The Oxidation of *N*-Benzylaziridine Catalyzed by Iron Porphyrin: Radical versus Electron Transfer Mechanism

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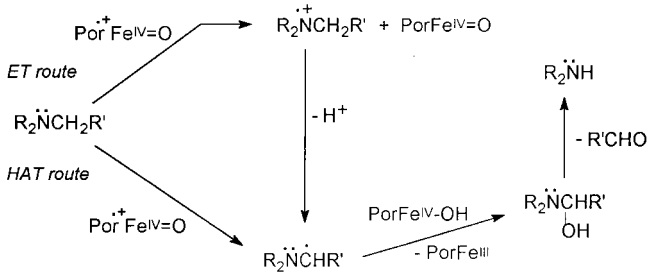
Abstract: A change in the mechanism of biomimetic oxidation of tertiary amines in response to appropriate structural features of the substrate, emerges from the investigation of the product pattern from *N*-benzylaziridine under bona fide radical or electron transfer conditions. This substrate is an amine endowed with a high oxidation potential as a result of steric constraint. Consequently, the hydrogen atom transfer route of oxidative N-dealkylation competes favorably with the electron transfer route, which is the mechanism observed for the reaction of conventional tertiary amines with metalloporphyrins and oxygen donors.

Keywords: aziridine · hydrogen abstraction · N-dealkylation · porphyrinoids · radical reactions

Introduction

The oxidative N-dealkylation of amines is one of the most important reactions catalyzed by monoxygenase enzymes such as cytochrome P450.[1a] Metalloporphyrin model compounds also catalyze this reaction.[1b] A possible mechanistic dichotomy exists between an electron transfer (ET) or a hydrogen atom transfer (HAT) route, as shown in Scheme 1.[1, 2]

So far, the ET pathway has received more general consensus both for enzymatic and biomimetic N-dealkylations on the basis of experimental evidence,[3] but mainly from the determination of the intramolecular H/D isotope effect.[4] However, the reliability of this mechanistic probe has been questioned, and kinetic deuterium isotope effect profiles for a series of substituted *N,N′*-dimethylanilines tended to the conclusion that the N-dealkylation reaction induced by cytochrome P450 occurs by the HAT mechanism.[5] A HAT mechanism is also suggested for the C-hydroxylation of alkanes and for the O-dealkylation of ethers catalyzed by cytochrome P450 and by metalloporphyrins.[1, 6] In contrast, a recent study of both intramolecular and intermolecular k_H/k_D profiles for the N-dealkylation of substituted *N,N′*-dimethylanilines has led to an endorsement of the ET route for the biomimetic process.[5d, 7] Another investigation also concluded in favor of the ET mechanism for N-dealkylation in a biomimetic process, on the grounds of a peculiar stereoelectronic effect observed with the probe substrate *N*-benzylpiperidine.[8] Thus, it would appear that the oxidation of tertiary amines by cytochrome P450 and by its chemical models occurs by different mechanisms!



Scheme 1. A possible mechanistic dichotomy for the oxidative N-dealkylation reaction of tertiary amines.

In view of such controversy about this important mechanistic problem,[3d, 5] we decided to undertake the investigation of one specific amine, *N*-benzylaziridine (**1**), as a representative mechanistic probe capable of distinguishing between the HAT and ET routes.[9] In fact, in a bona fide ET process such as electrochemical oxidation, **1** is reported to form a peculiar product [**2**; Eq. (1)][10] which is not a direct product of *N*-dealkylation. This process has no counterpart in other tertiary amines, which are the commonly employed substrates in previous studies of the biooxidation of amines.[3–8] Thus, the very detection of the tetrameric product **2** in a biomimetic

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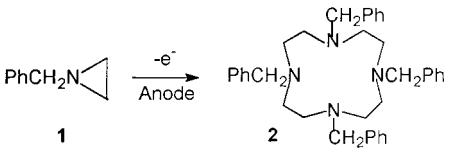

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oxidation of **1** would provide support for the occurrence of the ET route in Scheme 1.

We describe here the results of the biomimetic and enzymatic oxidations of **1**, which are complemented by and compared with the reactions of **1** in bona fide radical (HAT) or ET processes.

Results and Discussion

Electron transfer processes: Compound **1** was synthesized and allowed to react under bona fide ET conditions with potassium 12-tungstocobaltate(III) ($Co^{III}W$). This is a widely recognized outer-sphere oxidizing agent in homogeneous solution,[11] and we have determined its E° redox potential in CH_3CN as 1.49 V versus SCE (see Experimental Section). Reaction of **1** with $Co^{III}W$ (substrate:oxidant molar ratio of 300:1) in CH_3CN**:**H_2O 5**:**1 (with AcOK as a proton scavenger) gave 104% of the dimeric product *N,N′*-dibenzylpiperazine (**3**), 70% of tetramer **2**, and 5% of PhCHO (Table 1, entry 1; see [Eq. (2)]). The yields are expressed with respect to the oxidant, which is, in defect with respect to the substrate, and therefore yields exceeding 100% support a chain process. Similar results were obtained in an oxidation carried out in the less polar solvent CH_2Cl_2 (entry 2), where the oxidant is sparingly soluble, but with experimental conditions more comparable to the apolar conditions typical of the enzymatic site of cytochrome P450. Under these conditions, whenever the oxidation of **1** was conducted in the presence of catalytic amounts of tetramer **2** (**1**:**2** 100:1 molar ratio), the production of the tetramer was significantly enhanced (entry 3). A similar effect was observed for the addition of dimer **3** (**1**:**3** 50:1 molar ratio; entry 4).

We also attempted to duplicate as closely as possible the anodic oxidation conditions in the literature,[10] and obtained 24% of tetramer **2**, accompanied by the unexpected formation of dimer **3** (100%); no PhCHO was detected (Table 1, entry 5). Once again, when the anodic oxidation of **1** was repeated in the presence of catalytic amounts of **2** (**1**:**2** 100:1 molar ratio), the production of the tetramer was enhanced (entry 6). We suggest that, both under anodic conditions and in the presence of $Co^{III}W$, **1** is oxidized to the radical cation

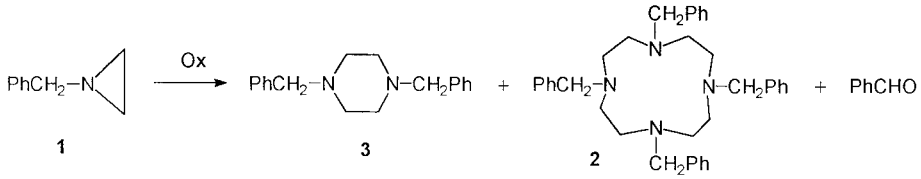

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Table 1. Comparison of the results of the various procedures of oxidation [see Eq. (2)].

Entry	Oxidant (**Ox**)	Conditions	**1**:**Ox**:Fe^{III}TPP (molar ratios)	Recovd. **1** [%]	Yield of products [%] (based on the **Ox**, unless otherwise stated) PhCHO	**3**	**2**
			ET Oxidation				
1	$K_5Co^{III}W_{12}O_{40}$	[a]	300:1:–	86	5	104	70
2	$K_5Co^{III}W_{12}O_{40}$	[b]	100:1:–	85	14	70	2
3	$K_5Co^{III}W_{12}O_{40}$	[c]	100:1:–	72	–	16	95
4	$K_5Co^{III}W_{12}O_{40}$	[d]	100:1:–	85	7	90	45
5	anodic oxidn.	[e]	–	68	–	100[l]	24[l]
6	anodic oxidn.	[f]	–	62	–	122[l]	183[l]
7	*hν*/*p*-$C_6H_4(CN)_2$	[g]	1:0.3:–	90	0.1	0.9	ca. 150
8	iodine	[h]	4.2:1:–	78	40	344	192
			HAT Oxidation				
9	*t*BuOOH	[i]	100:10:1	55	13	5	–
10	*t*BuOOH	[b]	100:1:1	90	44	61	–
11	*hν*/AIBN	[j]	100:1:–	90	traces	10	–
			Biomimetic Oxidation				
12	PhIO	[i]	100:10:1	89	5	16	–
13	PhIO	[i]	6.2:1:0.01	43	8	15	–
14	PhIO	[b]	100:1:1	93	25	55	–
15	$NaIO_4$	[k]	100:1:1	88	25	39	–

[a] Homogeneous solution in MeCN/H_2O (5:1, w/w) with added AcOK (40/1, molar, vs. **Ox**); reaction time 1 h. [b] Heterogeneous solution in CH_2Cl_2; reaction time 1.5 h. [c] As in [b], but with added **2** (**1**/**2** = 100/1, molar). [d] As in [b], but with added **3** (**1**/**3** = 50/1, molar). [e] At 1.3 V (vs. SCE) fixed potential; electricity consumption: 2.4 mF mol^{-1}; in a 0.07 M Et_4NClO_4 solution in CH_2Cl_2. [f] As in [e], but with added **2** (**1**/**2** = 100/1, molar). [g] In 9:1 CH_3CN/MeOH, over K_2CO_3; reaction time 1.5 h; $\lambda = 350$ nm. Yields are versus *p*-dicyanobenzene. [h] Homogeneous solution in $CDCl_3$ at 22 °C; reaction time 1 h; $[\mathbf{1}]_0 = 0.16$ M, $[I_2]_0 = 0.04$ M; iodine conversion = 5%. Yields are versus reacted I_2. [i] Heterogeneous solution in benzene; reaction time 1.5 h. [j] Homogeneous solution in benzene; reaction time 1.5 h; $\lambda = 350$ nm; yields are versus $2 \times [AIBN]_0$. [k] As in [i], with added $(PhCH_2)(n\text{-}C_{12}H_{25})NMe_2Cl$ (1/20, molar, vs. **Ox**). [l] Calcd. versus the amount of electricity passed through the cell. Yields of **2** and **3** are from ^{1}H NMR spectroscopy.

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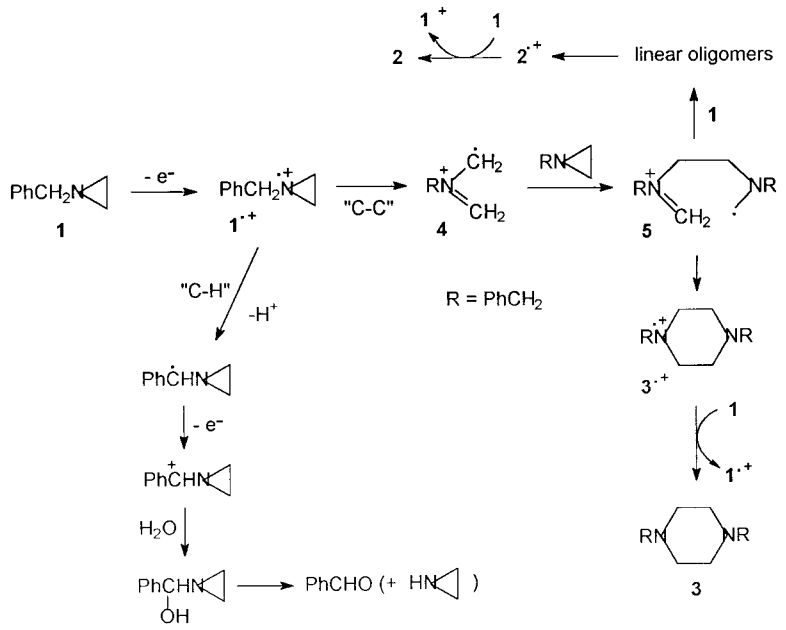
1$^{\bullet+}$. This would undergo a fast cleavage of the C–C bond of the aziridine ring,[12] in competition with deprotonation from the benzylic C–H bond (Scheme 2). Further oxidation of the benzylic radical resulting from the latter step would lead to PhCHO (a minor product), while dimeric and tetrameric products **3** and **2** would originate from the ring-opened radical



Scheme 2. A possible rationalization of the reaction pathways of the electron transfer (ET) route.

cation **4**. A possible attack of the latter on another molecule of **1** in a S_H2-type process[13, 14] would give the dimeric distonic radical cation **5**, which could partition between oligomerization and cyclization to **3**$^{\bullet+}$. The latter would yield **3** coupled with the oxidation of another molecule of **1** to **1**$^{\bullet+}$ in a chain process, thus explaining yields exceeding 100% with respect to the oxidant (or to the Coulomb passed in the electrochemical experiments). At the same time, oligomerization of **5**, followed by cyclization, would give **2**$^{\bullet+}$ and then **2** (Scheme 2).

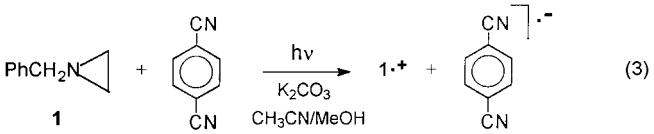
While the routes described in Scheme 2 must be taken as tentative at this stage, no precise mechanistic description has previously been offered for the formation of **2**, nor has the formation of **3** ever been reported under anodic oxidation conditions [Eq. (1)].[10] We were able to form dimer **3** and in quantities greater than tetramer **2**, both in the electrochemical process and in reaction with $Co^{III}W$, unless **2** was added as a catalyst from the beginning of the reaction. Prevalence of **3** over **2** is reasonable, since formation of **2** occurs at a later stage (Scheme 2). It is conceivable that there are differences in the local concentration of the precursor **1** under anodic and $Co^{III}W$ reaction conditions. In fact, double layer effects in the electrochemical reaction are likely to enhance the „effective" concentration of monomer **1** in the very region where the radical cation **4** is generated: this would have beneficial effects on the efficient occurrence of S_H2 oligomerization steps, ultimately affording **2**. Accordingly, when the oxidation of **1** with $Co^{III}W$ is carried out at a 2:1 oxidant:substrate molar ratio, that is, *without excess* substrate, **2** is not observed, only traces of **3** are detected, and PhCHO is obtained (24% yield, with a 51% recovery of **1**). This confirms that the S_H2 process (a bimolecular event) takes place successfully only in the presence of a high concentration of monomer. It is also observed that the yield of products in the anodic oxidation depends very much on the amount of Coulomb passed, and on the use of a divided or undivided cell, overoxidation steps being responsible for unwanted consumption of some of the products. Finally, it should be stressed that separation of **3** from **2** during work-up is not easy, and **3** could have escaped identification from the previous investigators.[10]

The catalytic effect of added **2** (or **3**) on the formation of **2** itself is also worth an explanation. The oxidation potential of **2** (and of **3**) is lower than that of **1**,[15] and the anodic oxidation is run at a fixed potential,[10] with a value between that of **2** and **1**.[16] Since the products are easier to oxidize than the reagent, substantial loss of the products from overoxidation may occur, unless a large excess of **1** is present. Excess **1**, instead, pushes the reaction to become a chain process (see Scheme 2), because mass law drives **2**$^{\bullet+}$ (or even **2**$^{2+}$)[15] to take an electron from **1**, to yield **2** (or **2**$^{\bullet+}$) and **1**$^{\bullet+}$ in an homogeneous oxidation step in the bulk of the solution: this, in part, protects **2** from further and extensive cleavage/addition events that would lead to higher order polymers. In this way the oxidation of **1** to **2** runs more efficiently.[10a] The oxidation of **1** is also made easier by the availability of catalytic amounts of the more easily oxidizable **2** from the very beginning of the process; **2** then acts as a mediator and would induce a sort of redox catalysis.[10] A similar explanation also holds for the addition of catalytic amounts of **3** to the reaction medium.

It is known that precursor **1** may undergo acid-induced ring cleavage to produce either **2** or **3**,[17] thereby simulating the formation of the products that we ascribe to an ET route in Scheme 2. However, AcOK is present in our experiments as a proton scavenger, and in a blank experiment in the absence of $Co^{III}W$ we recovered more than 95% of unchanged **1**, with no formation of **2** or **3**. Similarly, possibility of an acid-induced formation of **2** was excluded in the case of the anodic oxidation of **1**.[10] These control experiments would ensure that production of **2** and **3**, both in the electrochemical and in the $Co^{III}W$ reactions, is firmly linked to an ET pathway triggered on **1**.

We have also exploited a literature procedure for the photoinduced generation of radical cations,[18] by adapting it to our present case. Reaction of **1** in a 9:1 CH_3CN/MeOH solvent mixture, with heterogeneous K_2CO_3 as a proton

scavenger, in the presence of *p*-dicyanobenzene as a photosensitizer under irradiation at 350 nm, resulted in a substantial conversion to **2**, while **3** and PhCHO were produced only in minute amounts (entry 7). We infer that electron transfer from the donor **1** to the excited state of the acceptor *p*-dicyanobenzene leads to the formation of $\mathbf{1}^{\bullet+}$ [Eq. (3)],[12c, 18] which then reacts further as shown in Scheme 2.



(3)

One peculiar reaction that we have also discovered is one where iodine reacts with **1** in an apolar solvent at room temperature. Benzaldehyde, **2**, and **3** are all produced in considerable amounts (entry 8). We suggest that a charge transfer complex is formed between I_2 and **1** to produce $\mathbf{1}^{\bullet+}$ [cf. Eq. (3)] which would cleave immediately,[12d] reacting as shown in Scheme 2.[19] Evidence for the formation of charge transfer complexes between I_2 and **1**, **2**, and **3** is obtained from ^{1}H NMR spectroscopy, by the observation of a shift of the resonances of the corresponding $PhCH_2$ signals (of about 0.1 ppm). More details on this reaction will be disclosed in a future publication.

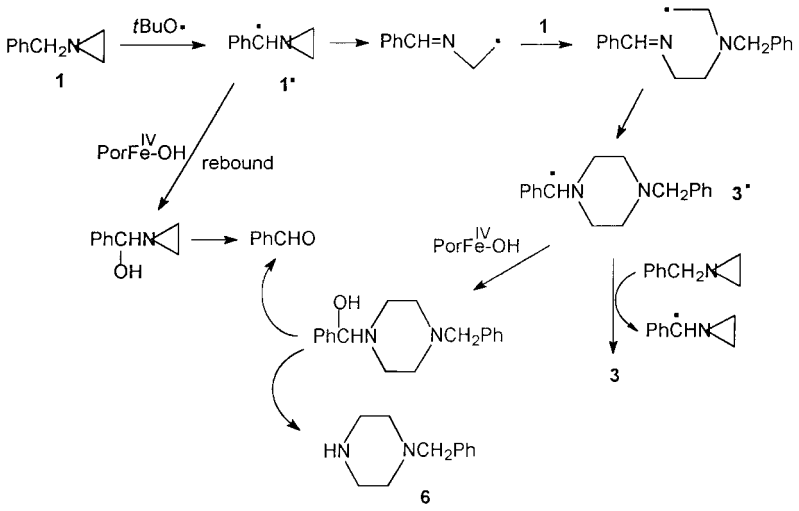
Hydrogen abstraction processes: The reactivity of **1** under bona fide radical conditions was then investigated by making use of a conventional and commercially available metalloporphyrin. Iron(III) tetraphenylporphyrin chloride (Fe^{III}TPPCl), on reaction with *t*BuOOH as the oxidant, is known to produce $PorFe^{IV}$-OH and *t*BuO$^{\bullet}$;[20] the latter abstracts hydrogen atom from the substrate[20, 21] while $PorFe^{IV}$-OH replenishes OH$^{\bullet}$ to the intermediate radical site.[1] Reaction of **1** with *t*BuOOH/Fe^{III}TPPCl (substrate**:***t*BuOOH**:**porphyrin 100**:1:**1 molar ratio) in benzene gave 13% of PhCHO and 5% of **3** (entry 9, Table 1). A higher conversion was obtained in CH_2Cl_2 (entry 10). No traces of **2** were found, not even by LC-MS analysis. Increasing the amount of *t*BuOOH was not profitable, since it decreased the yield of **3**, possibly by overoxidation.[22] As another example of a radical-induced HAT process, we tentatively used photolysis of azobisisobutyronitrile (AIBN) in the presence of an excess of **1** (entry 11). Formation of **3** was observed, while no traces of **2** were detected. Clearly, PhCHO is not expected in this particular case, owing to the lack of any species capable of replenishing OH$^{\bullet}$ to the benzylic radical site.

The formation of **3** in these two radical processes is tentatively explained in Scheme 3, and involves firstly an intermolecular S_H2-type event and then intramolecular addition of the carbon radical to the imine to give $\mathbf{3}^{\bullet}$. It is important to stress that the results of the bona fide ET and HAT processes, while having the formation of product **3** in common, do differ in the formation of product **2**, which is obtained only under ET conditions. This would suggest that the oligomerization events of Scheme 2 must compete more efficiently with the intramolecular event, to lead to $\mathbf{3}^{\bullet+}$, than in the corresponding „radical" case, which gives $\mathbf{3}^{\bullet}$. We have also delineated a possible route to the formation of PhCHO and *N*-benzylpiperazine **6** from overoxidation of **3**.[22] When an excess of *t*BuOOH is avoided and the normal substrate**:** *t*BuOOH**:**porphyrin 100/1/1 molar ratio is used, no significant formation of **6** is observed. This is reasonable, because **3** is produced in a medium rich in precursor **1**, in general, where further oxidation of **3** would be statistically unlikely. Therefore, the amount of PhCHO reported in experiments 9 and 10 largely (if not exclusively) derives from $\mathbf{1}^{\bullet}$, rather than from $\mathbf{3}^{\bullet}$ (Scheme 3).

Biomimetic and enzymatic reactions: Finally, the oxidation of **1** was attempted under typical biomimetic conditions,[1b, 21] namely, with Fe^{III}TPPCl and PhIO as the oxidant in benzene, at a substrate**:**PhIO**:**porphyrin molar ratio of 100:10:1 (entry 12, Table 1). Benzaldehyde (5%) and dimer **3** (16%) were obtained, but no tetramer **2** was produced. A better result was obtained in CH_2Cl_2 (entry 14). Once again, increasing the relative amount of the oxidant gave overoxidation of the products (entry 13).[23] When PhIO was substituted with $NaIO_4$ as the oxidant, in combination with a phase-transfer agent, PhCHO and **3** were again the only products formed (entry 15).

Unfortunately, the attempted oxidation of **1** with cytochrome P450 proceeded to a disappointingly low extent and only PhCHO could be observed (0.1% yield). Similarly, oxidation of **1** with horseradish peroxidase (HRP) gave only 0.3% of PhCHO, but 0.7% of **3** was also detected, while **2** was absent. Blank experiments, run without HRP or without H_2O_2, gave no trace of any product formation.

The results reported here support our expectation that **1** could provide useful hints for resolving the mechanistic



Scheme 3. A possible rationalization of the reaction pathways of the hydrogen atom transfer (HAT) route.

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dichotomy shown in Scheme 1. In fact, the nature of the reaction products significantly depends on the mechanism at play. Whenever care is taken to avoid acid-induced ring opening of **1**, efficient dimerization (to **3**) and, above all, tetramerization (to **2**) appear as the salient features of bona fide ET reactions, as opposed to the absence of product **2** under bona fide radical conditions. When compared with these two prototypical reactive types, absence of **2** in the biomimetic reactions appears consonant with the occurrence of a HAT route. This conclusion is safer in the biomimetic case, since in the enzymatic process with cytochrome P450 any dimerization and oligomerization event would be hampered by the fact that only one molecule of **1** enters the enzymatic pocket at a time.[1] Absence of **2** or **3** under these conditions is therefore not diagnostic for the operating mechanism. However, it is known that in oxidations with HRP the substrate does not enter the enzymatic pocket,[1, 24] but interacts with the edge of the heme group of HRP. Subsequent reactions of the radical cation of the substrate occur in the medium where, in the presence of other molecules of substrate, dimerization events could occur. In keeping with this difference in the reactive behavior of the two enzymes, and within the limits of the low conversion to products, and the lower concentration of substrate employed in our enzymatic experiments, the production of **3** in the reaction with HRP could be traced to a minor contribution from the ET route.[5d]

Although we conclude in favor of the operation of the HAT mechanism for the biomimetic oxidation of **1**, we point out, however, that the occurrence of either one of the routes of Scheme 1, and the consequent ongoing mechanistic debate, could be justified as follows. Whenever a biomimetic or peroxidase-induced oxidation of trialkyl- or aryldialkylamines is attempted, an ET mechanism would operate,[2, 3, 7] with formation of the radical cation of the substrate and ensuing deprotonation at C_α. In this scenario, stereoelectronic effects with suitable substrates would appear.[8] However, for substrates endowed with a high oxidation potential, the HAT mechanism can catch up and take over. For example, removal of an electron from the nitrogen lone-pair of **1** is more difficult than for normal amines, because of the strain in the three-membered ring,[25] which results in a more positive oxidation potential.[15] Consequently, a mechanistic changeover can occur with **1**, and this is why it becomes an interesting and telling tertiary amine from a mechanistic viewpoint. Similarly, N,N′-disubstituted amides represent substrates where the HAT mechanism of oxidation has consistently been reported, as a result of their oxidation potentials (in the 1.9–2.3 V vs. SCE range) being higher than that of trialkylamines.[26] Finally, it must be added that, since the radical cation of an amine is a rather weak acid (pK_a between 8 and 15),[27] there is an additional reason why the ET step reported in Scheme 1 might turn out to be unproductive. In some instances, the deprotonation of the amine radical cation would be not fast enough to compete with the back-ET step.[5d, 12c] In such a case, the HAT mechanism would take over.[28] Conversely, if this deprotonation occurs efficiently (for example, in the active site of cytochrome P450),[29] then the ET process would be productive. This point has been stressed recently,[7] along with other points to justify the mechanistic difference observed between the enzymatic and biomimetic routes of N-dealkylation of tertiary amines, on the grounds of the different polarity of the reactive environment, and also on the conceivable steric constraint present in the enzymatic pocket.

Experimental Section

General: NMR spectra were recorded in $CDCl_3$ at 300 MHz on a Varian Gemini 300BB or a Bruker AC300 instrument; chemical shifts δ are reported in ppm with respect to TMS as internal standard. LC-MS spectra were obtained by direct injection in the source of a Fison Instruments VG-Platform electrospray mass spectrometer. Determination of oxidation potentials and preparative oxidations were carried out with a AMEL 5000 potentiostat.

Materials: Dry CH_2Cl_2 was obtained by refluxing and distilling over P_2O_5. Methanol (HPLC grade) was purchased from Carlo Erba. Iron(III) tetraphenylporphyrin chloride and *t*BuOOH (70% in H_2O) were from Aldrich. Iodosylbenzene was prepared by basic hydrolysis of iodosylbenzene diacetate as previously reported,[8] stored at $-20\,^\circ C$ and titrated every 3 months. The $Co^{III}W$ salt, $K_5[Co^{III}W_{12}O_{40}]\cdot 11\,H_2O$, was synthesized as previously reported.[8]

***N*-Benzylaziridine (1)**: Compound **1** was prepared according to a conventional procedure[30] by the conversion of commercial (Aldrich) *N*-(2-hydroxyethyl)benzylamine into *N*-(2-bromoethyl)benzylamine hydrobromide. Under alkaline conditions, this then reacted to give **1** (38% overall yield); b.p. 56–58 °C at 2 Torr (ref. [31] 86–88 °C at 12 Torr). ^1H NMR: $\delta =$ 7.3 (s, 5H, Ph), 3.39 (s, 2H, $PhCH_2$), 1.86 and 1.31 ($2\times$ m, 2×2H, CH_2-CH_2).

1,4,7,10-Tetrabenzyl-1,4,7,10-tetraazacyclododecane (2): Compound **2** was obtained from cleavage of **1** by *p*-toluenesulfonic acid in boiling EtOH for 6 h;[17a] m.p. 143–144 °C (from EtOH; ref. [17a] 142–143 °C). ^1H NMR: $\delta = 7.3-7.2$ (m, 5H, Ph), 3.43 (s, 2H, $PhCH_2$), 2.69 (s, 4H, CH_2-CH_2).

1,4-Dibenzylpiperazine (3): Compound **3** was obtained in 92% yield from benzylation of piperazine with $PhCH_2Cl$ in refluxing EtOH for 24 h;[32] m.p. 91–92 °C (from EtOH; ref. [33] 92 °C). ^1H NMR: $\delta = 7.3-7.2$ (s, 5H, Ph), 3.51 (s, 2H, $PhCH_2$), 2.48 (br. s, 4H, CH_2-CH_2).

***N*-Benzylpiperazine (6)**: Compound **6** was obtained from monobenzylation of piperazine (9.7 g, 0.05 mol) with $PhCH_2Cl$ (6.3 g, 0.05 mol) in EtOH (30 mL) for 24 h.[34] After removal of the solvent, a 10% aqueous solution of NaOH was added (30 mL), and the slurry worked-up with diethyl ether. Distillation gave 3 g of **6**, b.p. 120 °C at 7 Torr (ref. [34] 154–160 °C at 18 Torr); from the higher boiling fraction, 0.5 g of **3** was also obtained after recrystallization from EtOH. ^1H NMR: $\delta = 7.34-7.30$ (m, 5H, Ph), 3.49 (s, 2H, $PhCH_2$), 2.84 (t, J = 4.9 Hz, 4H, C3 and C5), 2.41 (br. s, 4H, C2 and C6), 1.58 (s, 1H, NH).

Oxidation procedures: a) Oxidations with the ET agent potassium 12-tungstocobaltate(III) ($Co^{III}W$)[11b] were conducted in a 5:1 (by weight) CH_3CN/H_2O deareated mixed solvent (2 mL) which contained $Co^{III}W$ (8.2 mg, 2.5 μmol), AcOK (100 μmol), and **1** (100 mg, 750 μmol) for 60 min at room temperature under nitrogen. Addition of the internal standard (acetophenone), work-up with $CHCl_3$, and concentration to a small volume preceded GC injection on a methyl silicone column (25 m × 0.25 mm) for quantitative analysis of the more volatile products **3** and PhCHO. Use of HPLC was attempted for the determination of **2**; for example, a MeOH/H_2O 85/15 mixed eluent containing 0.03 M sodium heptanesulfonate (pH 4 for a NaH_2PO_4 buffer solution) on a reverse-phase C18 column. However, problems of clogging of the solution in the injection loop prevented any reliable determination. Preparative tlc or microcolumn chromatography were also attempted, but no reproducible results were obtained. Finally, use of a wide-bore (10 m × 0.55 mm) methyl silicone GC column at 250 °C (isothermal conditions) with acetophenone as the internal standard, proved to be viable and reliable for the quantitative determination of **2**. Occasional injection of the crude (taken up in CH_3CN) in the source of an electrospray LC-MS instrument, gave evidence for the *m*/*z* signals of **3** and **2**, as confirmed by injection of the authentic samples; these signals were accompanied by weaker signals, consistent with pentameric and hexameric higher homologues. No other major products were detected. In a typical case (conditions [a] in Table 1) we obtained 0.06 μmol of PhCHO (5%

yield: requires 2 moles of Co^{III}), 2.6 μmol of **3** (104% yield: requires 1 mole of Co^{III}), and 1.8 μmol of **2** (70% yield: requires 1 mole of Co^{III}). In a blank experiment, run in the absence of $Co^{III}W$, about 95% of **1** was recovered and no significant conversion into products was obtained, which confirmed the absence of unwanted reaction pathways (such as heterolytic) under the chosen experimental conditions.

b) Anodic oxidations were conducted in a divided cell connected to a nitrogen line.[10] In the anodic compartment, which contained a 0.07 M solution of Et_4NClO_4 in CH_2Cl_2 (40 mL), **1** (100 mg, 750 μmol) was added. The working electrode (anode) was a Pt foil (6 cm^2 surface), the counter electrode (cathode) was a Pt foil (1 cm^2 surface). The potential was measured versus a SCE electrode, equipped with a salt bridge which contained the same 0.07 M solution of Et_4NClO_4 in CH_2Cl_2, and separated from the anodic compartment by a glass frit. The electrolysis was set at +1.3 V. Passivation of the working electrode caused a rapid decrease of the current intensity. After the passage of 0.8 Coulomb, the electrolysis was stopped, the anolyte was added to the internal standard and brine, and worked-up with $CHCl_3$. Analysis by GC (as above) allowed determination of the yield of **3** and **2**. An even more reliable quantitative determination of the product yield was obtained by ^1H NMR spectroscopy. In this case, the organic solution was evaporated, diluted with $CDCl_3$, and the NMR spectrum measured. This enabled the integration of the peaks of the PhCH_2 group of **2** ($\delta = 3.43$) with respect to that of an internal standard (cyclohexane; $\delta = 1.43$). Under these conditions, the shifts of the PhCH_2 group of **3** and of **1** were at $\delta = 3.51$ and 3.39, respectively, as confirmed by measurement of the pure samples. The same experimental procedure was followed for the electrolysis of **1** in the presence of **2** (**2**:**1** 1/100 molar ratio).

c) The unprecedented oxidation of **1** (0.18 mmol) with I_2 (0.045 mmol) was conducted in $CDCl_3$ (1.1 mL) at room temperature, and directly analyzed by ^1H NMR spectroscopy (see above). Under these conditions, as a result of the formation of charge transfer complexes with iodine, the shifts of the Ph-CH_2 group of **3** and of **1** were at $\delta = 3.70$ and 3.49, respectively, as confirmed by the addition of pure samples to the reaction mixture.

d) Oxidations with the radical agent tBuO$^{\cdot}$ were conducted in benzene (2 mL)[21] with 1.75 mmol of **1**, either 17.5 or 175 μmol of tBuOOH, and 17.5 μmol of iron(III) tetraphenylporphyrin chloride, with stirring under nitrogen for 1 h. Addition of the internal standard and direct GC injection of the reaction solution (no work-up) for quantitative analysis of the products followed. Whenever a final work-up was carried out, in keeping with the conditions reported for procedure a), no substantial difference in the yields was observed. Further analysis of the reaction crude by direct injection in the electrospray LC-MS gave evidence of **3**, but no **2** or higher homologues. In a blank experiment, run without porphyrin, no products were detected and 95% of **1** was recovered.

e) A radical process was also induced on **1** (1.87 mmol) in benzene (2 mL) by the photolysis of AIBN (9 μmol) under irradiation with 16 350 nm lamps in a Rayonet RPR 100 photochemical reactor under nitrogen for 90 min. GC analysis followed.

f) Under biomimetic conditions,[21, 35] **1** (1.87 mmol), PhIO (either 19 or 180 μmol), and iron(III) tetraphenylporphyrin chloride (18 μmol) in benzene (2 mL) were stirred at room temperature under nitrogen for 90 min. GC analysis followed. Once again, further analysis of the reaction crude by direct injection in the electrospray LC-MS gave evidence of **3**, but no **2**. Blank experiments gave no conversion to products in the absence of either PhIO or the porphyrin, with substantial recovery of **1**.

g) A biomimetic reaction was also run as in f) by replacing PhIO with $NaIO_4$ (20 μmol); benzyl dimethyldodecylammonium chloride (0.9 μmol) was also added for solubility reasons.[36]

h) In the reaction of **1** with cytochrome P450, rat liver microsomes (40 mg of protein), NADPH generating system (10 μmol of $NADP^+$, 100 μmol of G6P, and 12 units of G6P-DH), and substrate (20 μmol) were incubated in a phosphate buffer (7 mL; pH 7.4, 0.1 M) at 36 °C for 2 h. The reaction products were extracted as described,[37] and analyzed by GC; 40% of **1** was recovered, along with trace amounts of PhCHO (0.6 μmol). In the absence of either microsomes or the NADPH generating system, no conversion to PhCHO was obtained, and 98% of the substrate was recovered.

i) For the reaction of **1** with HRP, 1 mg of the pure enzyme (Sigma) was dissolved in a buffered aqueous solution (1 mL) of phosphate (pH 6; 0.02 M); 20 μL of this solution (ca. 0.5 nmol) was added to 75 μmol of **1** in the buffered phosphate solution (5 mL);[38] then a 1.7% aqueous solution of H_2O_2 (90 μmol) was slowly added in 40 min by a motor-driven syringe. Work-up and analysis as in h).

j) Following a literature procedure,[18] reaction of **1** (1.55 mmol) with 0.55 mmol of *p*-dicyanobenzene, in a 9:1 CH_3CN/MeOH solution (2 mL) was performed in a quartz cuvette; the solution was stirred over K_2CO_3 at room temperature, while irradiated in a Rayonet RPR 100 photochemical reactor, fitted with 16 350 nm lamps. After 1.5 h irradiation, the reaction was worked-up with water and diethyl ether, and analyzed by GC as above.

Determination of the redox potentials: The oxidation potentials of compounds **1**, **2**, and **3** (1.2 mM) were determined by cyclic voltammetry vesus SCE in a 0.05 M Bu_4NPF_6 solution of anhydrous CH_2Cl_2 at 25 °C, with platinum electrodes and ferrocene as the internal reference compound. The waves were irreversible, even at a 100 Vs^{-1} sweep rate, so that only E^p values are given. For **1** we measured an E^p of 1.7 V, which is high for a tertiary amine, but reasonable in view of the steric constraint of the three-membered ring.[12c, 25b] For **2** we had two waves (E^p 0.9 and 1.4 V), while for **3** only the first wave was clearly resolved (E^p ca. 1.4 V). The reduction potential of $Co^{III}W$ (at 1.5 mM) was obtained as a reversible wave at $E^\circ = 1.49$ V versus SCE in a 0.1 M Et_4NBF_4 solution of anhydrous CH_3CN at 25 °C, with platinum electrodes and ferrocene as the reference compound. The experiments were conducted in a divided cell connected to a nitrogen line.

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[1] a) *Cytochrome P-450; Structure, Mechanism, and Biochemistry* (Ed.: P. R. Ortiz de Montellano), Plenum, New York, **1986**; b) B. Meunier, *Chem. Rev.* **1992**, *92*, 1411; c) M. Sono, M. P. Roach, E. D. Coulter, J. H. Dawson, *Chem. Rev.* **1996**, *96*, 2841.

[2] F. P. Guengerich, T. L. Macdonald, *Acc. Chem. Res.* **1984**, *17*, 9.

[3] a) R. H. Böcker, F. P. Guengerich, *J. Med. Chem.* **1986**, *29*, 1596; b) F. P. Guengerich, R. J. Willard, J. P. Shea, L. E. Richards, T. L. Macdonald, *J. Am. Chem. Soc.* **1984**, *106*, 6446; c) T. L. Macdonald, W. G. Gutheim, R. B. Martin, F. P. Guengerich, *Biochemistry* **1989**, *28*, 2071; d) F. P. Guengerich, C.-H. Yun, T. L. Macdonald, *J. Biol. Chem.* **1996**, *271*, 27321.

[4] G. T. Miwa, J. S. Walsh, G. L. Kedderis, P. F. Hollenberg, *J. Biol. Chem*. **1983**, *258*, 14445.

[5] a) J. P. Dinnocenzo, S. B. Karki, J. P. Jones, *J. Am. Chem. Soc.* **1993**, *115*, 7111; b) S. B. Karki, J. P. Dinnocenzo, J. P. Jones, K. R. Korzekwa, *J. Am. Chem. Soc.* **1995**, *117*, 3657; c) J. I. Manchester, J. P. Dinnocenzo, L. Higgins, J. P. Jones, *J. Am. Chem. Soc.* **1997**, *119*, 5069; d) Y. Goto, Y. Watanabe, S. Fukuzumi, J. P. Jones, J. P. Dinnocenzo, *J. Am. Chem. Soc.* **1998**, *120*, 10762.

[6] a) F. P. Guengerich, *J. Biol. Chem.* **1987**, *262*, 8459; b) E. Baciocchi, M. Crescenzi, O. Lanzalunga, *J. Chem. Soc. Chem. Commun.* **1990**, 687; c) Y. Urano, T. Higuchi, M. Hirobe, *J. Chem. Soc. Perkin Trans. 2*, **1996**, 1169; d) E. Baciocchi, F. D'Acunzo, C. Galli, O. Lanzalunga, *J. Chem. Soc. Perkin Trans.* **1996**, 133; e) P. H. Toy, M. Newcomb, P. F. Hollenberg, *J. Am. Chem. Soc.* **1998**, *120*, 7719; f) E. Baciocchi, S. Belvedere, M. Bietti, O. Lanzalunga, *Eur. J. Org. Chem*. **1998**, *1*, 299; g) S. Shaik, M. Filatov, D. Schröder, H. Schwarz, *Chem. Eur. J.* **1998**, *4*, 193.

[7] E. Baciocchi, O. Lanzalunga, A. Lapi, L. Manduchi, *J. Am. Chem. Soc.* **1998**, *120*, 5783.

[8] M. Bietti, A. Cuppoletti, C. Dagostin, C. Florea, C. Galli, P. Gentili, H. Petride, C. Russo Caia, *Eur. J. Org. Chem*. **1998**, 2425.

[9] For a somewhat related approach, see: H. Chen, M. J. de Groot, N. P. E. Vermeulen, R. P. Hanzlik, *J. Org. Chem*. **1997**, *62*, 8227.

[10] a) R. Kossai, J. Simonet, G. Dauphin, *Tetrahedron Lett*. **1980**, *21*, 3573; b) *J. Electroanal. Chem*. **1982**, *139*, 207.

  0947-6539/99/0510-2998 $ 17.50+.50/0

[11] a) L. Eberson, *J. Am. Chem. Soc.* **1983**, *105*, 3192; b) E. Baciocchi, M. Crescenzi, E. Fasella, M. Mattioli, *J. Org. Chem. Soc.* **1992**, *57*, 4684.
[12] a) X.-Z. Qin, F. Williams, *J. Phys. Chem.* **1986**, *90*, 2292; b) C. J. Rhodes, *J. Chem. Res.(S)* **1989**, 218; c) E. Hasegawa, S. Koshii, T. Horaguchi, T. Shimizu, *J. Org. Chem.* **1992**, *57*, 6342; d) C. Gaebert, C. Siegner, J. Mattay, M. Toubartz, S. Steenken, *J. Chem. Soc. Perkin Trans. 2* **1998**, 2735.
[13] S_H2 ring cleavage of cyclopropane by radical attack is well documented.[14] Similar events for the aziridine ring have not been reported but are likely in view of the comparable ring-strain.
[14] M. L. Poutsma in *Free Radicals, Vol. 2* (Ed.: J. K. Kochi), Wiley, New York, **1973**, p. 144.
[15] Electrochemical characterization of **1** gives a higher oxidation potential ($E^p = 1.7$ V) than **2** ($E^p = 0.9$ and 1.4 V) and **3** ($E^p \approx 1.4$ V), all data being versus SCE. Our E^p value for **1** compares well with that reported in ref. [12c].
[16] It can be observed that also the oxidation potential of $Co^{III}W$ (1.49 V) is intermediate between that of **1** and those of **2** and **3** (in ref. [15] and Experimental Section).
[17] a) G. R. Hansen, T. E. Burg, *J. Heterocyclic Chem*. **1968**, *5*, 305; b) E. J. Goethans, *Adv. Poly. Sci*. **1977**, *23*, 103; c) C. R. Dick, *J. Org. Chem*. **1967**, *32*, 72; **1970**, *35*, 3950.
[18] J. Lee, J. S. U, S. C. Blackstock, J. K. Cha, *J. Am. Chem. Soc*. **1997**, *119*, 10241.
[19] We are aware of a comparable oxidation of derivatives of anthracene to radical cations by the use of I_2 in pyridine: L. M. Tolbert, Z. Li, S. R. Sirimanne, D. G. VanDerveer, *J. Org. Chem*. **1997**, *62*, 3927. The oxidation potential of anthracene is comparable to that of **1** (see ref. [15]).
[20] T. C. Bruice, *Acc. Chem. Res.* **1991**, *24*, 243.
[21] J. R. Lindsay Smith, D. N. Mortimer, *J. Chem. Soc. Perkin Trans. 2* **1986**, 1743.
[22] Reaction of **3** with *t*BuOOH/Fe^{III}TPPCl (**3**:oxidant 10:1 molar ratio) gave 23% of PhCHO and 5% of *N*-benzylpiperazine **6**.
[23] Reaction of **3** with Fe^{III}TPPCl/PhIO (**3**:oxidant 10:1 molar ratio) gave 36% of PhCHO and 21% of *N*-benzylpiperazine **6**.
[24] a) R. Z. Harris, S. L. Newmyer, P. R. Ortiz de Montellano, *J. Biol. Chem*. **1993**, *268*, 1637; b) M. A. Ator, P. R. Ortiz de Montellano, *J. Biol. Chem*. **1987**, *262*, 1542.
[25] a) P. Asmus, M. Klessinger, *Angew. Chem.* **1976**, *88*, 343; *Angew. Chem. Int. Ed. Engl*. **1976**, *15*, 310; b) C. Cauletti, M. L. Di Vona, P. Gargano, F. Grandinetti, C. Galli, C. Lillocci, *J. Chem. Soc. Perkin Trans. 2*, **1986**, 667.
[26] a) L. R. Hall, R. T. Iwamoto, R. P. Hanzlik, *J. Org. Chem*. **1989**, *54*, 2446; b) L. R. Hall, R. P. Hanzlik, *J. Biol. Chem*. **1990**, *265*, 12349.
[27] a) S. F. Nelsen, J. T. Ippoliti, *J. Am. Chem. Soc*. **1986**, *108*, 4879; b) J. P. Dinnocenzo, T. E. Banach, *J. Am. Chem. Soc*. **1989**, *111*, 8646; c) S. Das, C. von Sonntag, *Z. Naturforsch*. A **1986**, *41*, 50S.
[28] This explanation had already been advanced in a preliminary form: E. Baciocchi, A. Cuppoletti, C. Galli, R. P. Hanzlik, 4th North America Meeting, International Society for the Study of Xenobiotics; Raleigh, NC, October 23–27, 1994; Book of Abstracts, p. 264; see note 30 in ref. [7].
[29] There is evidence for specific base catalysis in N-dealkylation reactions by cytochrome P450, see: O. Okazaki, F. P. Guengerich, *J. Biol. Chem.* **1993**, *268*, 1546.17.
[30] H. W. Heine, B. L. Kapur, *J. Am. Chem. Soc*. **1955**, *77*, 4892.
[31] A. T. Bottini, J. D. Roberts, *J. Am. Chem. Soc*. **1958**, *80*, 5203.
[32] D. R. Smith, J. W. Curry, R. L. Eifert, *J. Am. Chem. Soc*. **1950**, *72*, 2969.
[33] S. Gabriel, R. Stelzner, *Chem. Ber*. **1896**, *29*, 2384.
[34] R. Baltzly, J. S. Buck, E. Lorz, W. Schoen, *J. Am. Chem. Soc*. **1944**, *66*, 263.
[35] P. Inchley, J. R. Lindsay Smith, R. J. Lower, *New J. Chem*. **1989**, *13*, 669.
[36] E. Baciocchi, O. Lanzalunga, A. Lapi, *Tetrahedron Lett*. **1995**, *36*, 3547.
[37] a) R. Amodeo, E. Baciocchi, M. Crescenzi, O. Lanzalunga, *Tetrahedron Lett*. **1990**, *31*, 3477; b) E. Baciocchi, O. Lanzalunga, F. Marconi, *Tetrahedron Lett*. **1994**, *35*, 9771.
[38] a) E. Baciocchi, O. Lanzalunga, S. Malandrucco, *J. Am. Chem. Soc*. **1996**, *118*, 8973; b) S.-I. Ozaki, P. R. Ortiz de 0Montellano, *J. Am. Chem. Soc*. **1994**, *116*, 4487; c) S.-I. Ozaki, P. R. Ortiz de Montellano, *J. Am. Chem. Soc*. **1995**, *117*, 7056; d) S. Colonna, N. Gaggero, G. Carrea, P. Pasta, *J. Chem. Soc. Chem. Commun*. **1992**, 357.

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