proposed was the indolium-5-olate **10b**, generated by cyclisation of the intermediate *o*-quinone **7b** and aromatisation of the resulting cyclohexadienone **9b** (Scheme 2). This product **10b** is not a catechol



In formulae **6**-**11**; **a**, R = Me;
b, R = nPr;
c, $R_2 = (CH_2)_5$;
d, R = H.

Scheme 2 *Reagents*: i, O_2 + deoxytyrosinase; ii, met-tyrosinase; iii, An_2TeO; iv, MeI–K_2CO_3.

and we concluded that it cannot lead to redox activation of tyrosinase [eqn. (1)].[1] Material isolated from the pre-activated enzyme reaction media had a ^{1}H NMR spectrum consistent with the proposed betaine structure **10b** and we required at this stage authentic samples of this novel indolium-5-olate and related products for structure confirmation and studies of their properties. In this paper we describe the preparation of examples of novel heterocyclic betaines,[2] including compound **10b**, by cyclisation of chemically generated *o*-quinones, together with a pulse radiolysis study of the mechanisms and kinetics of these reactions. We also describe the investigation of three dopamine derivatives designed to explore the possibility of using tyrosinase mediated betaine formation *in vivo* as a method of selectively activating anti-cancer pro-drugs.

Results and discussion

Although intramolecular cyclisations of primary amino groups onto *o*-quinones to form 2,3-dihydro-5,6-dihydroxy-1*H*-indole derivatives are well known (*e.g.* **2**→**3**, Scheme 1),[5] *a priori* it was not obvious that tertiary amines would readily cyclise in a similar manner. In fact, during the course of our work, Jagoe and co-workers[7] in the report of a study of the binding of oxidised catechols to cysteine residues in Src family SH2 domains assumed that *o*-quinone **7b** is unlikely to cyclise to a 2,3-dihydro-1*H*-indole derivative although their observation that compound **8b**, like dopamine **8d**, was inactive in their assay is entirely consistent with cyclisation. However, our search of the literature showed that this type of cyclisation had previously been encountered by Robinson and Sugasawa,[8] and independently by Schöpf and Thierfelder,[9] during studies of the biogenesis of morphine alkaloids, but simple systems were not investigated.

Preparation of indolium-5-olates 10

Synthesis of precursor catechols **8** from 2-(3,4-dimethoxyphenyl)ethylamine was achieved using standard methods and in each case the 3,4-dimethoxyphenyl function was converted to the catechol using 48% HBr and the resulting tertiary amine hydrobromide salt converted to the free base using aqueous sodium bicarbonate. There are many reagents for oxidising catechols to *o*-quinones but our interest in hypervalent reagents[10] directed our attention to dianisyltellurium oxide ($An_2Te{=}O$)(DAT)[11] which has been shown to be selective for catechol oxidation in the presence of a wide variety of other functional groups including amines.

A preliminary study of the oxidation of *N*,*N*-dimethyldopamine **8a** using one equivalent of DAT in $CDCl_3$–MeOH solution was monitored using ^{1}H NMR spectroscopy and the results were extremely promising. All the proton signals associated with the reactants rapidly disappeared and were replaced by signals corresponding to quantitative formation of the betaine **10a** and dianisyltellurium. When the reaction was repeated on a preparative scale the betaine **10a** was isolated as a reddish brown solid in 91% yield. Oxidation of the amines **8b**,**c** similarly gave the betaines **10b**,**c** in high yield. NMR spectroscopy fully supported the assignment of the 2,3-dihydro-1*H*-indolium-5-olate structure **10** to these products. Typically, the betaine **10b** showed only two aromatic protons (δ 6.63 and 6.67) consistent with cyclisation together with a pair of triplets (J 7.5 Hz) at δ 3.07 and 3.99 corresponding to the dihydroindole ring methylene groups: the methylene group at particularly low chemical shift is adjacent to the quaternary nitrogen atom. Equally significant are the chemical shifts and non-equivalence of the methylene protons of the *N*-*n*-propyl substituents. Thus the non-equivalent methylene protons on the α-carbon atoms appear at low field as a pair of doublets of triplets (δ 3.40 and 3.55) and the protons on the β-carbon atoms appear as a pair of multiplets at higher field (δ 1.40 and 1.65). We assume that in each case the protons at lower field are those pointing towards the aromatic ring and are deshielded by the ring current. A COSY spectrum fully supported the proposed proton–proton coupling in the betaine **10b** and additional structural confirmation was provided by the ^{13}C NMR spectrum. The NMR spectra of the betaines **10a**,**b** were identical with those of the tyrosinase oxidation products providing confirmation of the structures of the enzyme products.

As might be expected, the UV spectra of the betaines **10** show a small pH dependence. At pH 7.4 in phosphate buffer all the derivatives **10a**–**c** show an absorption at 290 nm with a shoulder at *ca.* 310 nm. At pH 6.5 this shoulder is absent. We attribute this change at lower pH to complete formation of salts, which have spectra identical to those of the iodides **11**. In aqueous solution the betaines **10** are clearly in equilibrium with these salts. We have represented the betaines **10** as the 5-olates on the understanding that in these tautomers resonance places negative charge closest to the quaternary centre. This assumption is supported by AM1 semi-empirical MO calculations.[1] However, we cannot eliminate the possibility that these tautomers are in equilibrium with the 6-olates as well as their salts.

§ The IUPAC name for tyramine is 2-(4-hydroxyphenyl)ethylamine.

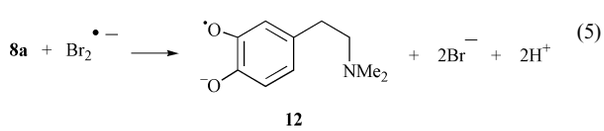
The betaines **10** are hygroscopic and difficult to recrystallise but further characterisation was achieved by conversion to the 5,6-dimethoxy iodides **11** using methyl iodide in the presence of potassium carbonate. In this way analytically pure samples of the colourless iodides **11a–c** were obtained after recrystallisation. Attempts to prepare the monomethyl iodides were unsuccessful and led to mixtures of mono- and dimethylated products that were difficult to separate.

The availability of the synthetic betaines **10** has enabled us to demonstrate experimentally that these betaines do not activate tyrosinase, in accord with our mechanistic conclusions.[1] We have previously shown that *N*,*N*-dimethyltyramine **6a** is oxidised at a negligible rate by tyrosinase unless the enzyme is activated by a catechol, which is presumptive evidence that the formation of the corresponding cyclic betaine **10a** is unable to abolish the lag-period of the enzyme.[1] However, to demonstrate unequivocally the inability of the betaines **10** to activate tyrosinase we have employed an oximetric assay using *N*,*N*-dimethyltyramine **6a** as substrate. In these experiments both pre-incubation with or addition to the incubation mixture of up to 125 μM of synthetic betaine **10a** failed to modify the rate of oxygen uptake. In contrast, small amounts (10 μM) of the corresponding catechol **8a** produced an immediate increase in oxygen utilization attributable to accelerated oxidation of the *N*,*N*-dimethyltyramine **6a**. Since the activation of tyrosinase is dependent on recruitment of met-enzyme by reduction of active site copper atoms,[5,6] we conclude that the lack of enzyme activation by the authentic betaine **10a** is a consequence of the significantly different redox properties of the betaine compared to the corresponding catechol **8a**.

Mechanism and kinetics of indolium-5-olate formation

The intermediate *o*-quinones **7** could not be detected by ^{1}H NMR spectroscopy because they cyclise too rapidly. We have used pulse radiolysis to identify *o*-quinone intermediates, *e.g.* **7a**, and study the mechanism and kinetics of their cyclisations. For these studies the *o*-quinones **7** were generated *in situ* by one-electron oxidation of the catechol to the semiquinone. The semiquinone then disproportionates to give the *o*-quinone.[12,13]

One-electron oxidation of the catechol **8a** was carried out by pulsed irradiation of an N_2O-saturated solution in the presence of KBr buffered to pH 7.0. Fig. 1 shows the changes in absorption spectrum 45, 315, 950 μs and 2.2 ms after the pulse. The initial spectrum at 45 μs is due to formation of the semiquinone **12** [eqn. (5)].



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The spectrum obtained when most of semiquinone **12** had decayed (2.2 ms) showed little evidence for formation of the expected *o*-quinone **7a** (λ_{max} 400 nm). However, a comparison of the decay of the semiquinone **12** at its peak (λ_{max} 310 nm) with the transmission-against-time curve observed at 400 nm over the same time scale (see inset to Fig. 1) provides evidence of a delayed growth at 400 nm over ~0.25 ms after the pulse. This is probably due to a short-lived *o*-quinone *i.e.* **7a** with a lifetime of a few milliseconds. The increase in the rate of decay of $Br_2^{\bullet-}$ observed at λ_{max} 360 nm on addition of the catecholamine **8a** led to measurement of a rate constant of 1.3×10^8 M^{-1} s^{-1} for the formation of the semiquinone **12** (eqn. (5)). The semiquinone absorption with a maximum at λ_{max} 310 nm (ε 11 900 dm^3 mol^{-1} cm^{-1}, based on thiocyanate dosimetry) decayed by disproportionation with a rate constant ($2k$) of 7.5×10^8 M^{-1} s^{-1}.

Since *o*-quinones derived from catecholamines can become longer-lived on changing from neutral to acid conditions,[12]

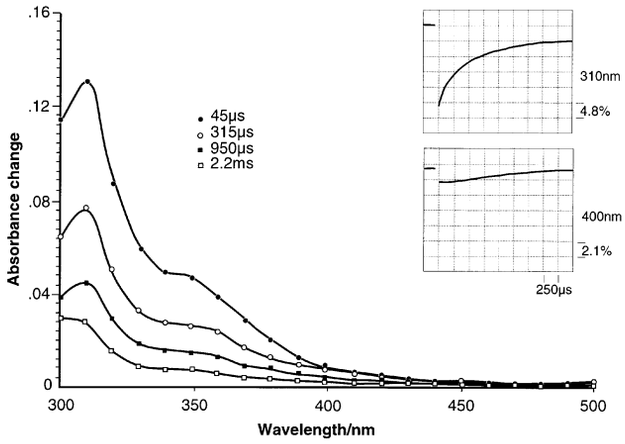


Fig. 1 Absorption changes at various times after pulse radiolysis of an N_2O-saturated aqueous solution of 0.58×10^{-3} M **8a** containing 0.1 M KBr and 10^{-2} M phosphate buffer, pH 7.0; dose ~14 Gy. Inset: time profiles of transmission changes.

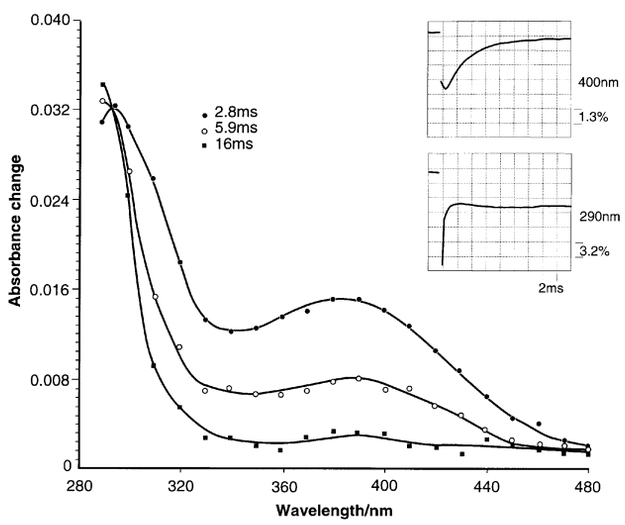


Fig. 2 Absorption changes at various times after pulse radiolysis of an N_2O-saturated aqueous solution of 0.82×10^{-3} M **8a** containing 0.1 M KBr and 10^{-2} M phosphate buffer, pH 6.2; dose ~30 Gy. Inset: time profiles of transmission changes.

the above experiment was repeated at pH 6.2. Fig. 2 shows the changes in absorption spectrum recorded 2.8, 5.9 and 16 ms after pulse radiolysis of compound **8a** and KBr in phosphate buffer at pH 6.2. The spectrum with wavelength maximum around λ_{max} 390 nm is attributed to the *o*-quinone **7a** (ε 1500 dm^3 mol^{-1} cm^{-1}) which decayed unimolecularly, with a rate constant of 300 s^{-1}, into a stable species absorbing increasingly below 300 nm.

It was not practicable to study the spectrum of the stable product below 300 nm using millimolar solutions of compound **8a** because of the strong parent absorption in this region. However, since $N_3^{\bullet}$ tends to react at least an order of magnitude faster with catechols than $Br_2^{\bullet-}$,[14] it was still possible with 1×10^{-4} M solutions of compound **8a** to obtain practically complete scavenging of the alternative one-electron oxidant $N_3^{\bullet}$ and make spectroscopic observations inside the region of parent **8a** absorption down to 250 nm. Accordingly, Fig. 3 shows the change in absorption spectrum 1.3 and 14 ms after pulse radiolysis of an N_2O-saturated solution of compound **8a** in the presence of NaN_3, buffered to pH 6.2 with phosphate. Care was taken to keep the azide concentration as low as possible in order to minimise the likelihood of nucleophilic addition of N_3^- to the *o*-quinone formed.[12] The spectrum measured at 1.3 ms is assigned largely to the *o*-quinone **7a**, together with a small amount of residual semiquinone **12**, and

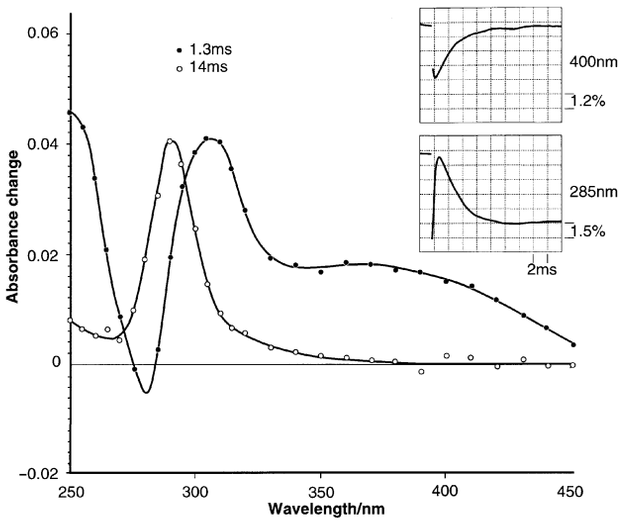


Fig. 3 Absorption changes 1.3 and 14 ms after pulse radiolysis of an N_2O-saturated aqueous solution of 1.0×10^{-4} M **8a** containing 3×10^{-3} M NaN_3 and 10^{-2} M phosphate buffer, pH 6.2; dose ~33 Gy. Inset: time profiles of transmission changes.

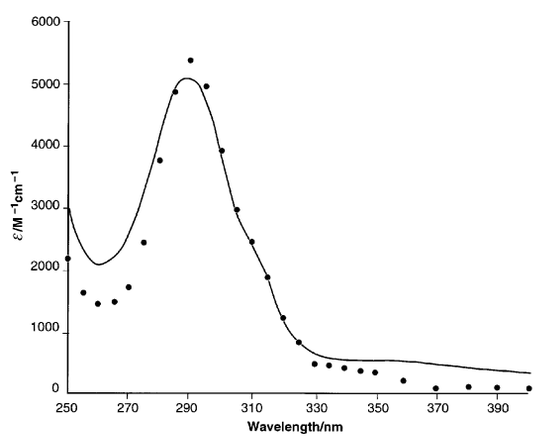
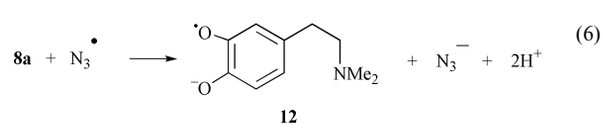


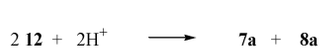
Fig. 4 Absolute absorption spectrum, measured at pH 7.4, of the final product of oxidation of the catechol **8a**: 2,3-dihydro-1,1-dimethyl-6-hydroxy-1*H*-indolium-5-olate **10a**. (The filled circles are from the pulse radiolysis experiments and the full line from measurements on the betaine prepared by $An_2Te{=}O$ oxidation).

that obtained at 14 ms is assigned to the final product. From the rate of formation of semiquinone at λ_{max} 310 nm, a rate constant of 5.8×10^9 M^{-1} s^{-1} was obtained for the reaction shown in eqn. (6).



(6)

It is suggested that the final product is the indolium-5-olate **10a**, resulting from the intramolecular cyclisation of the *o*-quinone **7a**, formed *via* the disproportionation reaction shown in eqn. (7), followed by reaction **7a**→**[9a]**→**10a** (Scheme 2).

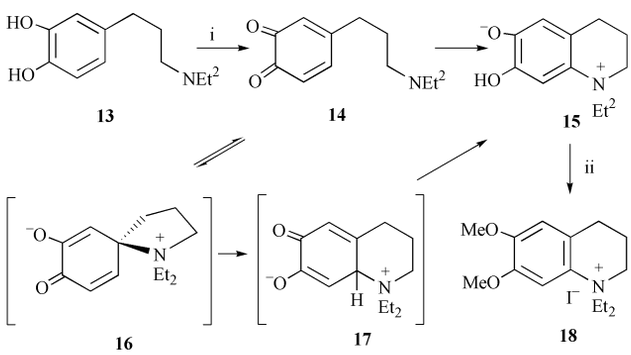


(7)

Based on thiocyanate dosimetry, and taking into account that the yield of the final product is half that of the initial yield of $N_3^{\bullet}$ radicals, the absolute spectrum of the radiolytically produced final product, corrected for parent **8a** depletion, was calculated and is presented in Fig. 4, together with the spectrum of an authentic sample of the betaine **10a** prepared by $An_2Te{=}O$ oxidation. It can be seen that the spectrum of the product generated pulse radiolytically (filled circles) matches rather well the spectrum of the isolated and fully characterised indolium-5-olate **10a** (full line).

Preparation of quinolinium-6-olate 15

The clean preparation of the indolium-5-olates **10** prompted us to investigate the preparation of quinolinium-6-olates, *e.g.* **15**, *via* an analogous oxidative cyclisation of the higher homologues. In this case we recognised that an alternative 5-*exo-trig* cyclisation of the intermediate *o*-quinone **14** to give a spirobetaine **16** might be favoured compared to six-membered ring formation (**14**→**15**)(Scheme 3). However, the cyclisation



Scheme 3 *Reagents*: i, An_2TeO; ii, $MeI–K_2CO_3$.

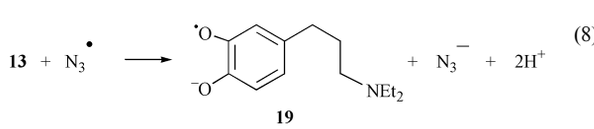
product **16** does not enjoy aromatic stabilisation and can be expected to equilibrate with the *o*-quinone precursor **14** or rearrange (**16**→**17**).

N,*N*-Diethyl-3-(3,4-dihydroxyphenyl)propylamine **13** upon treatment with one equivalent of DAT gave the 7-hydroxy-1,2,3,4-tetrahydroquinolinium-6-olate **15** quantitatively as monitored by ^1H NMR. There was no NMR evidence of the formation of the spirobetaine **16** during the reaction. On a preparative scale the betaine **15** was isolated in 84% yield as a reddish brown solid which was readily converted into the crystalline dimethoxy iodide **18**, mp 230–231 °C, in 90% yield. The structures **15** and **18** were fully supported by their spectroscopic properties: significantly only two aromatic protons are observed in the ^1H NMR spectra.

Mechanism and kinetics of quinolinium-6-olate formation

When the formation and cyclisation of the *o*-quinone **14** was studied using pulse radiolysis an interesting difference to the cyclisation of the dopamine derivatives **8** was observed. In particular, an additional intermediate was detected during the formation of the betaine **15** and we believe that this intermediate is the spirobetaine **16**.

One-electron oxidation of the catechol **13** was carried out by pulsed irradiation of an N_2O-saturated solution in the presence of NaN_3, buffered to pH 6.2 with phosphate. The initial product, which had completely formed 40 μs after the pulse, showed absorption maxima at λ 310 and 350 nm (Fig. 5). This species is almost certainly the semiquinone radical **19** formed *via* the reaction shown in eqn. (8).



(8)

From the pseudo first-order build-up of semiquinone absorption at 310 nm, a rate constant of 4.1×10^9 M^{-1} s^{-1} was obtained for the reaction shown in eqn. (8). Based on thiocyanate dosimetry, a molar absorption coefficient of 12 100 M^{-1} cm^{-1} was obtained for the semiquinone **19** at 310 nm which decayed bimolecularly with a rate constant ($2k$) of 4.1×10^7 M^{-1} s^{-1}.

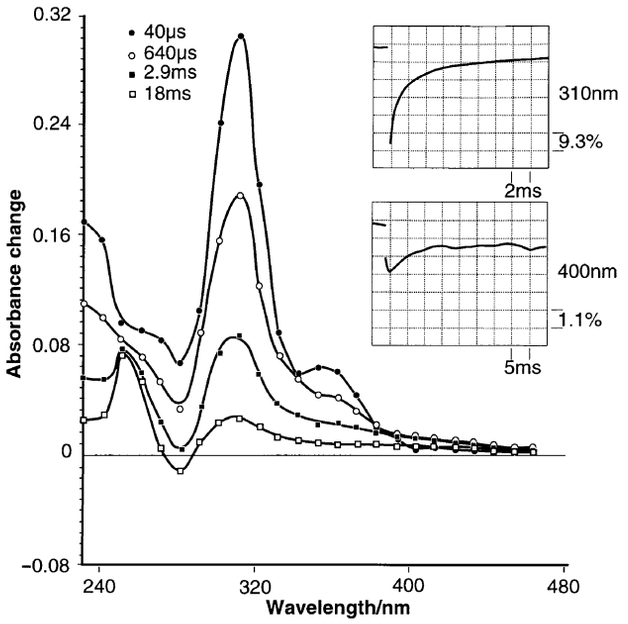


Fig. 5 Absorption changes at various times after pulse radiolysis of an N_2O-saturated aqueous solution of 0.96×10^{-4} M **13** containing 3×10^{-3} M NaN_3 and 10^{-2} M phosphate buffer, pH 6.2; dose ~30 Gy. Inset: time profile of transmission changes.

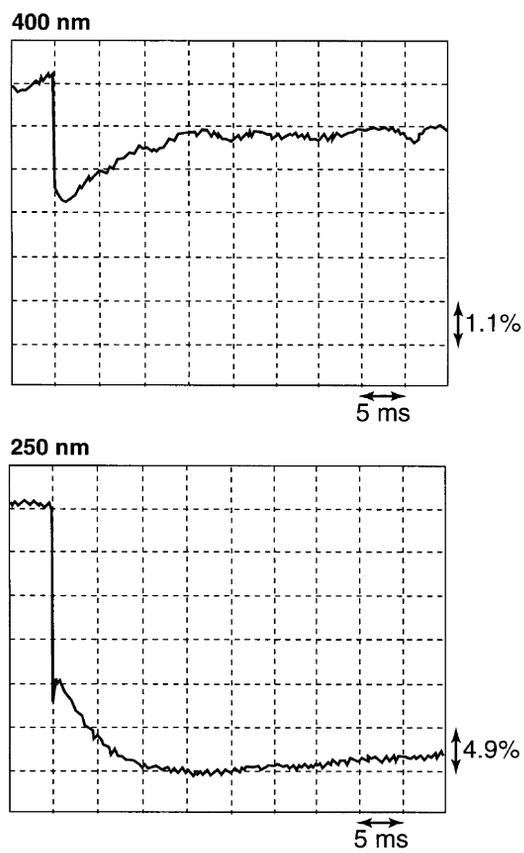


Fig. 6 Time profiles of transmission changes showing matching 1st order decay at 400 nm, and build-up at 250 nm, following pulse radiolysis of an N_2O-saturated aqueous solution of 1.0×10^{-3} M **13** containing 0.1 M KBr and 10^{-2} M phosphate buffer, pH 6.2; dose ~26 Gy.

As with the amine **8a**, there was evidence for an *o*-quinone (ε 1300 M^{-1} cm^{-1} at 400 nm) with a half-life of a few milliseconds growing in as the semiquinone **19** decayed (see inset to Fig. 5). The decay of the *o*-quinone in this case, however, instead of leading directly to a stable betaine, gave rise instead to a new transient absorption with a peak at 250 nm. The matching first order decay at 400 nm and build-up at 250 nm

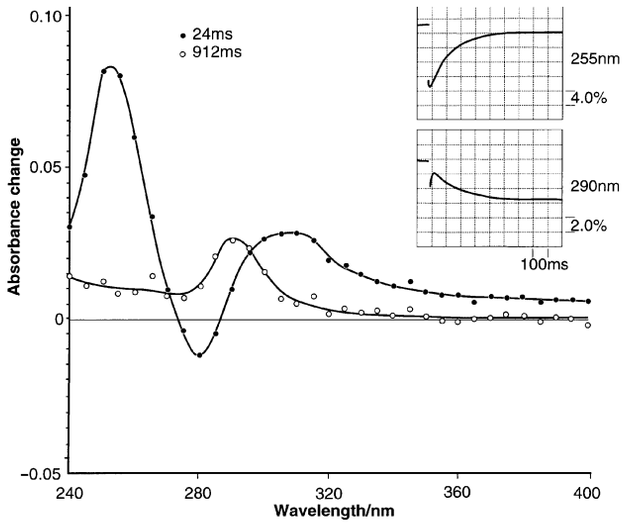


Fig. 7 Absorption changes 24 and 912 ms after pulse radiolysis of an N_2O-saturated aqueous solution of 1.0×10^{-4} M **13** containing 3×10^{-3} M NaN_3 and 10^{-2} M phosphate buffer pH 6.2; dose ~30 Gy. Inset: time profiles of transmission changes.

($k = 230$ s^{-1}) using $Br_2^{\bullet -}$ as oxidant is illustrated in Fig. 6. There is a window in the absorption of the catechol amine **13** in the λ 250 nm region allowing the use of the necessary higher concentrations (millimolar) [$k(Br_2^{\bullet -} + \mathbf{13}) = 2.4 \times 10^8$ M^{-1} s^{-1}] to ensure efficient $Br_2^{\bullet -}$ scavenging.

The species causing the difference absorption maximum at λ 250 nm decayed in turn, over several hundred milliseconds, unimolecularly, with a rate constant (k) of 7.1 s^{-1} at pH 6.2 (using $N_3^{\bullet}$ initiation), to give a stable product with a difference maximum at λ 290 nm (Fig. 7). This resembles the final product obtained on oxidation of compound **8a** and identified as the indolium-5-olate **10a**. Using the same assumptions as described earlier for the radiolytic formation of **10a**, the absolute spectrum of the final product obtained on oxidation of compound **13** was calculated, and is presented in Fig. 8a, together with the spectrum of an authentic sample of the quinolinium-6-olate **15** at the same pH. Tyrosinase catalysed oxidation of compound **13** at physiological pH (7.4)[1] also gave rise to a final product with the same UV spectrum as that produced by pulse radiolytic oxidation (Fig. 8b).

The transient spectrum with a difference maximum at 250 nm (Fig. 7) is assigned to the spirobetaine **16**. This maximum is similar to those of several more stable compounds with similar chromophores.[15–17] The lack of detection of an analogous spirobetaine intermediate in the oxidation of the lower homologue **8a** is understandable, since 4-membered ring formation is much less favoured. The species causing the difference maximum at λ 250 nm is unlikely to be due to the intermediate **17** as the analogous species **9** were not detected on oxidation of the dopamine derivatives **8**. Again, on the basis of thiocyanate dosimetry and that the spirobetaine yield is half that of the initial yield of $N_3^{\bullet}$, the absolute absorption spectrum of the spiro intermediate **16** at pH 6.2 was obtained and is illustrated in Fig. 9. The weak absorption of intermediate **16**, tailing into the visible, possibly due to a forbidden n–π* transition, is consistent with the presence of a cyclohexadienone component in the assigned structure.

The rapid first-order decay of the spirobetaine **16** leading to the final product **15** suggests that this occurs *via* 1,2-rearrangement to intermediate **17** followed by rapid aromatisation. 1,2-Rearrangement of spirointermediates are well known: an example is the rearrangement of carbocyclic spiro-species formed by oxidative radical coupling during the biosynthesis of some alkaloids. The novel 1,2-nitrogen rearrangement proposed in Scheme 3 is therefore fully in accord with expectation.

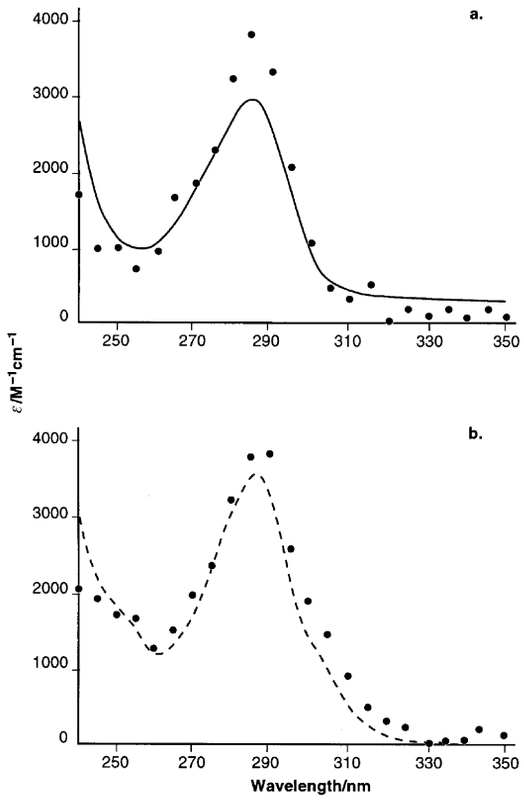


Fig. 8 Absolute absorption spectrum of 1,1-diethyl-7-hydroxy-1,2,3,4-tetrahydroquinolinium-6-olate **15**, which is the final product of oxidation of the catechol **13**, measured at: (a) pH 6.2 (the filled circles being from the pulse radiolysis experiments and the full line from measurements on the betaine prepared by $An_2Te{=}O$ oxidation); and, (b) pH 7.4 (the filled circles being from the pulse radiolysis experiments and the dotted line from the tyrosinase-catalysed oxidation of catechol **13**).

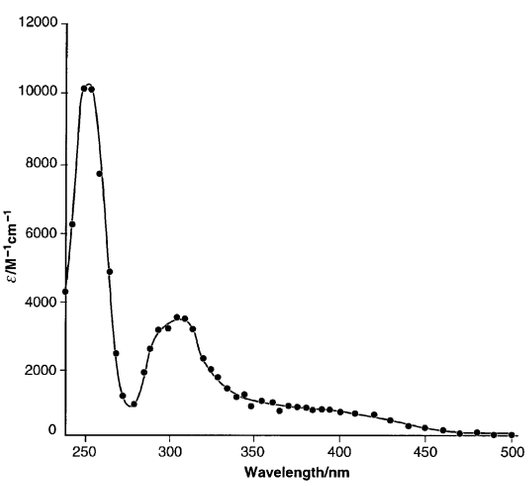
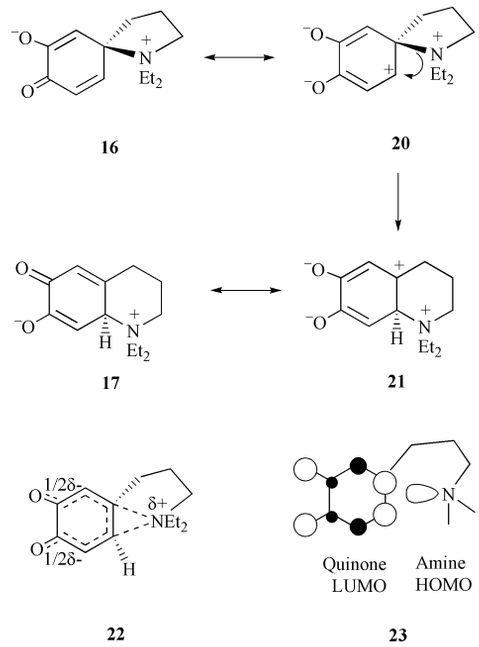


Fig. 9 Absolute spectrum, measured at pH 6.2, of the unstable spirobetaine **16**.

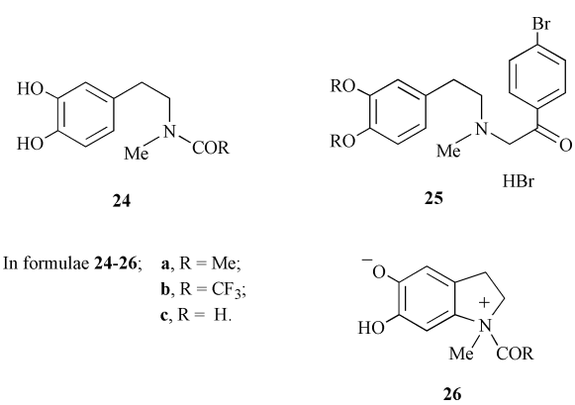
Consideration of the canonical forms **20** and **21** suggests that this reaction is symmetry allowed. Furthermore, the transition state **22** can be expected to be stabilised by a favourable interaction between the quinone LUMO and amine HOMO (**23**). However, we cannot exclude the possibility that the final product **15** is formed indirectly by ring opening of the spirobetaine followed by recyclisation (*i.e.* **16**→**14**→**17**→**15**).

Investigation of potential substrates for tyrosinase activated hydrolysis

Tyrosinase has limited occurrence in healthy human beings but significant amounts occur in malignant melanoma. This



limitation of its distribution essentially to invasive tissue provides in principle a mechanism for selectively activating anticancer pro-drugs within melanoma cells by employing *in vivo* oxidation of a phenol. In particular we envisage that if an anticancer drug were incorporated into a dopamine derivative of the general type **24** (or the corresponding tyramine derivative) then tyrosinase mediated betaine formation (**24**→**26**) might lead to rapid hydrolysis of the reactive quaternary amide **26** with selective release of the drug within the cancer cell.[18] Although we recognise that amide nitrogens are poor nucleophiles we believed that the reactivity of the *o*-quinone intermediates towards intramolecular cyclisation merited an initial investigation of simple amides and related species. Accordingly we prepared the novel amides **24a**,**b** and the aminoketone derivative **25c**. These compounds were prepared from *N*-methyl-2-(3,4-dimethoxyphenyl)ethylamine using standard methods and were fully characterised.



The catechols **24a**,**b** were rapidly oxidised by tyrosinase with a concurrent rise in the absorbance at 400 nm, consistent with formation of the corresponding *o*-quinone. Radiolytic oxidation of catechols **24a**,**b** also showed growth of a weak absorbance at 400 nm which is evidence of *o*-quinone formation as the corresponding semiquinones decay. This absorbance was stable for ⩾ 10 seconds. Chemical oxidation (DAT or DDQ) similarly led to *o*-quinone formation without any evidence of cyclisation when monitored by ^{1}H NMR. We conclude that under all these conditions cyclisation to form the *N*-acyl betaines **26** does not occur.

When the oxidation of the aminoketone **25c** by tyrosinase was investigated there was an initial rapid uptake of oxygen.

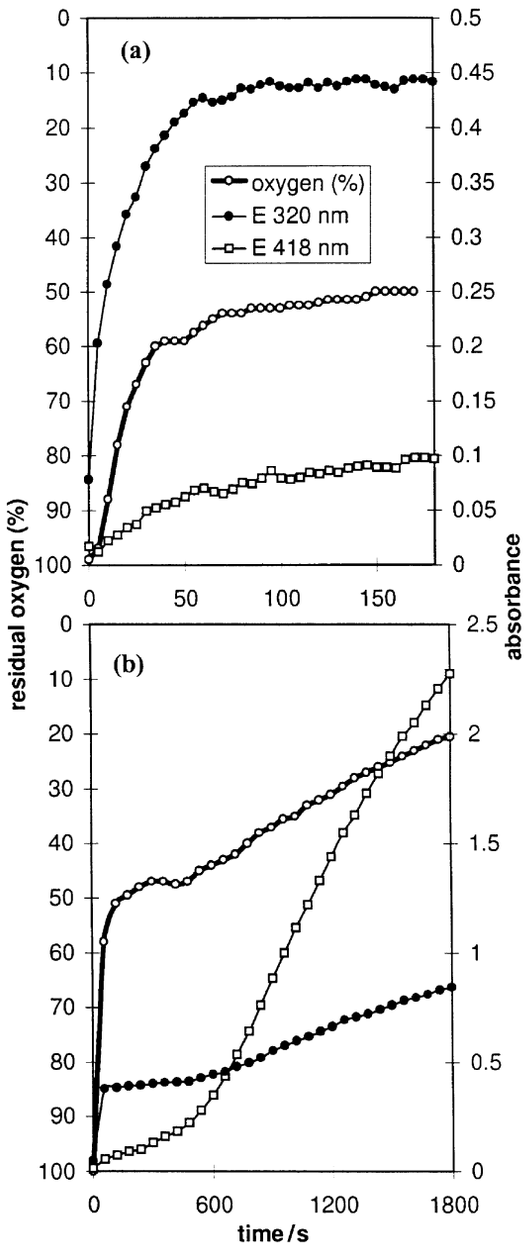
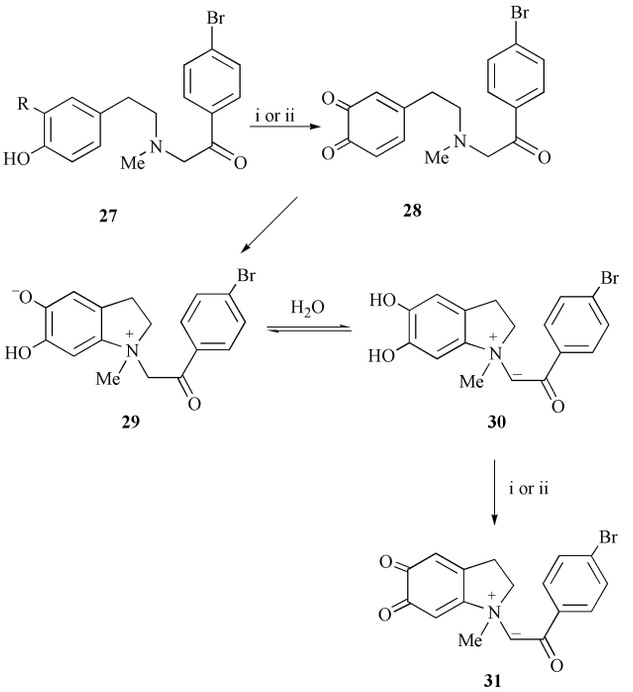


Fig. 10 Combined oximetry and spectrophotometric data of the tyrosinase-catalysed oxidation of the catechol **25c** showing (a) data for the initial phase oxidation up to 180 s and (b) the secondary phase up to 30 min.

This was associated with a shift in the peak absorbance from 266 to 278 nm, and, although not associated with any peak in the spectrum, a rise in absorbance at 320 nm (Fig. 10a). The oxygen stoichiometry of this reaction was found to be *ca*. 0.5 per mole of substrate. This stoichiometry is consistent with the rapid oxidation of the catechol **27** (R = OH) to the corresponding quinone **28** (Scheme 4). However, the absence of a quinone spectrum implies rapid conversion to another species with a peak absorbance in the 280 nm region, accounting for the shift in absorbance maximum from 266 nm. Comparison with the absorption spectrum at pH 7.4 of the related indolium-5-olate oxidation product **10b** of the catechol **8b**, which exhibits a peak at 290 nm with a marked shoulder between 310 and 320 nm,[1] suggests that the observed spectral changes in the initial phase of this reaction are due to formation of the betaine **29**. Subsequently, in a second experiment of longer duration a further phase of very slow oxygen utilisation, associated with a concurrent rise in an absorbance at 418 nm, was observed (Fig. 10b). This secondary oxidation is ascribed to the formation of the relatively stable *o*-quinone **31**. The



Scheme 4 *Reagents*: i, O_2 + deoxytyrosinase; ii, met-tyrosinase.

oxygen stoichiometry of this phase was also approximately 0.5 moles of oxygen per mole of substrate, which is consistent with the proposed transformation (Scheme 4).

Pulse radiolysis of the aminoketone **25c** under oxidative conditions at pHs 6 and 7 led to formation of the semiquinone (λ_{max} 310 nm) which decayed *via* a second-order process. A subsequent change in decay kinetics to first-order, detected at 400 nm, is consistent with the presence of the very short-lived *o*-quinone intermediate **28** ($\tau < 1$ ms), in agreement with the oximetry results. Furthermore, in a ^{1}H NMR study, chemical oxidation of the catechol **27** (R = OH) by one equivalent of DDQ resulted in disappearance of the *N*-Me singlet (δ 2.4) and appearance of a new singlet (δ 3.2). Significantly, after oxidation there was a complete absence of aliphatic protons in the region δ 2–3. Comparison with the ^{1}H NMR spectra of the authentic betaines **10** suggests that this observation is also entirely consistent with the formation of the betaine **29** *via* the short-lived *o*-quinone **28**.

The structure of the betaine **29** and its proposed oxidation by tyrosinase merits further comment. We have previously concluded that the indolium-5-olates **10** are not oxidised by tyrosinase and do not take part in redox exchange reactions with the *o*-quinone products of oxytyrosinase-catalysed oxidation of monohydric phenols to generate catechol substrates for met-tyrosinase. Since the mechanism of catechol oxidation by oxytyrosinase involves met-tyrosinase in an intermediate stage (eqn. (3)), which requires reactivation (eqn. (1)),[6] the enzymatic oxidation of the betaine **29** was unexpected. An important feature relevant to the oxidation of the betaine **29** is the presence of a particularly acidic proton on the carbon atom α to the carbonyl and ammonium groups. The betaine **29**, in contrast to the betaines **10**, can, therefore, be expected to be in equilibrium with the *N*-ylide tautomer **30**, which is a catechol derivative. It may be that it is this tautomer **30** that is further oxidised to the quinone **31**, which is itself a stabilised *N*-ylide.

Based on oximetry studies of the phenol **27** (R = H), we believe that the *N*-ylide tautomer **30**, in contrast to the betaine **29**, may be able to activate met-tyrosinase. Thus the phenol **27** (R = H) was oxidised without pre-activation and with almost no lag period, showing similar kinetics to those of the analogous catechol **25c**. The corresponding methyl ether was not oxidised. The ability of the betaine **29**, in contrast to the

betaines **10**, to equilibrate with an *N*-ylide tautomer **30**, which is a neutral catechol derivative, is therefore of special significance and fully accounts for the observed properties.

Although of some mechanistic interest, these preliminary studies of model systems that might be prototype pro-drugs activated *in situ* by tyrosinase were unsuccessful. In the systems that we have studied, *i.e.* **24** and **25**, either the nitrogen function was insufficiently nucleophilic for cyclisation to occur or *N*-ylide formation appears to stabilise the cyclic product and prevent hydrolysis. Nevertheless, these results are fully consistent with our conclusions on the mechanism of tyrosinase oxidation of phenols which are described in the previous section. We believe that it should be possible to design other derivatives that avoid these limitations and thus lead to useful pro-drugs and alternative strategies are under consideration.

In conclusion, our detailed studies of the formation of novel heterocyclic betaines by cyclisation of dopamine derivatives *via* intermediate *o*-quinones provide firm evidence that tyrosinase oxidises phenols to *o*-quinones in one step and not *via* an intermediate catechol. Catechols are formed by an indirect non-enzymic mechanism and this accounts for the characteristic lag period associated with tyrosinase oxidations. Whereas dopamine derivatives cyclise to the betaines in one step, the higher homologue forms the corresponding betaine derivative in a two step mechanism involving a spiro intermediate.

Experimental

Melting points were determined using a Reichert Kofler Block apparatus and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer 881 spectrophotometer with only major absorbances being quoted. Unless otherwise stated IR spectra were measured as KBr discs. ^{1}H NMR spectra were recorded at ambient temperatures using a JEOL GSX270 FT-NMR spectrometer with tetramethylsilane (TMS) as an internal reference, and were run in deuterated chloroform solution unless otherwise stated. Chemical shifts are quoted in parts per million and the following abbreviations are used: s = singlet; d = doublet; t = triplet; q = quartet; m = multiplet; br = broad. Elemental analyses were determined using a Perkin-Elmer 240 CHN Elemental Analyser. Low resolution mass spectra were recorded on an AEI MS12 Mass Spectrometer at 70 eV electron impact ionisation. Separations by column chromatography were carried out using aluminium oxide (150 mesh, Aldrich) deactivated with water to Brockmann grade IV unless otherwise stated. Flash chromatography was performed using silica gel (Janssen Chimica) 0.035–0.07 mm. Preparative radial (chromatotron) chromatography was carried out on a Harrison Research Ltd Chromatotron 7924 using a 2 mm plate with silica gel 60 PF_{254} containing gypsum (Merck). All solvents were predistilled and dried appropriately prior to use. Concentration and evaporation refer to the removal of volatile materials under reduced pressure on a Büchi Rotovapor. Substances stated to be identical were so with respect to mps, mixed mps and IR spectra.

The pulse radiolysis experiments were performed with a 9–12 MeV Vickers linear accelerator, using 50–200 ns pulses with doses up to ≈33 Gy, and quartz capillary cells of optical path 2.5 cm.[19,20] Absorbed doses were determined from the transient $(SCN)_2^{\bullet -}$ formation from air-saturated 10^{-2} M KSCN solutions, as described by Adams *et al.*,[21] but using the recently updated $G\varepsilon$ value of 2.59×10^{-4} m^2 J^{-1} obtained by Buxton and Stuart,[22] G being the radiation chemical yield of $(SCN)_2^{\bullet -}$, and ε its molar absorption coefficient (units of dm^3 mol^{-1} cm^{-1}) at 475 nm. Saturation of such solutions with N_2O results in a doubling of the $(SCN)_2^{\bullet -}$ yield. Generation of the one-electron oxidising species $Br_2^{\bullet -}$ or $N_3^{\bullet}$ was achieved by irradiating N_2O-saturated aqueous solutions of 100×10^{-3} M KBr or 3×10^{-3} M NaN_3. Under such conditions $Br_2^{\bullet -}$ or $N_3^{\bullet}$ radicals are formed within ~0.1 μs after the radiation pulse, *via* the following reactions:

$$H_2O \rightsquigarrow e^-_{aq} + HO^{\bullet}$$
$$e^-_{aq} + N_2O + H_2O \rightarrow HO^{\bullet} + N_2 + OH^-$$
$$HO^{\bullet} + Br^- \rightarrow HO^- + Br^{\bullet}$$
$$Br^{\bullet} + Br^- \rightarrow Br_2^{\bullet -}$$
$$HO^{\bullet} + N_3^- \rightarrow OH^- + N_3^{\bullet}$$

To monitor tyrosinase-catalysed oxidation simultaneous oximetric and spectrophotometric measurements were made using the apparatus and methods previously described.[1]

Preparation of indolium-5-olates 10

Unless specific details are given, catecholamines were prepared as hydrobromide salts using the literature method cited in each individual preparation. In each case the free amine was prepared by treating the salt with excess saturated aqueous sodium bicarbonate and extraction into $CHCl_3$.

2,3-Dihydro-1,1-dimethyl-6-hydroxy-1*H*-indolium-5-olate 10a. To a stirred solution of *N*,*N*-dimethyldopamine **8a**[23] (0.5 g, 0.003 mol) in $CHCl_3$–MeOH (9 : 1)(50 cm^3) under a nitrogen atmosphere was added dropwise (15 min) dianisyltellurium oxide (0.98 g, 0.003 mol) in $CHCl_3$–MeOH (9 : 1)(20 cm^3). The resulting red solution was then stirred at ambient temperature (30 min). The reaction mixture was partitioned with water (50 cm^3) and the organic phase was separated and washed with water (2 × 30 cm^3). The combined aqueous layers were then washed with $CHCl_3$ and the water was removed under reduced pressure to yield compound **10a** (0.45 g, 91%) reddish brown solid, mp 208 °C with some decomp. at 118 °C; ν_{max}/cm^{-1} 1622, 1492, 1305, 1266, 1152, 1041, 840; λ_{max} (0.1 M phosphate buffer): pH 7.4, 290 (ε 3360) and 310 (sh) (ε 1500) nm; pH 6.5, 286 (ε 3700) nm; δ_H (D_2O) 2.98 (t, *J* 7.0 Hz, 2H), 3.17 (s, 6H), 3.85 (t, *J* 7.0 Hz, 2H), 6.51 (s, 1H), 6.64 (s, 1H); δ_C (D_2O) 26.1 (t), 54.1 (q), 68.5 (t), 101.9 (d), 110.1 (d), 120.3 (s), 136.6 (s), 151.3 (s), 151.7 (s); *m*/*z* 179 (48%)($M^{\bullet +}$). The product was further characterised by conversion to the 5,6-dimethoxy iodide **11a**.

In a similar manner the following betaines were prepared from the amines **8b**[23] and **8c**[24] and subsequently converted to their iodides.

2,3-Dihydro-1,1-di-n-propyl-6-hydroxy-1H-indolium-5-olate **10b** (0.45 g, 90%) reddish brown solid, mp 115–120 °C; ν_{max}/cm^{-1} (KBr) 3422, 2971, 1491, 1385, 1297, 1267, 1152, 1037, 960, 862, 843; λ_{max} (0.1 M phosphate buffer): pH 7.4, 290 (ε 4120) and 312 (sh) (ε 1450) nm; pH 6.5, 290 (ε 3920) nm; δ_H (D_2O) 0.85 (t, *J* 7.3 Hz, 6H), 1.40 (m, 2H), 1.65 (m, 2H), 3.07 (t, *J* 7.5 Hz, 2H), 3.40 (dt, *J* 7.9 and 4.4 Hz, 2H), 3.55 (dt, *J* 7.9 and 4.4 Hz, 2H), 3.99 (t, *J* 7.5 Hz, 2H), 6.63 (s, 1H), 6.67 (s, 1H); δ_C ($CDCl_3$) 9.8 (q), 16.0 (t), 27.5 (t), 61.8 (t), 67.1 (t), 103.5 (d), 109.9 (d), 123.1 (s), 132.0 (s), 150.5 (s), 151.5 (s); *m*/*z* (FAB) 236.1639 (MH^+). $C_{14}H_{22}NO_2$ requires 236.1650.

2,3-Dihydro-6-hydroxyspiro[indole-1,1′-piperidin]-1-ylium-5-olate **10c** (0.40 g, 80%), red brown solid, mp 143 °C with some decomp. at 120 °C; ν_{max}/cm^{-1} (KBr) 3412, 1624, 1490, 1456, 1300, 1269, 1203, 1156, 1079, 1032, 864; λ_{max} (0.1 M phosphate buffer): pH 7.4, 288 (ε 3810) and 310 (sh) (ε 1800) nm; pH 6.5, 286 (ε 3800) nm; δ_H (D_2O) 1.50 (m, 1H), 1.87 (m, 5H), 3.00 (t, *J* 7.2 Hz, 2H), 3.22 (d, *J* 12.4 Hz, 2H), 3.47 (m, 2H), 3.91 (t, *J* 7.2 Hz, 2H), 6.59 (s, 1H), 6.74 (s, 1H); δ_C (D_2O) 15.0 (t), 16.2 (t), 20.7 (t), 55.1 (t), 57.5 (t), 97.4 (d), 105.0 (d), 115.7 (s), 131.5 (s), 146.1 (s), 147.4 (s); *m*/*z* 219 (2%)($M^{\bullet +}$).

Preparation of 5,6-dimethoxyindolium iodides 11

2,3-Dihydro-5,6-dimethoxy-1,1-dimethyl-1*H*-indolium iodide 11a. To compound **10a** (250 mg) and acetone (30 cm^3) was added sufficient water to ensure solution (1 or 2 drops). Methyl

iodide (2 cm^3) and potassium carbonate (300 mg) were then added and the resulting mixture was heated under reflux (24 h). After cooling the mixture was filtered and the solvent removed under reduced pressure to give a yellow solid that was recrystallised from ethyl acetate–methanol and identified as *compound* **11a** (390 mg, 83%), colourless crystals, mp 200–201 °C (Found: C, 42.82; H, 5.67; N, 4.03. $C_{12}H_{18}INO_2$ requires: C, 42.98; H, 5.41; N, 4.18%); ν_{max}/cm^{-1} 1600, 1512, 1464, 1433, 1354, 1310, 1268, 1244, 1225, 1166, 1064, 990, 968, 854, 840; δ_H (D_2O) 3.24 (t, *J* 7.1 Hz, 2H), 3.38 (s, 6H), 3.77–3.79 (2 × s, 6H), 4.09 (t, *J* 7.1 Hz, 2H), 6.99 (s, 1H), 7.16 (s, 1H).

In a similar manner the following iodides were prepared from the corresponding indolium-5-olate.

2,3-Dihydro-5,6-dimethoxy-1,1-di-n-propyl-1H-indolium iodide **11b** (95%), colourless crystals, mp 172–173 °C (Found: C, 49.11; H, 6.69; N, 3.57. $C_{16}H_{26}INO_2$ requires C, 49.10; H, 6.65; N, 3.58%); ν_{max}/cm^{-1} 1602, 1509, 1464, 1442, 1418, 1358, 1304, 1271, 1243, 1222, 1074, 996; λ_{max} (0.1 M phosphate buffer): pH 7.4, 284 (ε 4115); unchanged at pH 6.5; δ_H ($CDCl_3$) 0.95 (t, *J* 7.0 Hz, 6H), 1.31 (m, 2H), 1.65 (m, 2H), 3.19 (t, *J* 7.0 Hz, 2H), 3.82 (s, 3H), 4.0 (s, 3H), 4.12 (t, *J* 7.0 Hz, 4H), 4.23 (t, *J* 7.0 Hz), 6.70 (s, 1H), 7.61 (s, 1H). *2,3-Dihydro-5,6-dimethoxyspiro[indole-1,1′-piperidin]-1-ylium iodide* **11c** (78%), colourless crystals, mp 228–229 °C (Found: C, 47.88; H, 5.76, N, 3.73. $C_{15}H_{22}INO_2$ requires C, 48.00; H, 5.91; N, 3.73%); δ_H (D_2O) 1.50 (m, 1H), 1.8–2.0 (m, 5H), 3.18 (t, *J* 7.2 Hz, 2H), 3.39 (d, *J* 12.0 Hz, 2H), 3.65 (dt, *J* 4.6 and 12.3 Hz, 2H), 3.77 (s, 3H), 3.79 (s, 3H), 4.08 (t, *J* 7.2 Hz, 2H), 7.00 (s, 1H), 7.16 (s, 1H).

1,1-Diethyl-7-hydroxy-1,2,3,4-tetrahydroquinolinium-6-olate 15. To a stirred solution of *N*,*N*-diethyl-3-(3,4-dihydroxyphenyl)propylamine **13** (0.5 g, 0.002 mol), prepared from 3-(3,4-dimethoxyphenyl)propionic acid by the method of Ginos *et al.*,[24] in $CHCl_3$–MeOH (9:1)(50 cm^3) under a nitrogen atmosphere was added dropwise (15 min) dianisyltellurium oxide (0.8 g, 0.002 mol) in $CHCl_3$–MeOH (9:1)(20 cm^3). The resulting red solution was then stirred at ambient temperature (30 min). The reaction mixture was partitioned with water (50 cm^3) and the organic phase was separated and washed with water (2 × 30 cm^3). The combined aqueous layers were then washed with $CHCl_3$ and the water was removed under reduced pressure to yield compound **15** (0.42 g, 84%) reddish brown solid, mp 95–100 °C; ν_{max}/cm^{-1} (KBr) 3405, 1653, 1501, 1399, 1273, 1251, 1197, 1008; λ_{max} (0.1 M phosphate buffer): pH 7.4, 286 (ε 2830) and 308 (sh) (ε 1000) nm; pH 6.5, 284 (ε 2800) nm; δ_H (D_2O) 1.09 (t, *J* 7.0 Hz, 6H), 2.0 (m, 2H), 2.57 (t, *J* 7.0 Hz, 2H), 3.4–3.8 (m, 6H), 6.42 (s, 1H) and 6.63 (s, 1H); δ_c (D_2O) 3.0 (q), 12.4 (t), 19.5 (t), 50.0 (t), 56.8 (t), 102.2 (d), 109.9 (d), 116.2 (s), 122.5 (s), 144.7 (s), 146.0 (s); *m/z* 221 (26%)($M^{\bullet+}$). The product was further characterised by conversion to the 6,7-dimethoxy iodide **18**.

6,7-Dimethoxy-1,1-diethyl-1,2,3,4-tetrahydroquinolinium iodide 18. In the manner described for the preparation of compound **11a**, the betaine **15** (250 mg) was treated with MeI–K_2CO_3 in acetone to give a yellow solid that was recrystallised from ethyl acetate–MeOH and identified as *compound* **18** (380 mg, 90%), colourless crystals, mp 230–231 °C (Found: C, 47.54; H, 6.64; N, 3.51. $C_{15}H_{24}INO_2$ requires C, 47.73; H, 6.41; N, 3.71%); ν_{max}/cm^{-1} (KBr) 3425, 2973, 2940, 2362, 1613, 1514, 1443, 1259, 1233, 1222, 1181, 1056, 1019, 940, 904, 860, 813; δ_H (D_2O) 1.14 (t, *J* 7.1 Hz, 6H), 2.09 (m, 2H), 2.79 (t, *J* 6.4 Hz, 2H), 3.6–3.7 (m, 4H), 3.78 (s, 3H), 3.79 (s, 3H), 3.8–3.9 (m, 2H), 6.85 (s, 1H), 6.95 (s, 1H).

Preparation of catechols 24 and 25

***N*-Methyl-*N*-[2-(3,4-dihydroxyphenyl)ethyl]acetamide 24a.** Acetic anhydride (3.0 g) was added to *N*-methyl-2-(3,4-dimethoxyphenyl)ethylamine (1.5 g). Following an exothermic reaction, dichloromethane (5 cm^3) and K_2CO_3 (3.0 g) were added and the mixture was stirred (8 h). The resulting suspension was diluted with $CHCl_3$ (15 cm^3), filtered and the residue washed with a further portion of $CHCl_3$ (15 cm^3). Evaporation and drying under high vacuum gave a colourless liquid (1.8 g) that was identified as the desired amide and used without further purification. The amide was dissolved in CH_2Cl_2 (15 cm^3) and, with cooling at −80 °C, 1 M BBr_3 in CH_2Cl_2 solution (15 cm^3) was added dropwise. After standing overnight, water (3 cm^3) was added and the mixture evaporated under high vacuum to give a viscous oil. Flash chromatography (silica gel: ethyl acetate–cyclohexane–MeOH (8:2:1) followed by ethyl acetate–cyclohexane–MeOH–$MeCO_2H$ (40:10:1:1) as eluent) gave a colourless oil which solidified on grinding with Et_2O and was identified as *N-methyl-N-[2-(3,4-dihydroxyphenyl)-ethyl]acetamide* **24a** (400 mg, 25%), tiny crystals, mp 134–136 °C; ν_{max}/cm^{-1} 3254, 1590, 1528, 1445, 1412, 1293, 1236, 1118, 1040, 1018, 871 and 824; δ_H (d_6-DMSO) (both amide diastereoisomers observed) 1.76 and 1.94 (2s, 3H), 2.56 (dt, 2H), 2.77 and 2.88 (2s, 3H), 3.35 (m, 2H), 6.43 (m, 1H), 6.57 (m, 1H) and 6.62 (m, 1H); HRMS (EI) Found: $MH^{\bullet+}$, 210.1127; Calc. for $C_{11}H_{16}NO_3$: 210.1130.

In a similar manner compound **24b** was prepared from trifluoroacetic anhydride and *N*-methyl-2-(3,4-dimethoxyphenyl)-ethylamine.

N-Methyl-N-[2-(3,4-dihydroxyphenyl)ethyl]trifluoro-acetamide **24b** (469 mg, 29%), micro-crystals, mp 113–115 °C (Found: C, 50.32; H, 4.59; N, 5.09. $C_{11}H_{12}F_3NO_3$ requires C, 50.19; H, 4.56; N, 5.32%); ν_{max}/cm^{-1} 3368, 1684, 1618, 1533, 1450, 1374, 1196, 1151, 1084 and 952; δ_H (d_6-DMSO) (both amide diastereoisomers observed) 2.7 (2t, 2H), 2.98 and 3.00 (2q, J_{HF} 0.7 and 1.7 Hz, 3H), 3.50 (2t, 2H), 6.45 (m, 1H), 6.59 and 6.60 (2d, *J* 2.2 Hz, 1H), 6.63 and 6.65 (2d, *J* 7.8 Hz, 1H); *m/z* 263 (6%)($M^{\bullet+}$).

***N*-(4-Bromobenzoylmethyl)-*N*-[2-(3,4-dihydroxyphenyl)-ethyl]-*N*-methylammoniumbromide 25c.** A mixture of *N*-methyl-2-(3,4-dimethoxyphenyl)ethylamine (2.0 g) in toluene (50 cm^3) and $NaHCO_3$ (2.0 g) and $Na_2SO_3 \cdot 7H_2O$ (1.0 g) in water (20 cm^3) was stirred under a nitrogen atmosphere. 4-Bromophenacyl bromide (2.8 g) was added and stirring was maintained (4 h). The organic phase was washed with saturated Na_2HPO_4 solution (50 cm^3) and then shaken with aqueous 1 M HBr (20 cm^3) to precipitate a viscous yellow oil, which solidified on standing (2 days). This material was dissolved in warm $CHCl_3$ (10 cm^3) and hexane (20 cm^3) was added to give a yellow solid (2.70 g) that was identified as *compound* **25a** and used without further purification.

The product **25a** was suspended in CH_2Cl_2 (40 cm^3) and 1 M BBr_3 in CH_2Cl_2 (11 cm^3) was added dropwise (5 min) at room temperature. The resulting dark red–brown solution was allowed to stand at room temperature (2 days) after which a colourless solid had precipitated. Aqueous 1 M HBr (1 cm^3) was added and the solvent evaporated at room temperature. The residue was stirred with water (10 cm^3) and filtration gave a pale yellow solid (2.51 g). This material was recrystallised from water (200 cm^3) and identified as *compound* **25c** (1.46 g, 63%), tiny pale yellow crystals, mp 234 °C (Found: C, 45.58; H, 4.18; N, 2.86. $C_{17}H_{19}Br_2NO_3$ requires C, 46.05; H, 4.32; N, 3.16%); ν_{max}/cm^{-1} 3441, 3175, 2963, 1687, 1588, 1517, 1400, 1347, 1283, 1237, 1193, 1125, 1074, 1009, 961, 840, 824 and 790; δ_H (d_6-DMSO) 2.88 (t, 2H), 2.92 (s, 3H), 3.16 (br m, 2H), 5.06 (br m, 2H), 6.5–6.7 (m, 3H), 7.85–7.95 (q, 4H), 9.81 (br s, 1H); *m/z* 367 (18%)($MH^{\bullet+}$).

Preparation of *N*-(4-bromobenzoylmethyl)-*N*-[2-(4-hydroxy-phenyl)ethyl]-*N*-methylammonium bromide 27 (R = H). A solution of $NaHCO_3$ (2.8 g) and $Na_2SO_3 \cdot 7H_2O$ (1.0 g) in water (20 cm^3) was magnetically stirred under an atmosphere of

nitrogen. Toluene (50 cm^3) was added followed by *N*-methyl-4-methoxyphenylethylamine hydrochloride[25] (3.0 g) and then 4-bromophenacyl bromide (2.9 g). After further stirring (4 h) the organic phase was separated, washed with saturated Na_2HPO_4 solution (50 cm^3) and then stirred with aqueous HBr (3 cm^3 of 49% HBr made to 15 cm^3). After standing overnight the colourless precipitate was collected and washed with H_2O (5 cm^3) and then ether (2 × 5 cm^3) to give the desired tertiary amine (2.2 g, 50%); δ_H (d_6-DMSO) 2.96 (s, 3H), 3.02 (t, *J* 5.7 Hz, 2H), 3.35 (br s, 2H), 3.72 (s, 3H), 5.13 (s, 2H), 6.89 (d, *J* 8.5 Hz, 2H), 7.22 (d, *J* 8.5 Hz, 2H), 7.84 (d, *J* 8.7 Hz, 2H), 7.95 (d, *J* 8.7 Hz, 2H), 9.91 (br s, 1H). This material was demethylated without further purification using the following procedure.

The methyl ether (1.6 g) was suspended in CH_2Cl_2 and with magnetic stirring under an atmosphere of nitrogen, BBr_3 (4 cm^3, 1M in CH_2Cl_2) was added. The solid dissolved to give a gold coloured solution which after standing overnight was pale brown and a brown gum had precipitated. Hydrolysis by stirring with 1 M HBr gave a pale cream powder that was identified as the *phenol* **27** (R = H) (HBr salt) (1.13 g, 74%), mp 120–122 °C; ν_{max}/cm^{-1} (KBr) 3509, 3435, 3221, 2969, 1688, 1586, 1514, 1471, 1450, 1406, 1339, 1271, 1243, 1069, 1008, 964, 834, 814; δ_H (d_6-DMSO) 2.95 (s, 3H), 3.35 (m, 2H), 5.07 (br d, 1H), 5.19 (br d, 1H), 6.73 (d, *J* 8.3 Hz, 2H), 7.09 (d, *J* 8.3 Hz, 2H), 7.86 (d, *J* 8.3 Hz, 2H), 7.95 (d, *J* 8.3 Hz, 2H), 9.32 (v br s, 1H), 9.89 (br s, 1H); *m/z* (Electrospray) 348.0605 (MH^+; ^{79}Br). $C_{17}H_{19}NO_2Br$ requires 348.0599.

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