Aromatic iodination: a new investigation on the nature of the mechanism †

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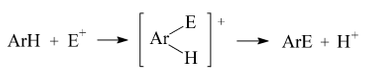
Received (in Cambridge, UK) 25th April 2001, Accepted 18th June 2001
First published as an Advance Article on the web 31st July 2001

Following a suggestion by the late Lennart Eberson, we have employed the ICl–HFP (HFP being hexafluoropropan-2-ol) system in iodination reactions, and found unambiguous evidence for the occurrence of an ET-mechanism of halogenation. The evidence is based on the use of 'intelligent' substrates, which make it possible to fix the boundaries between the occurrence of an ET-mechanism and of a conventional polar mechanism. In an 'intelligent' substrate, in fact, the nature of the product(s) changes significantly depending on the operating mechanism. The ICl–HFP combination is instrumental to the onset of a one-electron oxidation with electron-rich substrates, followed by halogenation. The most prominent example is that of the electron-rich substrate durene (1,2,4,5-tetramethylbenzene, DUR), when compared to mesitylene (1,3,5-trimethylbenzene, MES): with a 'conventional' iodination system (*i.e.*, I_2/Ag^+) and in common solvents, where the polar mechanism holds, durene is less reactive ($k_{MES}/k_{DUR} = 46 \pm 3$), but becomes more reactive ($k_{MES}/k_{DUR} = 0.23$) in HFP with ICl, where the ET-mechanism takes over. Other substrates also support the onset of ET-pathways in HFP. Finally, a preliminary survey of a biohalogenation reaction induced by laccase indicates the modest occurrence of a polar process of iodination with a few substrates.

Introduction

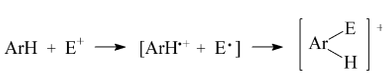
The debate over the mechanism of electrophilic aromatic substitution reactions, which are among the most important processes in organic chemistry, has continued in the recent literature. The 'conventional' polar route of substitution, *via* rate-limiting collapse of the electrophile (E^+) and the aromatic substrate (ArH) to form a σ-complex (Scheme 1),[1] has been



σ-complex

Scheme 1

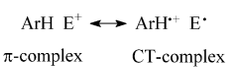
challenged by other formulations that originate from the electron transfer (ET) concept.[2] For example, in view of the oxidising character of some electrophiles, an alternative mechanism could involve an initial electron transfer step leading to a radical cation ($ArH^{\bullet+}$) that combines with $E^{\bullet}$ to give the same σ-complex as the polar pathway (Scheme 2).



radical pair σ-complex

Scheme 2

An even more subtle formulation of this point regards the transition state of the process as a resonance hybrid between the initial state and a radical pair, that would lead to the σ-complex.[3] This radical pair can be viewed as the excited state of a π-complex between the substrate and the electrophile (*viz.*, a charge-transfer complex, CT), where a shift of electron has occurred (Scheme 3) between the electron-donor and the electron-acceptor species, according to a Benesi–Hildebrand formulation.[4]



π-complex CT-complex

Scheme 3

Close scrutiny of many electrophilic reactions from an electrochemical approach led to the conclusion that the ET mechanism is not supported by the available facts,[5] with the possible exception of the case of the strongest oxidant among the electrophiles, *i.e.*, the nitronium ion NO_2^+, whenever it reacts with electron-rich aromatic substrates.[2c,5,6] Operation of the ET mechanism could also be excluded in the case of aromatic iodination,[7] and of some other electrophilic reactions,[8] by resorting to the determination of reactivity ratios for appropriate pairs of substrates.[9] Certainly, each electrophile would require a mechanistic assessment on its own, because the threshold of the oxidation potential of the substrate, beyond which a transition from the conventional polar route to the ET route becomes possible, will depend on the particular oxidation potential of the electrophile involved [eqns. (1) and (2)].

$$E^+ \text{ (strong oxidant)} + ArH \rightarrow \text{ET substitution (Scheme 2)} \quad (1)$$

$$E^+ \text{ (weak oxidant)} + ArH \rightarrow \text{polar substitution (Scheme 1)} \quad (2)$$

A recent investigation by the late Lennart Eberson of aromatic halogenation (mainly iodination with ICl),[10] has provided evidence for the involvement of radical cations in 1,1,1,3,3,3-hexafluoropropan-2-ol (HFP) with substrates of

† Dedicated to the memory of Professor Lennart Eberson

 DOI: 10.1039/b103758g

moderate-to-good electron richness. The explanation provided for the role of HFP was that this solvent fostered the ET mechanism by increasing both the oxidation power of the halogenating agent and also, because of its very weak nucleophilic character, the persistency of the $ArH^{\bullet+}$ species.[10] This finding stimulated us to expand our previous investigation of the iodination reaction towards the use of HFP as the solvent. The aim was to detect any transition between the competing mechanisms (*i.e.*, polar *vs.* ET) in this new reaction medium. To enable the nature of the operating mechanism to be appraised we studied the reactivity of our previous substrate pairs,[7] and also of other 'intelligent' substrates. Reaction of all these substrates with iodine will therefore be the common feature of the experiments reported below. A preliminary survey of a biohalogenation process, and its mechanism, is also described.

Results and discussion

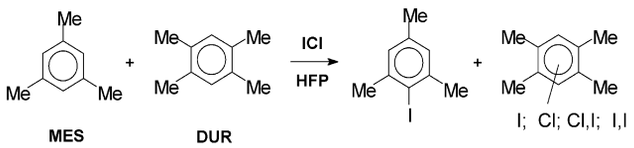
The following pairs of substrates are 'intelligent' as far as the competition between the polar and the ET mechanisms of halogenation is concerned because, within each pair, the relative substrate reactivity presents clearly contrasting trends depending on the mechanism.

Mesitylene *vs.* durene

The experimental σ-basicity (*viz.*, log K_B) of mesitylene is higher than that of durene, the values being −0.4 and −2.2, respectively.[11] MES should therefore be more reactive than DUR in a 'conventional' electrophilic aromatic substitution, where the structure of the transition state (TS) of the rate determining step resembles that of the σ-complex (Scheme 1).[9] Conversely, since the oxidation potential ($E°$) of DUR is lower than that of MES (2.07 and 2.35 V *vs.* NHE in TFA, respectively[12]), DUR should be more reactive than MES if the TS of the electrophilic process is structurally closer to the radical pair (Scheme 2).[9] Accordingly, when this intermolecular selectivity was determined for the most often studied electrophilic aromatic substitutions, MES : DUR reactivity ratios significantly >1 were in general obtained, both from competitive experiments and from absolute rate constant determinations.[9] This result supports a σ-complex structure for the TS of the investigated reactions, including the iodination reaction,[7,8b] and the operation of the conventional polar mechanism with these two particular substrates, or with substrates of comparable activation. Clearly, this finding does not preclude that the ET mechanism may take over for substrates easier to oxidise than the polyalkylbenzenes, such as the polymethoxybenzenes.[10,13]

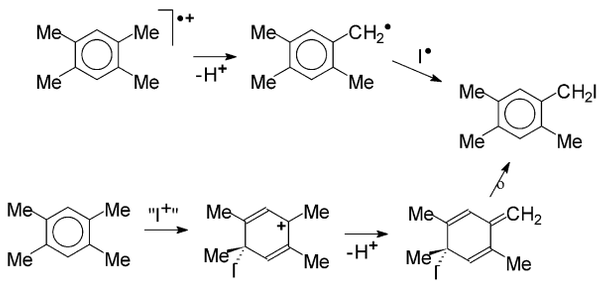
Our interest here is bound to determine whether the reported 'beneficial' effect of the HFP solvent[10] on the stability of the radical cation of the substrate can be such as to induce a shift of the mechanism of iodination, from polar to ET. In addition to the solvent, the use of ICl as the iodinating agent, in view of its higher oxidation potential with respect to that of I_2, particularly in HFP (E_{pc} 0.98 *vs.* 0.39 V, respectively),[10] would appear to be instrumental to the onset of an ET iodination. It has been found, however, that a competitive reaction of MES and DUR, with a deficit of ICl as the iodinating agent, in a AcOH–TFA–TFAA–CH_3CN 60 : 8 : 8: 24 v/v solvent mixture, did provide a k_{MES}/k_{DUR} reactivity ratio of 43 at room temperature,[8b] in keeping with the polar mechanism. This value is very close to those of other iodinating systems, based on molecular iodine,[7] that cluster around k_{MES}/k_{DUR} values of 45 ± 5. It is also in line with the value of 52 that can be obtained from the ratio of the absolute rate constants of reaction of MES and of DUR with ICl in AcOH,[14] emphasising the consistency between results originating from competitive experiments and from direct kinetic determinations.[9b]

The competition of MES and DUR for a deficit of ICl was repeated here in acetonitrile, and a k_{MES}/k_{DUR} value over forty was once more obtained (Table 1, entry 1). When the reaction was run in HFP, however, a completely different intermolecular selectivity was obtained (entry 2): 3-iododurene prevailed over 2-iodomesitylene, with chlorodurene, chloroiododurene and diiododurene also being detected in small amounts (Scheme 4).



Scheme 4

The possible interference from side-chain halogenation, resulting either from $DUR^{\bullet+}$ (ET route),[15] or from a polar *ipso*-addition, followed by side-chain functionalisation (Scheme 5),[16]



Scheme 5

was tested by subjecting the reaction products to exhaustive acetoxylation, to convert any benzylic haloarene (possibly co-eluting in the GC analysis with the nuclear counterpart) into a benzylic acetoxy-derivative: this acetoxylation had no effect on the nature of the products nor on the k_{MES}/k_{DUR} reactivity value.[9b,17,18]

The haloarenes were therefore truly nuclear-halogenated derivatives, and this supported an ET pattern of nuclear aromatic substitution in HFP [eqns. (3)–(5)], at least with DUR;

$$ArH + ICl \rightarrow ArH^{\bullet+} + I^{\bullet}\ (0.5\ I_2) + Cl^- \qquad (3)$$

$$ArH^{\bullet+} + I^{\bullet}\ (0.5\ I_2) \rightarrow Ar\text{–}I + H^+ \qquad (4)$$

$$ArH^{\bullet+} + Cl^- \rightarrow \rightarrow Ar\text{–}Cl + H^+ + e^- \qquad (5)$$

durene, the easier to oxidise substrate, becomes accordingly more reactive than mesitylene (k_{MES}/k_{DUR} = 0.23). In agreement with the operation of the ET mechanism is also the formation of chloro-derivatives from DUR, deriving from capture of $DUR^{\bullet+}$ by Cl^- [eqn. (5)].[10,13]

It is remarkable that another iodination of MES and DUR in HFP, run with I_2 and with a Ag^+ salt as a Lewis acid, provided the 'normal' k_{MES}/k_{DUR} value > 1 (entry 3). This result indicates that it is only the *whole* HFP–ICl system that is capable of inducing a polar → ET shift in the mechanism of aromatic iodination with this pair of substrates (compare Table 1, entries 1–3).

m-Dimethoxy-/*p*-dimethoxybenzene

The oxidation potential (E^p) of *p*-dimethoxybenzene (*p*-MeO) is lower than that of *m*-dimethoxybenzene (*m*-MeO) (1.34 and 1.55 V *vs.* SCE in TFA,[2b] respectively), both compounds being easier to oxidise than MES and DUR. Unfortunately, no σ-basicity data are available for these two dimethoxybenzenes, but the *meta*-isomer is much more reactive than the *para*-isomer in a typical electrophilic process such as the bromination reaction.[19] Analogous σ-basicity values can therefore be drawn with

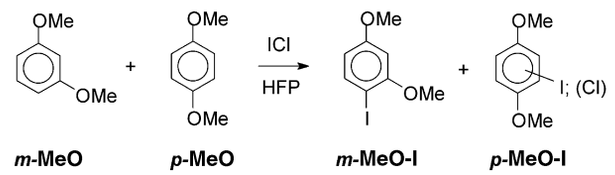
Table 1 Iodination reactions of the substrate pairs, or of other single substrates, at room temperature [a]

Entry number	Substrate/mmol	Halogenating species or oxidant/mmol	Solvent	Reaction time/h	Product(s)/mmol	Selectivity	Other products (traces)
1	MES (1.3) DUR (4.0)	ICl (0.45)	CH_3CN	0.5	I-MES (0.28) I-DUR (0.014)	$k_{MES}/k_{DUR} = 46 \pm 3$	
2	MES (1.3) DUR (3.9)	ICl (0.29)	HFP	0.5	I-MES (0.013) I-DUR (0.11)	$k_{MES}/k_{DUR} = 0.23 \pm 0.01$ [b]	Cl-DUR, I,I-DUR, Cl,I-DUR
3	MES (1.3) DUR (3.9)	CF_3CO_2Ag/I_2 (0.47)	HFP	0.5	I-MES (0.19) I-DUR (0.010)	$k_{MES}/k_{DUR} = 41 \pm 3$	
4	*m*-MeO (1.1) *p*-MeO (4.5)	ICl (0.48)	'Mixed' [c]	48	*m*-MeO-I (0.36) *p*-MeO-I (0.0025)	$k_{m\text{-MeO}}/k_{p\text{-MeO}} = 1420 \pm 150$	
5	*m*-MeO (1.1) *p*-MeO (4.0)	ICl (0.41)	HFP	48	*m*-MeO-I (0.11) *p*-MeO-I (0.004)	$k_{m\text{-MeO}}/k_{p\text{-MeO}} = 210 \pm 20$ (130 [d])	*p*-MeO-Cl (≈0.002 mmol)
6	MES (1.3) Naph (4.5)	ICl (0.41)	HFP	3	I-MES (0.33) I-Naph (~0.001)	$k_{MES}/k_{Naph} \geq 1700$	Cl-Naph
7	**1** (1.9)	I_2 (1.9)	$CHCl_3$	2	**2** (1.3)	—	
8	**1** (0.38)	I_2 (0.39)	$CHCl_3 : CH_3OH$ 3 : 1	4	**2** (0.22)	—	
9	**1** (0.96)	I_2 (0.95)	HFP	2	**2** (0.46) **3** (0.03)	—	benzidinic dimers
10	**1** (0.46)	$Co^{III}W$ (0.92) [e]	$H_2O : CH_3CN$ 1 : 5	4	**3** (0.33)	—	benzidinic dimers
11	**4** (0.41)	I_2 (0.33)	$CDCl_3 : CD_3OD$ 3 : 1	2	**5** (0.02) **6** (0.13)	—	
12	**7** (0.39)	ICl (0.40)	HFP	0.5	**8** (0.22)	—	
13	**7** (0.39)	ICl (0.40)	HFP	4	**8** (0.16) **9** (0.04)	—	bis-chlorinated product **10**

[a] The solvent was in the range of 1–8 ml. Experiments were run in duplicate. [b] From the nuclear monoiodinated products. [c] 'Mixed' stands for AcOH–$(CF_3CO)_2O$–CH_3CN 60 : 20 : 20 (v/v). [d] If the amount of *p*-MeO-Cl is also included. [e] Plus 0.9 mmol AcOK.

those of *m*- (−3.2) and *p*-xylene (−5.7).[11] Based on these considerations, an intermolecular selectivity *m*-MeO/*p*-MeO > 1 would support the polar mechanism, while the ET mechanism would cause a ratio of < 1.

Iodination of these two substrates by ICl in a AcOH–$(CF_3CO)_2O$–CH_3CN mixed solvent provided a $k_{m\text{-MeO}}/k_{p\text{-MeO}} = 1400$ (Table 1, entry 4), in agreement with the polar route and with a previous result.[8b] The iodination reaction with ICl has also been run in HFP. Even though *m*-MeO was again more reactive than *p*-MeO, the $k_{m\text{-MeO}}/k_{p\text{-MeO}}$ decreased to 210 (entry 5). The crucial observation was, however, that a chloro-substituted product appeared and accompanied that of iodo-substitution in the case of *p*-MeO,[13] but not of *m*-MeO (Scheme 6).



Scheme 6

Occurrence of the ET route of halogenation, delineated in [eqns. (4) and (5)], is therefore supported for *p*-MeO in HFP,[10,13] which accordingly gains in reactivity, while *m*-MeO continues to react by the polar mechanism.

Mesitylene *vs.* naphthalene

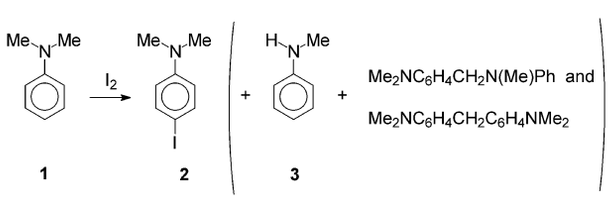
The ionisation potential of naphthalene (Naph; $E°$ 2.08 V)[20] is similar to that of durene, while its σ-basicity (*i.e.*, −4)[21] is much lower. Accordingly, a competitive iodination of MES and Naph with I_2 in a AcOH–CH_3CN 3 : 1 mixed solvent, with CF_3CO_2-Ag as the Lewis acid promoter, gave $k_{MES}/k_{Naph} \cong 2000$.[8b] The reaction has been repeated here with ICl in HFP. Although naphthalene was again much less reactive ($k_{MES}/k_{Naph} \geq 1700$; Table 1, entry 6), a small amount of 1-chloronaphthalene did accompany 1-iodonaphthalene this time. Therefore, the operation of the ET route, and of (eqn. 5), appears to play some role towards Naph in the ICl–HFP system.

From the foregoing it can be seen that the use of substrate pairs as a mechanistic tool has its advantages.[9] The substrates that follow have been investigated singularly, not pairwise. They are also mechanistically 'intelligent' in that the nature of the product(s) of their reaction with iodine does change significantly in response to a change of the reaction conditions, in a way that suggests a shift of the mechanism.

N,*N*-Dimethylaniline 1

Iodination of **1** with I_2 in $CHCl_3$ (or also in $CHCl_3$–CH_3OH 3 : 1 v/v) gave only *N*,*N*-dimethyl-*p*-iodoaniline (**2**) in good yield (Table 1, entries 7 and 8) (Scheme 7).

Repetition of the iodination of **1** with I_2, but in HFP as the



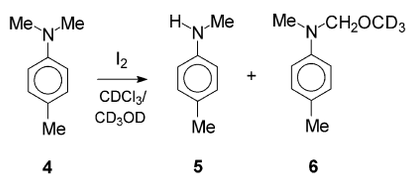
Scheme 7

solvent, gave instead small amounts of *N*-methylaniline **3**, besides **2**, and traces of benzidinic dimers (entry 9). While an aromatic substitution on the highly activated nucleus of **1** is expected for a polar route, formation of **3** is typical for the fragmentation of the radical cation of a tertiary amine.[22] In fact, independent reaction of **1** with potassium 12-tungstocobaltate(III) ($Co^{III}W$), an outer-sphere oxidising agent ($E°$ 1.49

V *vs.* SCE in CH_3CN),[22b] did yield **3** (entry 10), accompanied by the benzidinic dimers.[23] The mechanistic relevance of *N*-dealkylation steps resulting from the radical cation of tertiary amines is well documented, even in connection with some biomimetic processes.[22–24] Thus, the lack of formation of the *N*-dealkylation product in the iodination experiments run in conventional solvents (entries 7 and 8), in contrast to the appearance of **3** and of the benzidinic dimers on changing the solvent to HFP (entry 9), could endorse a change of the mechanism from polar to ET, and support the oxidation of **1** to $\mathbf{1}^{\bullet+}$ by I_2 in HFP, where molecular iodine becomes a stronger oxidant.[10] This would not exclude the possibility that the iodination mechanism (to **2**) has shifted from the polar to the ET route (see eqn. (4)) in HFP, with this electron-rich substrate (E^p of **1** is 0.76 V *vs.* SCE at 0.5 V s^{-1} in CH_3CN). It would not prevent either the dealkylation pathway (through $\mathbf{1}^{\bullet+}$) and the iodination pathway (through a polar σ-complex) from being independent and competing events.

N,*N*-Dimethyl-4-methylaniline 4

While the redox potential of this substrate is comparable (E^p 0.61 V *vs.* SCE at 0.5 V s^{-1} in CH_3CN) with that of **1**, its iodination at the *para* position is prevented by the presence of the methyl group, the reactivity of the *ortho*-positions being strongly depressed sterically by the bulkiness of the dimethylamino-substituent. Experimentally, no nuclear iodination occurred for the reaction of **4** with I_2 in a $CDCl_3$–CD_3OD (3 : 1, v/v) mixed solvent (Table 1, entry 11). Rather, some *N*-dealkylation took place (to **5**), while the main product (**6**) was that of capture of the solvent by the intermediate side-chain carbocation,[23a,25] originating from the radical cation of the precursor (Scheme 8).[26]



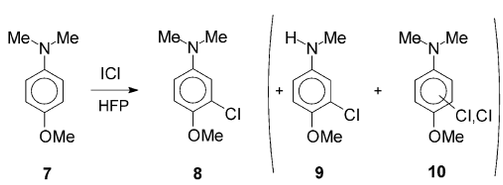
Scheme 8

Presumably, the interaction of I_2 with **4** provokes the formation of $\mathbf{4}^{\bullet+}$ even in this solvent of conventional activation. It is also likely that the conventional attack of 'I^+' at the *para*-position of **4**, where the presence of the methyl-substituent prevents substitution, becomes reversible; this would enable the concurrent oxidation of **4** to $\mathbf{4}^{\bullet+}$ by I_2 to take place, with ensuing *N*-dealkylation and functionalisation of the *N*-alkyl group as the only observable productive routes.[25]

N,*N*-Dimethyl-4-methoxyaniline 7

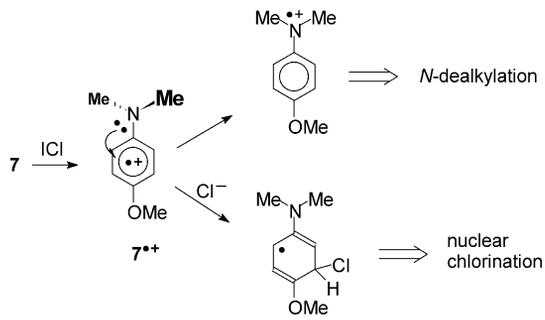
The redox potential of this substrate is even lower (E^p 0.45 V *vs.* SCE at 0.5 V s^{-1} in CH_3CN) than that of **4**, and the *para*-position is once again occupied.

Treatment of **7** with ICl in HFP (Scheme 9) gave only nuclear



Scheme 9

chlorination (to **8**) after 0.5 h (Table 1, entry 12); over a longer reaction time (4 h), the *N*-monomethyl-chloro-derivative **9** began to appear, along with the *N,N*-dimethyl-dichloro-derivative **10** (entry 13). This result is consistent with the intermediate formation of $\mathbf{7}^{\bullet+}$ and with a stereoelectronic problem associated with it. In fact, the bulky *N,N*-dimethylamino group in **7**, in order to relieve the steric interference with the two C–H bonds at the *ortho* positions, is likely to be tilted away from planarity with the aromatic ring (Scheme 10). This means that in



Scheme 10

$\mathbf{7}^{\bullet+}$ the radical cation is mainly localised onto the anisole-like ring, and further stabilised there by the combined action of the solvent HFP and of the methoxy-substituent. Attack by Cl^- on the ring, in keeping with the operation of eqn. (5), would accordingly have more chances to prevail over electron transfer from the nitrogen lone-pair of the dimethylamino group, with ensuing *N*-demethylation.

Conclusion

Molecular iodine is both a weak halogenating agent and an oxidant of moderate strength.[1,10] In general, and with substrates of moderate-to-good activation, the polar mechanism of iodination prevails (see the MES–DUR or *m*-MeO/*p*-MeO probes).[7–9] However, whenever the use of the non-nucleophilic HFP solvent strongly favours the formation of the radical cation of the substrate,[10] the onset of ET pathways, and in particular of the ET-route of halogenation (Scheme 2), can be documented (see Schemes 4 and 6). The elucidation of the remarkable role of solvent HFP in this mechanistic dichotomy represents an opportunity to acknowledge the contributions of the late Lennart Eberson to the field of physical-organic chemistry.[2f] The mechanistic usefulness of our 'intelligent' substrates is also confirmed.

Biohalogenations

Halogenation reactions also occur in Nature: the haloperoxidase enzymes are active in this respect.[27,28] Another enzyme that could seemingly induce iodination reactions is laccase. This is a multi-copper oxidase, expressed by ligninolytic fungi, which catalyses the oxidation of phenols,[29] but has also been shown to function as an iodide oxidase.[30] In fact, in line with a reported oxidation potential of around 0.7 V,[29,31] laccase can oxidise I^- to I_2 ($E°$ 0.7 V *vs.* NHE),[32] while the other halide ions, being more difficult to oxidise,[32] act as inhibitors.[30] It appeared useful to investigate the potential of the I^-/laccase system as a novel method of iodination; however, in view of the weak electrophilicity of molecular iodine,[1] this could be attempted only with very electron-rich substrates. The possibility could then exist that the enzyme directly oxidises the electron-rich substrate *before* iodination, thereby providing a new entry into the mechanistic dichotomy delineated in Schemes 1 and 2. The substrates investigated were: (i) *p*-methylphenol **11** (*viz. p*-cresol), as a model of tyrosine, in view of the role of iodine in the thyroid[33] where this amino acid plays an important metabolic role; (ii) 1,2,4,5-tetramethoxybenzene **12**, in view of its low oxidation potential (0.81 V *vs.* SCE),[34] which is comparable to

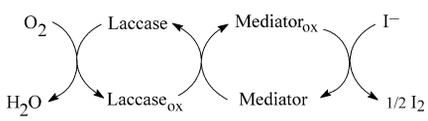
Table 2 Biohalogenation experiments using the I^-/laccase system[a]

Entry number	Substrate (60 μmol)	Halogenating species (60 mmol)	Product/μmol
1	**11**	I^-	phenolic dimers (<1%)
2	**12**	I^-	—
3[b]	**12**	I^-	quinones (<1%)
4	**13**	I^-	—
5	*m*-MeO	I^-	*m*-MeO-I (2.4)
6[b]	*m*-MeO	I^-	*m*-MeO-I (7.2)
7[bc]	*m*-MeO	I^-	*m*-MeO-I (6.4)

[a] At room temperature, for a 72 h reaction time. [b] In the presence of ABTS (20 μmol). [c] In the presence of $Cu(OAc)_2$ (60 μmol).

that of laccase; (iii) 1,4-dimethoxy-2-methylbenzene **13** ($E°$ 1.4 *vs.* SCE),[10] for its methyl group that could enable the detection of side-chain radical iodinations; (iv) *m*-dimethoxybenzene (*m*-MeO), for its high reactivity in the iodination reactions seen above.

The iodination of these compounds, with laccase and I^-, was carried out in a buffered acidic medium (pH 3.5) as required for optimal laccase activity.[29,30] While the change of the colour of the solution, subsequent to the addition of the enzyme, did support conversion of (some) iodide ion into molecular iodine, the results reported in Table 2 show that conversion into iodinated products was negligible. In particular, substrate **11** (entry 1) underwent the typical oxidative coupling of phenols induced by laccase.[29] It has been suggested that the low reactivity of the I^-/laccase system can be overcome by the use of suitable mediators of the enzyme.[29,30,35] For example, ABTS (2,2′-azinobis(3-ethylbenzthiazoline-6-sulfonate)) has shown promise as an efficient mediator of the oxidase activity of laccase,[36] and we accordingly attempted to foster the production of molecular iodine by reacting **12** with I^- and laccase in the presence of ABTS (Scheme 11; Table 2, compare entries 2 and 3).



Scheme 11

The effect was only a small increase in the modest production of quinonic derivatives from **12**. A slightly more encouraging result was obtained in the case of *m*-MeO, where the addition of ABTS did promote more nuclear iodination than in the reaction without mediator (compare entries 5 and 6). The enzyme, though, did not appear to provide any suitable polarisation of the molecule of iodine[7] so as to push the ensuing electrophilic attack forward. The additional use of a Lewis acid (possibly inert toward the enzyme) such as $Cu(OAc)_2$ was therefore attempted (entry 7), but no significant difference was obtained. We conclude that the efficiency of the I^-/laccase system is not sufficient to deserve further attention as a biohalogenation procedure.

Experimental

^{1}H NMR spectra were taken at 200 MHz on a Bruker AC 200 instrument, and at 300 MHz on a Varian Gemini A 300A instrument. GLC analyses were performed on a VARIAN 3400 instrument, fitted with a 30 m × 0.25 mm methyl silicone gum capillary column. GC-MS analyses were carried out on a HP 5892 GC, equipped with a 12 m × 0.2 mm methyl silicone gum capillary column, and coupled with a HP 5972 MSD instrument operating at 70 eV.

Materials

1,1,1,3,3,3-Hexafluoropropan-2-ol (HFP) was from Aldrich, while the other solvents were from Carlo Erba. ICl was from Aldrich. Most substrates were either commercial, or available from earlier work.[7,8,22] *N,N*-Dimethyl-4-methoxyaniline (**7**) had been obtained from methylation of commercial *N*-methyl-4-methoxyaniline (Aldrich).[24c] Potassium 12-tungstocobaltate(III) ($Co^{III}W$) was available from previous investigations.[22] Laccase from a strain of *Trametes villosa* was a gift from Novo Nordisk Biotech. The enzyme was purified by chromatography on Q-Sepharose.[35] The activity (10^4 units ml^{-1}) was then determined spectrophotometrically by the standard reaction with ABTS.[37]

Products

2-Iodomesitylene, 3-iododurene, the iodo-derivatives of *p*- and *m*-dimethoxybenzene and the *N*-(trideuteriomethoxymethyl)-derivative (*i.e.*, **6**) of *N,N*-dimethyl-*p*-toluidine (**4**) were available form previous investigations,[7,8,26] while 1-iodonaphthalene and *N*-methyl-*p*-toluidine (**5**) are commercial (Aldrich) products. Mono- and di-methylation of commercial 3-chloro-*p*-anisidine (Aldrich) with MeI provided analytical samples of **9** (*m*/*z* 171 and 173) and **8** (*m*/*z* 185 and 187), respectively. Compound **10** was characterised only by its MS spectrum (*m*/*z* 219 and 221). A sample of 4-iodo-*N,N*-dimethylaniline (**2**) was obtained from reaction of **1** with I_2 in $CHCl_3$,[38] and crystallised from ethanol–water, m.p. 75–77 °C (lit.[39] m.p. 79 °C); ^{1}H NMR δ 7.45 and 6.48 (dd, 4H, aromatic *para* protons), 2.92 (s, 6H, Me_2N), *m*/*z* 247.

Determination of oxidation potentials

Cyclic voltammetry determinations were carried out with an Amel 5000 potentiostat in a 0.1 M Bu_4NBF_4 acetonitrile solution with a platinum working electrode (planar disk, ø 1 mm) and a saturated calomel electrode (SCE); ferrocene was employed as the reference compound. Irreversible oxidation potentials (E^p) were determined for compounds **1**, **4**, and **7**, and the corresponding values (at 500 mV s^{-1}) are reported in the text.

Iodination reactions

The iodination of compound **7** is described in detail; the other reactions were performed analogously. A solution of **7** (25 mg; 0.16 mmol) and ICl (30 mg; 0.18 mmol) in 2 ml HFP was stirred at room temperature for 0.5 or 4 h. Addition of the internal standard and conventional work-up with diethyl ether preceded GC analysis for quantitative determination of the iodination products **8**, **9** and **10**.

Reactivity studies in competition experiments

The yield of products from the competitive experiments was determined by GC with respect to an appropriate internal standard, suitable response factors being employed to convert the GC areas into mmols of products. Experiments were run in duplicate, and the conditions described in previous publications were in general followed.[7,8b] The competition experiment of MES and Naph is reported in detail as an example. A solution of naphthalene (575 mg; 4.5 mmol) and mesitylene (156 mg; 1.3 mmol) was prepared in 4 ml of HFP; ICl (0.41 mmol) was then added, and the solution stirred at room temperature for 3 h. Addition of the internal standard and conventional work-up with CH_2Cl_2 preceded GC analysis for quantitative determination of the iodination products. The intermolecular reactivity was calculated from the molar amounts of the two nuclear iodinated products by the use of the standard equation for competitive reactions[40] and by taking into account the statistical factors of the two competing substrates (3 to 4, respectively, for MES and Naph). Minute amounts of 1-chloronaphthalene,

even smaller than those of the accompanying 1-iodonaphthalene, were detected.

Biohalogenation reactions

In a typical reaction with laccase,[30] 3 ml of a 0.1 M phosphate buffer solution at pH 3.4 was placed in a vial; 60 μmol of substrate, 60 μmol of NaI and, if present, 20 μmol of ABTS as a laccase mediator, were added. Finally, 1.5 μl of a solution of purified laccase (with an activity of 10^4 units ml^{-1}) was added. After the chosen reaction time at room temperature, the reaction was worked up and analysed as in the previous case.

Acknowledgements

The authors are indebted to Novo Nordisk Biotech, Inc., for a generous gift of laccase, and to Dr F. Xu (Novo Nordisk Biotech, 1445 Drew Avenue, Davis, CA 95616, USA) for suggestions and advice.

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