Novel heterocyclic betaines relevant to the mechanism of *tyrosinase*-catalysed oxidation of phenols

John Clews,[a] Christopher J. Cooksey,[b] Peter J. Garratt,[b] Edward J. Land,[c] Christopher A. Ramsden*[a] and Patrick A. Riley[d]

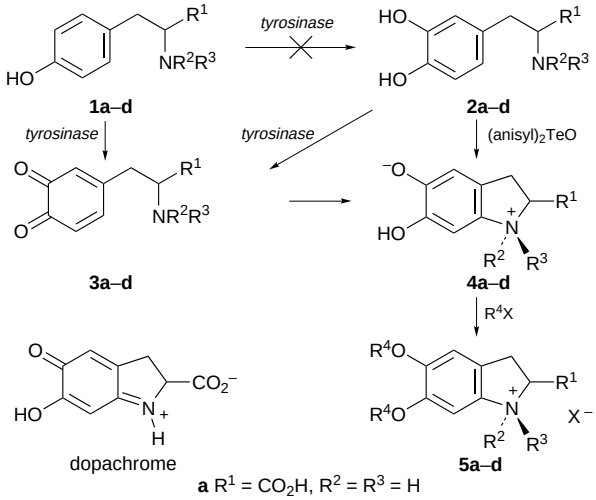
[a] *Department of Chemistry, Keele University, Keele, Staffordshire, UK ST5 5BG*
[b] *Department of Chemistry, Christopher Ingold Laboratories, UCL, 20 Gordon Street, London, UK WC1H 0AJ*
[c] *CRC Department of Biophysical Chemistry, Paterson Institute for Cancer Research, Christie Hospital NHS Trust, Wilmslow Road, Manchester, UK M20 9BX*
[d] *Windeyer Institute, UCL Medical School, 46 Cleveland Street, London, UK W1P 6DB*

Betaines formed by dianisyltellurium oxide oxidation of *N*,*N*-dialkyldopamines are identical to the products formed by *tyrosinase* oxidation of *N*,*N*-dialkyltyramines or *N*,*N*-dialkyldopamines and provide evidence that *tyrosinase* does not act as a *tyrosine hydroxylase*; oxidations of higher homologues of *N*,*N*-diethyldopamine are also described.

Tyrosinase [EC 1.14.18.1] catalyses the formation of *ortho*-quinones **3** from both phenolic (**1**) and catecholic (**2**) substrates (Scheme 1):[1] the mechanism of the phenol oxidation (**1** → **3**) has been the subject of disagreement.[2] There is a lag phase during initial *tyrosinase*-catalysed oxidation of monohydric phenols **1** because the enzyme requires activation by a catechol: this process is believed to involve reduction of Cu^{II} ions in the active site. Once formed, initially by largely unactivated enzyme, quinone derivatives of primary amines, such as dopaquinone **3a**, undergo rapid cyclisation and subsequent disproportionation with a second molecule of quinone to give a catechol amine (*e.g.* **2a**) (eqn. 1) which then activates more enzyme. The catechol **2** is not, therefore, formed directly by the

2 dopaquinone (**3a**) → dopa (**2a**) + dopachrome (1)

enzyme acting as a *tyrosine hydroxylase*, as is widely claimed,[2] but by a subsequent sequence of non-enzymic reactions. We have recently reported oximetry studies that firmly support the exclusive operation of this indirect mechanism of auto-activation of *tyrosinase*.[3] We now report novel chemical studies relevant to these conclusions.

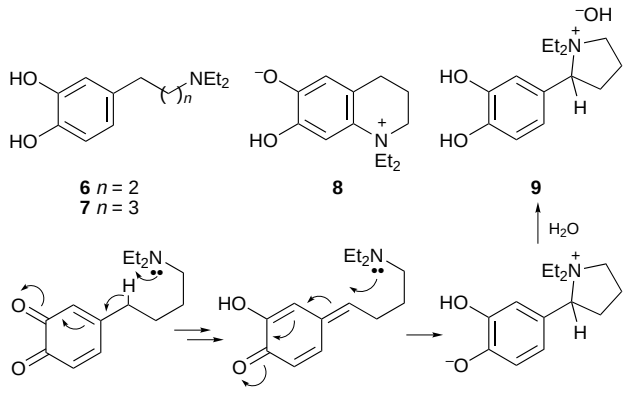


a $R^1 = CO_2H$, $R^2 = R^3 = H$
b $R^1 = H$, $R^2 = R^3 = Me$
c $R^1 = H$, $R^2 = R^3 = Pr$
d $R^1 = H$, $R^2–R^3 = (CH_2)_5$

Scheme 1

A priori it is not clear whether the *ortho*-quinone of a tertiary amine **3** (R^2 and $R^3 \neq H$) will cyclise. Other workers,[4] in a study of protein binding of oxidised catechols, have recently expressed the view that formation of 2,3-dihydroindole derivatives (*e.g.* **3** → **4**) *via* *N*,*N*-dialkylquinones **3** is unlikely. However, a close examination of the literature reveals that this type of cyclisation was encountered by Robinson and Sugasawa[5] during studies of chloranil oxidation of laudanosaline but, as far as we are aware, this remains the only example. In this context, we have observed[3] that *tyrosinase* oxidises *N*,*N*-di-*n*-propyldopamine **2c** with an oxygen stoichiometry of 0.5 to give a stable product that is not an *ortho*-quinone. Similar oxidation of *N*,*N*-dimethyltyramine **1b** by pre-activated *tyrosinase* gives a similar product with an oxygen stoichiometry of 1.0: no enzymic oxidation of this phenolic precursor **1b** occurred without pre-activation by a trace of dopa **2a**. Spectroscopic evidence suggested that these enzymic products are the betaines **4b** and **4c** formed by rapid cyclisation and aromatisation of the initially formed *ortho*-quinones **3** (Scheme 1). Significantly, oxidative cyclisation of the tertiary amines **1** does not lead to catecholic products that can function as *tyrosinase* activators. Since there is no autocatalysis using the tyramine precursor **1b** we have concluded that direct formation of catechols by *tyrosinase* acting as a hydroxylase (*e.g.* **1b** → **2b**) does not occur. We now describe the chemical synthesis and characterisation of the indol-1-ium-5-olates **4b**–**d** and products obtained by oxidation of the higher homologues **6** and **7**.

Our recent interest in hypervalent oxidising agents[6] led us to investigate the use of dianisyltellurium oxide (DAT), which has been shown to be particularly mild and selective for quinone formation.[7] Oxidations were monitored in deuterated solvents *via* ^1H NMR spectroscopy. Amine **2c** was rapidly and quantitatively transformed to the betaine **4c** upon treatment with 1 equiv. of DAT in CH_2Cl_2–MeOH (9 : 1) solution. The water soluble betaine **4c**, obtained as a crystalline solid, mp 115–120°C (90%), was easily separated from the accompanying dianisyltelluride by $CHCl_3$–water partitioning and the proposed structure is fully supported by its spectroscopic properties. The ^1H NMR (D_2O) spectrum exhibits two aromatic protons (singlets at δ 6.42 and 6.46) indicating formation of the second ring at C-5 of the catechol ring. Further evidence of ring formation is provided by the non-equivalence of each pair of methylene protons (CH_aH_b) of the *N*-*n*-propyl substituents (NCH_2CH_2Me) which, as a result of the quaternary nitrogen atom, are also significantly shifted downfield and appear as pairs of multiplets at δ 3.16 and 3.35 and at δ 1.19 and 1.40. A COSY spectrum confirmed the expected proton coupling. A high resolution mass spectrum of compound **4c** confirmed the constitution of the molecular ion (*m*/*z* 235). The UV spectrum of the betaine **4c** in 0.1 M phosphate buffer was pH dependent [pH 7.4: λ_{max} 290 (ε 4122) and 312(sh) nm (1453); pH 6.5: λ_{max} 290



Scheme 2

nm (ε 3923)] and this change is attributed to the formation of the salt **5c** (R^4 = H) at low pH.

Attempts to monomethylate the betaine **4c** using MeI were unsuccessful and gave mixtures. However, use of MeI and solid K_2CO_3 in acetone gave exclusively the dimethoxy iodide **5c** (R^4 = Me, X = I), mp 172–173 °C (95%).† Significantly, the UV spectrum of this salt **5c** was pH independent [λ_{max} 284 nm (ε 4115)] and showed no shoulder at higher wavelength. In a similar manner the betaines **4b**,**d** were prepared, characterised and converted to their dimethoxy iodides **5b**,**d** (R^4 = Me, X = I). The synthetic products **4b**,**c** were found to be identical in all respects to the material produced by *tyrosinase* oxidation of amines **1b** and **2c**.[3]

Oxidation of the higher homologue **6** resulted in a similar quantitative cyclisation giving the tetrahydroquinolin-1-ium-6-olate **8** which was obtained as a crystalline solid, mp 95–100 °C (84%). The ^{1}H NMR [δ_H(D_2O) 6.42 and 6.63 (s, 2 × arom H), 3.4–3.8 (m, 3 × CH_2N$^+$), 2.57 (t, CH_2Ar), 2.0 (m, $CH_2CH_2CH_2$) and 1.09 (t, 2 × CH_3)] and UV [pH 7.4: λ_{max} 286 nm (ε 2831)] spectra are analogous to those of the betaines **4** and fully consistent with structure **8**. Evidence of spirocyclisation was not detected by NMR spectroscopy. Methylation (MeI–K_2CO_3) gave the expected 6,7-dimethoxy iodide as a crystalline solid mp 230–231 °C (90%).

A different mode of reaction occurred when the 4-alkylamine chain was extended by an additional methylene unit. Again, clean formation of a single product was observed by ^{1}H NMR spectroscopy when the amine **7** was treated with 1 equiv. of DAT and after isolation this was identified as the quaternary salt **9**, mp 118–120 °C (86%) [*m/z* 236.1642 ($C_{14}H_{22}N_1O_2$), M − OH$^-$]. In particular the ^{1}H NMR spectrum (D_2O) showed non-equivalent ethyl groups [δ_H 0.99 and 1.25 (t, 2 × CH_2CH_3)] and a low field pseudo-triplet at δ_H 4.60 corresponding to the methine proton. There was no evidence of cyclisation to a seven-membered betaine. We rationalise the formation of the product **9** by an isomerisation of the initially formed *ortho*-quinone **10** to the quinomethane **11**, assisted by intramolecular deprotonation (Scheme 2). The quinomethane **11** then undergoes a 5-*exo*-*trig* cyclisation giving the observed product **9** *via* the betaine **12**. This cyclisation (**11** → **12**) is analogous to that proposed for the formation of the tetrahydrofuran ring in the biosynthesis of lignans (*e.g.* pinoresinol and olivil)[8] and for the epimerisation of profisetinidins.[9] The formation of a quinomethane intermediate *via* an *ortho*-quinone (**10** → **11**) is also relevant to the role of *quinone isomerase* in the sclerotization of insect cuticles.[10]

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Footnotes and References

* E-mail: cha33@cc.keele.ac.uk

† New compounds were characterised by spectroscopy and elemental analysis.

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