Chemical and microsomal oxidation of tertiary amides: regio- and stereoselective aspects

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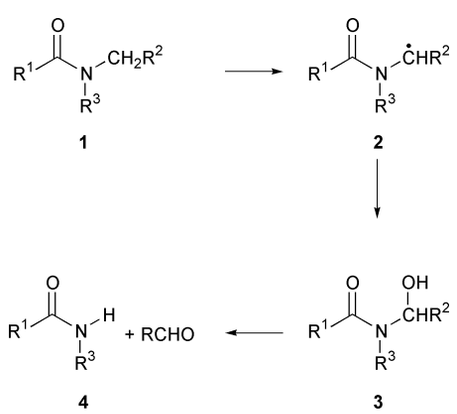
The conformationally restricted tertiary amides *N*-methyl-2-pyrrolidone **6**, *N*-methyl-2-piperidone **7** and *N*-methyl-ε-caprolactam **8** were oxidised by 5,10,15,20-tetraphenylporphyrinatoiron(III) chloride/*tert*-butyl hydroperoxide (TPPFe/Bu^{t}OOH) and by phenobarbital-induced rat liver microsomes. The products were the *N*-demethylated lactams together with the analogous *N*-methylimides and norimides. For the TPPFe/Bu^{t}OOH reaction ring oxidation is preferred to *N*-demethylation, paralleling the relative stabilities of the corresponding intermediate carbon-centred radicals as calculated by the AM1 semi-empirical method. In contrast, the microsomal reaction of the *N*-methyllactams strongly favours *N*-demethylation, demonstrating that hydrogen atom abstraction from the alkyl group *Z* to the amide carbonyl oxygen atom is preferred. The chiral tertiary amides *N*-methyl-*N*-(1-phenylethyl)benzamide **9** and *N*-methyl-5-phenyl-2-pyrrolidone **10** were also oxidised by TPPFe/Bu^{t}OOH and by phenobarbital-induced rat liver microsomes. Using TPPFe/Bu^{t}OOH, loss of the secondary alkyl group of **9** is preferred by a factor of *ca.* 6. Similarly, ring oxidation of **10** is favoured over demethylation by a factor of 9. For the microsomal reaction of (*R*)-**9** dealkylation is preferred over demethylation by a factor of 1.7, whereas for (*S*)-**9** demethylation is favoured by a factor of 1.25. For the microsomal reaction of (*R*)-**10** and (*S*)-**10** ring oxidation at the 5-position of the pyrrolidone ring is preferred over demethylation by factors of *ca.* 4 and 9 for the two isomers, respectively, and the (*S*)-enantiomer undergoes ring oxidation 2–3 times more readily than the (*R*)-enantiomer. For both **9** and **10** there is negligible stereochemical influence of the chiral centre upon the *N*-demethylation reaction. The results show that the stereochemical preference of the microsomal *N*-dealkylation reaction is modest.

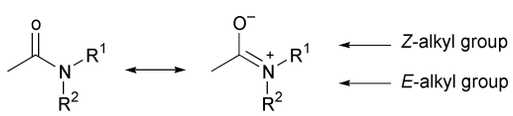
Tertiary amides **1** undergo oxidative dealkylation by cytochrome P450 enzymes to give the secondary amide **4** by way of the carbinolamide **3** (Scheme 1).[1–3] The carbon-centred radical **2**



Scheme 1 Pathway for the oxidative dealkylation of tertiary amides ($R^3 \neq H$).

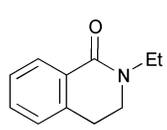
is an intermediate in this process,[4] formed presumably by hydrogen atom abstraction from an *N*-alkyl group by the haem iron(V)–oxo species that is the active oxidant of the P450 enzymes.[5] Two issues that have yet to be addressed about this oxidative dealkylation process are the regiochemistry and stereochemistry of the hydrogen atom abstraction. Herein we report an investigation of these issues.

Restricted rotation about the amide C–N bond of tertiary amides renders the two alkyl groups different (structure **A**).



A

However, the energy barrier to rotation is relatively small, *ca.* 65–85 kJ mol^{-1},[6] and, although the analogous value is unknown for an amide bound in the P450 active site, it is known that the rotation rate increases with decreasing solvent polarity and that the P450 active site is non-polar.[7] Acyclic amides, therefore, are unable to provide insights into the regioselectivity of the dealkylation process, although semi-empirical AM1 SCF MO calculations imply that the carbon-centred radical formed from the *E*-alkyl group is 4–5 kJ mol^{-1} more stable than the corresponding radical from the *Z*-alkyl group.[3,8] Consequently, we have chosen to examine the conformationally rigid lactams **5**–**8**.



5

As far as we are aware, the stereochemistry of amide dealkylation has not been studied. In general, however, the stereoselectivity of oxidations effected by cytochrome P450 enzymes appears to vary widely, depending both on the substrate and the isoform of the P450 enzyme involved. Thus, *S*-oxidation of achiral sulfides to chiral sulfoxides gives rise to (*R*) : (*S*) product selectivities between 0.96 and 1.4 for CYP102[9] and 0.25 and <0.01 for CYP108.[5,10] Olefin epoxidation similarly exhibits a range of stereoselectivities.[5,11–14] As for

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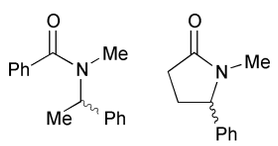
hydroxylation of aliphatic carbon atoms, phenylethane undergoes CYP2B6 enzyme-catalysed formation of phenylethanol *via* loss of the *pro*-(*S*) hydrogen atom four times more readily than the alternative abstraction of the *pro*-(*R*) hydrogen.[15] For the oxidation of ethanol to acetaldehyde, however, hydrogen abstraction α to the oxygen atom is not stereoselective.[16] Nicotine, (*S*)-*N*-methyl-2-(3-pyridyl)pyrrolidine, also undergoes hydroxylation α to the pyrrolidine nitrogen atom, as occurs in amide dealkylation, by human liver microsomes; diastereoselective (9 : 1) abstraction of the *pro*-(*R*) ring C-5 hydrogen atom, the proton that lies in a *trans* relationship across the pyrrolidine ring to the pyridyl group, is observed.[17] By way of contrast, only a modest stereoselectivity (1.4) was reported for the mechanism-based inhibition of CYP2B and CYP1A enzymes by the enantiomers of *N*-(1-phenylethyl)-1-aminobenzotriazole.[18]

In the present study we have examined the (*R*) and (*S*) enantiomers of the acyclic amide **9**, in which rotation about the C–N bond is allowed, and the cyclic amide **10**, in which the amide conformation is fixed. Compound **10** is an analogue of cotinine, a major nicotine metabolite,[19] in which the 3-pyridyl group of cotinine is replaced by phenyl. Compounds **9** and **10** have the potential to provide information on the stereoselectivity of the dealkylation (ring oxidation in **10**) of both the chiral and non-chiral alkyl groups.



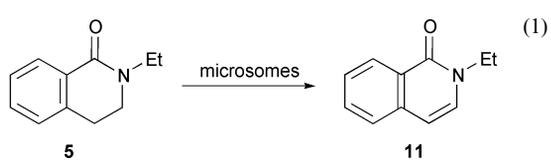
9 **10**

Our approach was as follows; microsomal incubations were compared with a chemical oxidation system (tetraphenylporphyrinatoiron(III) chloride/*tert*-butyl hydroperoxide (TPPFe/Bu^{t}OOH)) known to involve a hydrogen atom abstraction mechanism involving the *tert*-butoxyl radical.[20] Significantly, the *tert*-butoxyl radical has been proposed as a suitable chemical model for P450 reactions.[21] AM1 semi-empirical SCF MO calculations were also performed to assess the relative stabilities of the putative carbon-centred radicals involved in these reactions. Throughout, we have used a phenobarbital-induced rat liver microsomal P450 enzyme preparation. Phenobarbital induces P450 enzymes (primarily P450 2B1) that catalyse amide dealkylation,[4,7,8,22] and the microsomal preparation has been found to give essentially the same results as purified P450 2B1, at least as far as kinetic deuterium isotope effects are concerned.[7]

Results and discussion

Regioselectivity

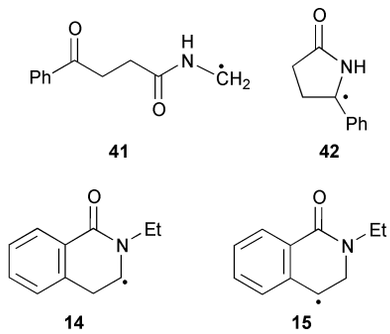
Microsomal metabolism of **5** afforded the dehydro derivative **11** as the sole product [eqn. (1)]. No evidence for any product of



(1)

5 **11**

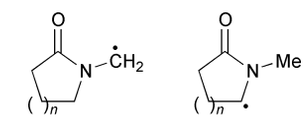
N-deethylation was found. The α-hydroxylated compound **12**, a potential intermediate in the conversion of **5** to **11**, also rapidly decomposed to the dehydro derivative **11** under the microsomal reaction conditions. Though these results would appear to imply that the *E*-alkyl group undergoes preferential reaction it is also possible that **11** could be derived from **13**, the product of benzylic oxidation (a well-known reaction of cytochrome P450[23]). Furthermore, the intermediate radicals **14** and **15** may



41 **42**

14 **15**

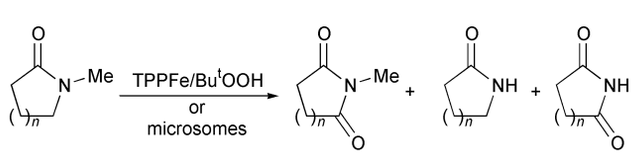
not give rise to the hydroxylated products **12** and **13** but undergo aromatisation to **11** directly by a further hydrogen atom abstraction. Consequently, we turned our attention to the lactams **6**–**8**. The relative stabilities of the relevant exocyclic and endocyclic radicals, **16**–**21**, corresponding to these lactams are



16 *n* = 1 **17** *n* = 1
18 *n* = 2 **19** *n* = 2
20 *n* = 3 **21** *n* = 3

contained in Table 1. In all cases the endocyclic radical *E* to the carbonyl oxygen atom is the thermodynamically favoured radical. The energy differences between the endocyclic and exocyclic radicals are larger than those observed for *N*,*N*-dimethylamides,[3] but are of similar magnitude to those observed for *N*-ethyl-*N*-methyl- (20.9 kJ mol^{-1}) and *N*-butyl-*N*-methyl- (19.2 kJ mol^{-1}) benzamides.[8] Thus, it would appear that the preference for the endocyclic radical is a result of its secondary nature. Using $\Delta\Delta H_f$ values, the following approximate preferences for endocyclic to exocyclic radical formation can be calculated: **6**, 12; **7**, 10^5; **8**, 7.4×10^3.

Oxidation of the lactams **6**–**8** by TPPFe/Bu^{t}OOH gave rise to the *N*-methylimides **22**, **25**, and **28** (the products of ring oxidation), the norlactams **23**, **26**, and **29** (the products of oxidative demethylation), and the norimides **24**, **27**, and **30** (the products of both ring oxidation and oxidative demethylation) (Scheme 2).



6 *n* = 1 **22** *n* = 1 **23** *n* = 1 **24** *n* = 1
7 *n* = 2 **25** *n* = 2 **26** *n* = 2 **27** *n* = 2
8 *n* = 3 **28** *n* = 3 **29** *n* = 3 **30** *n* = 3

Scheme 2 The products of biomimetic and microsomal oxidation of lactams **6**–**8**.

The formation of the norimides can arise *via* two pathways, namely the oxidative demethylation of the *N*-methylimides or the ring oxidation of the norlactams. To assess the contributions of these latter two reactions, compounds **22**, **25**, **28** and **23**, **26**, **29** were subjected to the same experimental conditions as **6**–**8** (Scheme 3). These revealed that the norimide **24** is formed from *both* the *N*-methylimide **22** and the norlactam **23**, the rate of formation of **24** from **22** being *ca.* 1.6 times greater than **23** (Table 2). In contrast, norimides **27** and **30** were formed only from the *N*-methylimides **25** and **28**; no formation from the corresponding norlactams **26** and **29** was observed.

The relative yields of the products of oxidation of **6**–**8** are contained in Table 3. For lactams **7** or **8** reaction at the ring carbon is expressed by **25** + **27** or **28** + **30** whereas demethylation is expressed by **26** or **29**. For lactam **6**, however, the formation of **24** needs to be partitioned in a ratio of 1.6 : 2.6 for

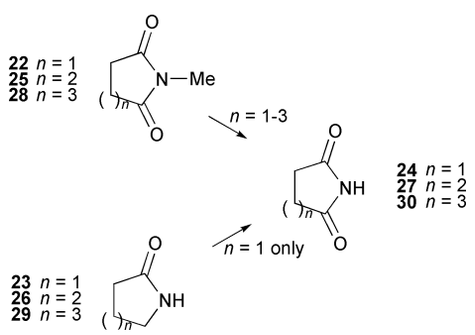
Table 1 Heats of formation, ΔH_f, calculated by the AM1 SCF MO method for amides **6**–**10**, **38** and **39** and their corresponding radicals **16**–**21**, **31**–**34**, **41** and **42**

	ΔH_f/kJ mol^{-1}		$\Delta H_f(\Delta\Delta H_f)^a$/kJ mol^{-1}		$\Delta H_f(\Delta\Delta H_f)^a$/kJ mol^{-1}
6	−168.5	**16**	−69.2 (99.3)	**17**	−75.4 (93.1)
7	−201.9	**18**	−100.8 (101.1)	**19**	−129.3 (72.6)
8	−215.9	**20**	−112.0 (103.9)	**21**	−134.1 (81.8)
9	85.1	**31**	180 (94.9)	**32**	151.1 (66.0)
10	−42.8	**33**	56.9 (99.7)	**34**	11.7 (54.5)
38	−57.2	**41**	19.8 (77)		
39	−65.8			**42**	−4.9 (60.9)

^{a} $\Delta\Delta H_f = \Delta H_f$ (radical) − ΔH_f (amide).

Table 2 Initial rates for the formation of succinimide **24** from *N*-methylsuccinimide **22** and pyrrolidone **23** using TPPFe/Bu^{t}OOH in CH_2Cl_2 at 30 °C

	Initial rate/mM h^{-1} (mmol TPPFe)$^{-1}$		
[**22** or **23**]/mM	From **22**	From **23**	Rate ratio
0.5	0.788 ± 0.09	0.454 ± 0.04	1.74
3.0	3.12 ± 0.10	1.93 ± 0.04	1.62
5.0	4.74 ± 0.25	2.99 ± 0.10	1.59
10.0	5.11 ± 0.07	3.26 ± 0.09	1.57



Scheme 3 Pathways for the formation of the norimides **24**, **27** and **30**.

ring oxidation to 1 : 2.6 for demethylation (as implied by the data in Table 2). Clearly, the overwhelming preference is for ring oxidation, and the relative selectivity order for ring oxidation over demethylation, *viz.* **7** > **8** > **6**, follows the relative order of radical stability calculated by the AM1 method.

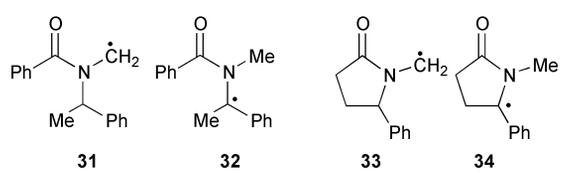
Oxidation of the lactams **6**–**8** by liver microsomes obtained from phenobarbital-induced rats gave the same three products as the TPPFe/Bu^{t}OOH oxidation (Scheme 2). Independent incubation of compounds **22**, **25**, **28** and **23**, **26**, **29** revealed that the norimide products **27** and **30** were formed exclusively by *N*-demethylation of the *N*-methylimides **25** and **28**, whereas **24** was formed from both **22** and **23**. The data in Table 4 reveal that **24** is formed *ca.* 5 times more rapidly from **22** than from **23**, which is almost certainly a result of the greater lipophilicity of the former.[8] The initial rates for the microsomal reactions of **6**–**8** are given in Table 5. These data were analysed using the Michaelis–Menten equation. For **7** and **8**, ring oxidation was determined by using the sum **25** + **27** and **28** + **30**, while demethylation was measured by **26** and **29**; for **6**, ring oxidation was determined by using the sum of the initial rate of formation of **22** plus $\frac{5}{6}$ of the initial rate for the formation of **24**, while demethylation was determined from the formation of **23** plus $\frac{1}{6}$ of the initial rate for the formation of **24**. ‡ Such analysis gives the V_{max} and V_{max}/K_m data in Table 6.

‡ It is clear from the data in Table 4 that the selectivity between ring oxidation and *N*-demethylation diminishes with increasing substrate concentration. This is unsurprising as the two substrates would be expected to exhibit different K_m values for the enzyme system. However, use of the average selectivity value will have only a small affect on the partitioning of the norimide formed during the metabolism of **6**.

These data show that, in contrast to the TPPFe/Bu^{t}OOH system, (i) the pyrrolidone system **6** undergoes reaction most readily and (ii) demethylation is preferred to ring oxidation. Indeed, if we take the TPPFe/Bu^{t}OOH reaction as providing a measure of the underlying chemical preference for reaction at the methyl or ring positions, a reasonable assumption given that it mirrors the AM1 semi-empirical calculations, then a comparison of the microsomal and biomimetic results reveals a very large preference for demethylation in the microsomal reactions. Thus, we conclude that microsomal dealkylation is regioselective for the *Z*-alkyl group and that such selectivity must arise from protein substrate interactions in the enzyme active site rather than from any underlying chemistry of the haem unit.

Stereoselectivity

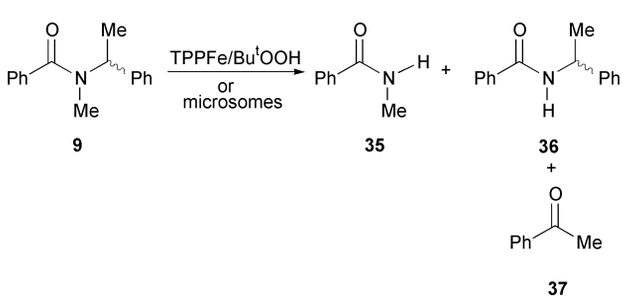
The relative stabilities of the carbon-centred radicals **31**–**34**



31 **32** **33** **34**

derived from **9** and **10**, as calculated by the AM1 method, are given in Table 1. As expected, the radical produced by hydrogen atom abstraction from the benzylic position is thermodynamically favoured over similar abstraction from the methyl group. Comparison of the data for **6** and **10** shows that the effect of the 5-phenyl group is to stabilise the endocyclic radical by *ca.* 38 kJ mol^{-1}.

Oxidation of **9** by TPPFe/Bu^{t}OOH gave *N*-methylbenzamide **35** (the product of dealkylation), *N*-(1-phenylethyl)benzamide **36** (the product of demethylation) and acetophenone **37** (the co-product of dealkylation) (Scheme 4). The relative product



9 **35** **36** + **37**

Scheme 4 The products of oxidative dealkylation of **9**.

yields are contained in Table 7 and these show that (i) the extent of acetophenone formation is identical to that for *N*-methylbenzamide, and (ii) allowing for the differing number of hydrogen atoms available for reaction, loss of the secondary alkyl group is favoured in this system by a factor of *ca.* 13.5.

Microsomal dealkylation of (*R*)-**9** and (*S*)-**9** also gave compounds **35**–**37**. The initial rates for these reactions are contained

Table 3 Relative product yields for the oxidation of lactams **6**–**8** by TPPFe/Bu^{t}OOH in CH_2Cl_2 at 30 °C

		Relative yield			
	Conc./mM	**22** or **25** or **28**	**23** or **26** or **29**	**24** or **27** or **30**	Ring oxidation : demethylation [a]
6	0.1	13	1	4.7	8.5
	1	13	1	2.2	11.6
	10	12.6	1	0.7	15.8
7	0.1	5400	1	3450	13275
	1	4760	1	2160	10380
	10	3175	1	955	6195
8	0.1	179	1	193	558
	1	327	1	305	948
	10	213	1	121	501

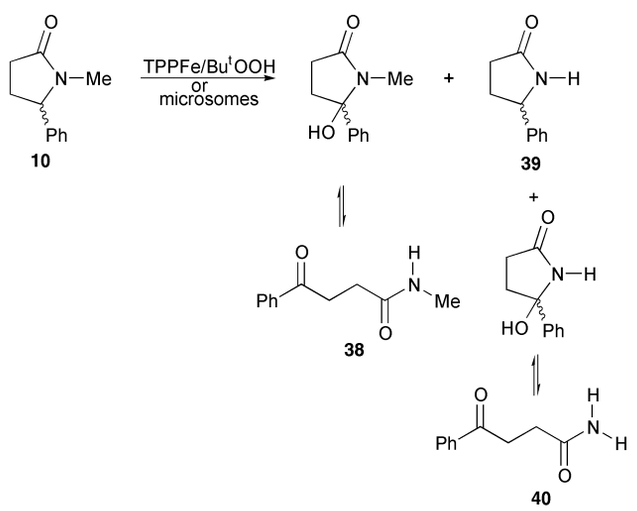
[a] Statistically corrected for three methyl and two ring oxidisable hydrogen atoms.

Table 4 Initial rates for the formation of **24** from either **22** or **23** catalysed by rat liver microsomes at 37 °C

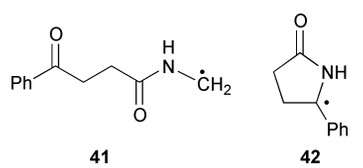
	Initial rate/mM h^{-1} (nmol P450)$^{-1}$		
[**22** or **23**]/mM	From **22**	From **23**	Rate ratio
0.05	0.35 ± 0.02	0.066 ± 0.008	5.4
0.5	3.41 ± 0.26	0.66 ± 0.03	5.2
1.0	6.70 ± 0.36	1.31 ± 0.23	5.1
3.0	18.0 ± 0.94	3.82 ± 0.56	4.7
5.0	27.2 ± 1.32	6.20 ± 0.85	4.4

in Table 8 and they reveal that (i) the rates of formation of *N*-methylbenzamide **35** and acetophenone **37** are identical, (ii) the rates of demethylation of (*R*)-**9** and (*S*)-**9** are virtually identical and (iii) the dealkylation of (*R*)-**9** is slightly faster than the dealkylation of (*S*)-**9**. Fitting the initial rate data to the Michaelis–Menten equation gives the values of V_{max} and V_{max}/K_m contained in Table 6. Allowing for the relative number of hydrogen atoms available for demethylation and dealkylation, these data reveal a preferential loss of the secondary alkyl group. Nevertheless, a comparison of the dealkylation : demethylation ratio for the microsomal reaction with that for the TPPFe/Bu^{t}OOH reaction reveals that demethylation in the microsomal system is enhanced by a factor of 7–16. A similar factor of 16 in favour of microsomal demethylation has been observed for *N*-isopropyl-*N*-methylbenzamide.[24] Presumably the slightly smaller factor observed for **9** is a result of the stabilising effect of the phenyl group on the radical intermediate which partially offsets the steric hindrance to hydrogen atom abstraction from the secondary alkyl group. The data indicate that (i) the chiral alkyl group has little affect on the rate of microsomal demethylation and (ii) the (*R*)-alkyl group is removed between 1.5–2 times more rapidly than the (*S*)-alkyl group. Clearly, little stereoselectivity is expressed in the conformationally mobile system in **9**.

Oxidation of the conformationally rigid system **10** by TPPFe/Bu^{t}OOH gave rise to three compounds: *N*-methyl-4-oxo-4-phenylbutanamide **38** (the product of ring hydroxylation), 5-phenyl-2-pyrrolidone **39** (the product of demethylation), and 4-oxo-4-phenylbutanamide **40** (the product of both ring hydroxylation and demethylation) (Scheme 5). The relative product yields for the formation of these products are given in Table 7. Both **38** and **39** independently gave the secondary product **40**; the initial rates for the formation of **40** from **38** and **39** contained in Table 9 reveal that the major pathway to **40** from **10** is *via* the *N*-demethylated product **39**. This is consistent with the AM1 data in Table 1 which show that the ring radical **42** is thermodynamically more stable than the methylene radical **41**. Although the rate ratio for the formation of **40** from **38** and **39** varies with substrate concentration, the formation of **40** during oxidation of **10** is small. Therefore, without introducing significant error we can assume that the product **40** can be used



Scheme 5 The products of oxidative dealkylation of **10**.



together with **39** as a measure of demethylation, and the data in Table 7 show that ring oxidation (dealkylation) is preferred to demethylation.

Microsomal metabolism of (*R*)-**10** and (*S*)-**10** gave identical products to the TPPFe/Bu^{t}OOH reaction. Initial rates for the formation of **38**–**40** are contained in Table 10. The secondary product **40** is formed from both **38** and **39**, but, in contrast to the TPPFe/Bu^{t}OOH reaction, the major route to **40** is *via* **38** (Table 11). Partitioning the data in Table 10 for the formation of **40** according to the average ratios in Table 11 and fitting the resultant data to the Michaelis–Menten equation gives rise to the values of V_{max} and V_{max}/K_m contained in Table 6. Several features are worthy of note. First, for either enantiomer ring oxidation is preferred to demethylation, as observed for the acyclic system **9**. Again, the radical-stabilising effect of the phenyl group must counteract steric hindrance to hydrogen atom abstraction. Second, there is no stereochemical effect of the chiral *N*-alkyl group upon the *N*-demethylation process. Third, ring oxidation of the (*S*)-enantiomer is the more rapid by a factor of between 1.8 and 3.6. Thus, even with the conformationally rigid amide **10** the stereoselectivity of the microsomal dealkylation is modest. This implies that the phenobarbital-induced P450 isoenzymes have low stereochemical requirements of the substrate, more especially so

Table 5 Initial rates for the microsomal oxidation of lactams **6**–**8** at 37 °C

	Initial rate[a]/ mM h^{-1} (nmol P450)$^{-1}$								
[Lactam]/mM	**6** → **22**	**6** → **23**	**6** → **24**	**7** → **25**	**7** → **26**	**7** → **27**	**8** → **28**	**8** → **29**	**8** → **30**
0.05	0.119	0.10	0.025	0.006	0.121	0.0003	0.003	0.05	0.00004
0.5	0.927	0.982	0.21	0.059	1.06	0.003	0.021	0.506	0.0004
3.0	5.76	5.43	1.15	0.312	6.36	0.016	0.118	3.40	0.003
5.0	9.02	8.51	1.97	0.476	7.40	0.026	0.19	5.10	0.004
10.0	13.0	14.8	3.85	0.786	10.3	0.041	0.389	11.3	0.009

[a] Values are the average of 3 determinations and are reproducible to ±8%.

Table 6 Values of V_{max} and V_{max}/K_m at pH 7.4 and 37 °C for the microsomal oxidation of amides **6**–**10**

	Ring oxidation or		Demethylation		Ring oxidation or dealkylation : demethylation[a]		Demethylation preference[b]	
	V_{max}[c]	V_{max}/K_m[d]	V_{max}[c]	V_{max}/K_m[d]	V_{max}	V_{max}/K_m	V_{max}	V_{max}/K_m
6	47.7	2.43	62.8	2.06	1.14	1.8	10.5	6.7
7	2.4	0.13	14.9	3.25	0.24	0.06	4.1×10^4	1.7×10^5
8	1.71	42.8	40.1	1140	0.043	0.038	1.8×10^4	1.8×10^4
(*R*)-**9**	0.205	0.07	0.309	0.122	2	1.7	6.8	8
(*S*)-**9**	0.144	0.034	0.296	0.13	1.5	0.8	9	16.4
(*R*)-**10**	3.21	0.55	2.18	0.72	4.4	2.3	1.4	2.6
(*S*)-**10**	5.88	1.97	1.88	0.75	9.4	7.9	0.6	1.3

[a] Statistically corrected. [b] Calculated by dividing the ring oxidation : demethylation ratio for the TPPFe/Bu^{t}OOH reaction by the ratio for the microsomal reaction. [c] In mM h^{-1} (nmol P450)$^{-1}$. [d] In h^{-1} (nmol P450)$^{-1}$.

Table 7 Relative product yields for the oxidative dealkylation of **9** and **10** by TPPFe/Bu^{t}OOH in CH_2Cl_2 at 30 °C

	Relative yield[a,b]				Relative yield[a,b]			
[**9** or **10**]/mM	**35**	**36**	**37**	Dealkyl^n : Demethyl^n[c]	**38**	**39**	**40**	Dealkyl^n : Demethyl^n
3.0	4.0	1	4.0	12	2.3	1	0.04	6.6
5.0	4.5	1	4.5	13.5	2.1	1	0.04	6.0
10.0	5.1	1	5.1	15.3	1.9	1	0.05	5.4

[a] Average of 3 determinations ±5%. [b] Identical values for both enantiomers. [c] Statistically corrected.

Table 8 Initial rates for the microsomal dealkylation of (*R*)-**9** and (*S*)-**9** at pH 7.4 and 37 °C

	Initial rate[a]/μM h^{-1} (nmol P450)$^{-1}$					
	From (*R*)-**9**			From (*S*)-**9**		
[**9**]/mM	**35**	**36**	**37**	**35**	**36**	**37**
0.5	30.0	51.0	33.0	15.2	53.0	14.1
1.0	52.2	87.5	50.2	27.5	90.2	29.0
3.0	104	168	106	59.6	168	58.0
5.0	129	205	131	77.7	203	79.0
10.0	159	247	160	101	241	103

[a] The average of three experiments (±10%).

Table 9 Initial rates for the formation of compound **40** from **38** and **39** by reaction with TPPFe/Bu^{t}OOH in CH_2Cl at 30 °C

	Initial rate[a]/mM h^{-1} (mmol TPPFe)$^{-1}$		
[**38** or **39**]/mM	From **38**	From **39**	Rate ratio
0.5	0.06	2.76	46
3.0	0.31	6.71	21.6
5.0	0.47	7.58	16.1
10.0	0.76	8.39	11

[a] Average of three experiments (±5%).

given that the enantiomers of **9** and **10** that are most rapidly dealkylated have opposite configurations.

Experimental

NMR spectra were recorded in $CDCl_3$ solutions using JEOL JNM-EX 400 and FX90Q spectrometers; chemical shifts are given in ppm relative to Me_4Si and *J* values are given in Hz. Mass spectra were recorded using a VG Mass Lab 25–250 spectrometer; GCMS employed a Hewlett-Packard 5809-A chromatograph equipped with a 20 m × 0.5 mm id BP5 column connected to the spectrometer. Infrared spectra were recorded using a Nicolet 205 FTIR spectrometer. Gas chromatography was performed using direct on-column injection into a Perkin-Elmer 8410 chromatograph equipped with a 20 m × 0.5 mm id BP1 (0.1 μm film thickness) aluminium column and FID detection at 250 °C. The injection temperature was 210 °C. HPLC was performed using a Varian 5000 instrument equipped with a Jones 25 cm × 5 mm C-18 column, a Cecil 2112 variable wavelength detector and a Waters integrator. Diode array HPLC was performed using a Varian 9060 polychrom detector. Elemental analyses were obtained from Medac Ltd., Brunel Science Centre, Englefield Green, UK TW20 0JZ.

Substrates and products

N-Methyl-2-pyrrolidone **6**, *N*-methyl-2-piperidone **7**, *N*-methyl-ε-caprolactam **8**, *N*-methylsuccinimide **22**, 2-pyrrolidone **23**,

succinimide **24**, 2-piperidone **26**, glutarimide **27**, ε-caprolactam **29**, *N*-methylbenzamide **35**, acetophenone **37**, glutaric anhydride, adipamide, adipoyl chloride, pyroglutamic acid, (*S*)-(−)-1-phenylethylisocyanate, 3-benzoylpropionic acid, (*R*)-(+)- and (*S*)-(−)-1-phenylethylamine, tetraphenylporphyrinatoiron(III) chloride, *tert*-butyl hydroperoxide, $NADP^+$, glucose-6-phosphate and glucose-6-phosphate dehydrogenase were obtained commercially. *N*-Ethyl-3,4-dihydroisoquinolin-1(2*H*)-one **5**,[25] (*S*)-*N*-methyl-*N*-(1-phenylethyl)benzamide (*S*)-**9**,[26] *N*-ethylisoquinolin-1(2*H*)-one **11**,[25,27] 3,4-dihydroisoquinolin-1(2*H*)-one,[28] *N*-ethylisoquinoline-1(2*H*),3(4*H*)-dione,[29] *N*-methylglutarimide **25**,[30] *N*-methyladipimide **28**,[31] adipimide **30**,[32] (*R*)- and (*S*)-*N*-(1-phenylethyl)benzamides (*R*)-**36** and (*S*)-**36**,[33,34] *N*-methyl-4-oxo-4-phenylbutanamide **38**,[35] (*R*,*S*)-5-phenyl-2-pyrrolidone (*R*,*S*)-**39**,[36] and 4-oxo-4-phenylbutanamide **40**[37,38] are known compounds, were synthesised by literature methods and were analytically (C, H, N ± 0.3%) and chromatographically (GC and/or HPLC) pure.

***N*-Ethyl-3,4-dihydro-3-hydroxyisoquinolin-1(2*H*)-one 12.** Sodium borohydride (80 mg) was added to an ice-cold solution of *N*-ethyl-4*H*-isoquinoline-1,3-dione (0.4 g) in chloroform–methanol (1 : 1 v/v, 20 cm^3).[39] After 2 h, aqueous 2 M ammonium chloride (50 cm^3) was added and the solution extracted with dichloromethane (3 × 20 cm^3). The combined organic extracts were dried (Na_2SO_4), evaporated and the resultant solid recrystallised from diethyl ether–dichloromethane: mp 115–117 °C; ν_{max}/cm^{-1} 3333, 1635; δ_H 1.28 (3H, t, *J* 7.5, CH_3), 3.07 (1H, d, *J* 16.5, 4-*H*), 3.31 (1H, dd, *J* 3.3, 16.5, 4-*H*), 3.05–3.5 (1H, br s, O*H*), 3.57 (1H, dq, *J* 7.5, 15, *N*-CH), 3.93 (1H, dq, *J* 7.5, 15, *N*-CH), 5.19 (1H, s, 3-*H*), 7.11 (1H, d, *J* 7.5, 5-*H*), 7.33 (1H, t, *J* 7.5, 7-*H*), 7.43 (1H, t, *J* 7.5, 6-*H*), 8.01 (1H, d, *J* 7.5, 8-*H*); *m*/*z* (%) 191 (M^+, 32), 173 ($M^+ - H_2O$, 89), 145 ($M^+ - H_2O - C_2H_4$, 100), 128 (74), 118 (87). $C_{11}H_{13}NO_2$ requires: C, 69.1; H, 6.9; N, 7.3%. Found: C, 69.2; H, 6.8; N, 7.2%.

(*R*)-*N*-Methyl-*N*-(1-phenylethyl)benzamide (*R*)-9. Butyllithium (15 mmol) was added under nitrogen to (*R*)-*N*-(1-phenylethyl)benzamide (13 mmol, 2.92 g) dissolved in THF (30 cm^3). After 10 min, iodomethane (100 mmol) in THF (5 cm^3) was added. After 45 min, ethanol (5 cm^3) was added, the solvent removed and the residue dissolved in ethyl acetate (30 cm^3). The organic phase was washed with water (2 × 10 cm^3), dried ($MgSO_4$) and concentrated to give a crude product that was purified by column chromatography (ethyl acetate–*n*-hexane 3 : 1) on silica gel: ν_{max}/cm^{-1} 3090, 3060, 1640; δ_H 1.52 (3H, d, *J* 7, C-Me), 2.61 (3H, s, *N*-Me), 5.4 (1H, q, *J* 7, α-C*H*), 7.05 (5H, s, Ar), 7.15 (5H, s, Ar); *m*/*z* 239 ($M^{+\bullet}$, 30), 224 ($M^{+\bullet}$ − Me, 10), 105 ($PhCO^+$, 100), 77 (Ph^+, 45). $C_{16}H_{17}NO$ requires: C, 80.3; H, 7.1; N, 5.85%. Found: C, 80.1; H, 7.2; N, 5.7%.

(*R*)- and (*S*)-*N*-Methyl-5-phenyl-2-pyrrolidone (*R*)-10 and (*S*)-10. (*R*)- or (*S*)-5-Phenyl-2-pyrrolidone, (*R*)- or (*S*)-**39** (3 mmol, 0.48 g) was added to a suspension of sodium hydride (3.3 mmol, 0.079 g) in THF (50 cm^3). Iodomethane (15 mmol, 2.13 g) was added and the reaction left at room temperature for 48 h. The solvent was removed, the residue resuspended in ethyl acetate (20 cm^3), washed with water (2 × 10 cm^3) and dried ($MgSO_4$). The organic layer was concentrated and the residue purified by column chromatography (ethyl acetate–hexane 3 : 1) on silica gel. Both enantiomers were oils: ν_{max}/cm^{-1} 1720, 1660, 1580; δ_H 2.76 (4H, m, 3-H_2 and 4-H_2), 3.2 (3H, s, *N*-Me), 4.6 (1H, t, *J* 7, 5-H), 7.05 (5H, s, Ph); *m*/*z* 175 ($M^{+\bullet}$, 70), 145 (50), 117 ($M^{+\bullet}$ − MeNCO, 60), 98 ($M^{+\bullet}$ − Ph, 40), 77 (Ph^+, 40). $C_{11}H_{13}NO$ requires: C, 75.4; H, 7.4; N, 8.0%. Found for (*R*)-**10**: C, 75.8; H, 7.65; N, 8.3% and for (*S*)-**10**: C, 75.6; H, 7.8; N, 8.2%.

(*R*)- and (*S*)-5-Phenyl-2-pyrrolidone (*R*)-39 and (*S*)-39. A solution of racemic 5-phenyl-2-pyrrolidone (10.1 mmol, 1.7 g) and (*S*)-1-phenylethyl isocyanate (11 mmol, 1.62 g) in toluene (10 cm^3) was refluxed for 24 h. After cooling the reaction mixture was washed with water (2 × 10 cm^3) dried ($MgSO_4$) and concentrated. The mixture of diastereomeric *N*-{1-[(*S*)-phenylethyl]carbamoyl}-5-phenyl-2-pyrrolidones was separated by chromatography on silica gel.[40] We found that DCM–acetonitrile (93 : 3) as eluant gave a better separation but the same order of elution as DCM–hexane (1 : 1). The initially eluting diastereoisomer, mp 99–100 °C, has the (5*S*)-configuration, and the lower R_f-diastereoisomer, a colourless oil, has the (5*R*)-configuration.[40] Hydrolysis of the individual *N*-carbamoylpyrrolidone diastereoisomers was achieved following the literature procedure,[40] and the desired (*R*)-**39** and (*S*)-**39** were obtained after recrystallisation from diethyl ether–ethanol (1 : 1). Analysis using the NMR chiral resolving agent (*S*)-2,2,2-trifluoro-1-(9-anthryl)ethanol revealed that each enantiomer was >99% stereochemically pure. $C_{10}H_{11}NO$ requires: C, 74.5; H, 6.8; N, 8.7%. Found for (5*R*)-**39**: C, 74.5; H, 7.0; N, 8.55%; for (5*S*)-**39**: C, 74.45; H, 6.9; N, 8.4%.

Table 10 Initial rates for the microsomal metabolism of (*R*)-**10** and (*S*)-**10** at pH 7.4 and 37 °C

	Initial rate[a]/μM h^{-1} (nmol P450)$^{-1}$					
	From (*R*)-**10**			From (*S*)-**10**		
[**10**]/mM	**38**	**39**	**40**	**38**	**39**	**40**
0.5	161	301	97	598	254	282
1.0	313	529	165	1106	541	426
3.0	806	1065	300	2389	918	645
5.0	1144	1334	357	3058	1153	720
10.0	1636	1648	414	3843	1425	788

[a] The average of three experiments (±10%).

Chemical oxidation

A solution TPPFe (2 mM) in dry dichloromethane (2.5 cm^3) was kept at a constant temperature of 30 °C. *tert*-Butyl hydroperoxide (225 mg) was added and the solution left for 10 min prior to the addition of the appropriate quantity of substrate. Initial substrate concentrations were between 0.5 and 10 mM. Aliquots (100 μl) of the reaction mixture were withdrawn

Table 11 Initial rates for the formation of **40** from **38** and **39** by phenobarbital induced rat liver microsomes at pH 7.4 and 37 °C

	Initial rate[a]/μM h^{-1} (nmol P450) $^{-1}$				
[**38** or **39**]/mM	From **38**	From (*R*)-**39**	From (*S*)-**39**	**38**/(*R*)-**39**	**11**/(*S*)-**39**
0.5	164	9.8	21.8	16.7	7.5
1.0	288	17.6	39	16.4	7.4
5.0	736	48	105	15.4	7.0
10.0	914	61	134	15.0	6.8

[a] Average of 3 experiments (±10%).

at timed intervals and added to ethanol (200 μl) to terminate the reaction. 1 M Methanolic KOH (200 μl) was added the solutions were left for 10 min then acidifed with 1 M methanolic HCl (200 μl). The samples were evaporated to dryness and resuspended in acetonitrile for GC analysis or methanol–water (1 : 1) for HPLC analysis (see below).

Microsomal incubations

Liver microsomes from phenobarbital-induced rats were obtained from the Institute of Cancer Research, Sutton, Surrey, UK. The P450 content of these microsomes was 1.95 nmol mg^{-1} microsomal protein. Incubations with these microsomes were performed in 0.1 M pH 7.4 phosphate buffer using microsomal concentrations of 1.5 mg protein cm^{-3} in the presence of an NADPH generating system comprising $NADP^+$ (1.25 mmol cm^{-3}), $MgCl_2$ (6 mmol cm^{-3}), glucose-6-phosphate (6.25 nmol cm^{-3}) and of glucose-6-phosphate dehydrogenase (2.5 units cm^{-3}). The incubations were maintained at 37 °C and reactions were initiated by addition of substrate (to give a substrate concentration in the range 0.5–10 mM). Aliquots (100 μl) were withdrawn at timed intervals and added to a 10% (w/v) solution of trichloroacetic acid (200 μl) to precipitate protein. After centrifugation (4500 rpm, 5 min) the supernatant was evaporated to dryness and analysed for products as described below. Only products of *N*-dealkylation or ring oxidation adjacent to the nitrogen atom were detected. No evidence for the competing formation of phenols (from oxidation of the aromatic groups) or of ring oxidation at positions other than next to the amide nitrogen atom was obtained. For the kinetic reactions the supernatant was treated with 1 M sodium hydroxide (200 μl) for 10 min, acidified with 1 M HCl (200 μl) for 10 min, evaporated to dryness then analysed as described below.

Analytical methods

The GC analysis of **6**–**8** and **22**, **25** and **28** employed direct injection of the sample under isothermal conditions at 90 °C. Analysis of **23**, **24**, **26**, **27**, **29** and **30** required derivatisation of the sample as follows. The dried sample was dissolved in acetonitrile (100 μl); a portion (50 μl) of this solution was added to *N*-*tert*-butyldimethylsilyl-*N*-methyltrifluoracetamide (50 μl), the mixture sonicated for 5 min then heated at 100 °C for 2 h. After cooling, the sample was analysed using a thermal gradient: 2 min at 90 °C, 90 to 140 °C at 5 °C min^{-1}, 140 °C for 1 min.

Reactions involving compound **5**, **9** and **10** were analysed by HPLC. A gradient comprising two eluants, A and B, was employed: eluant A, 5% acetonitrile in 0.05 M pH 2 phosphate buffer; eluant B, 60% acetonitrile in 0.05 M pH 2 phosphate buffer. The gradient was as follows: 0–15 min, 60% B to 100% B; 15–18 min, 100% B; 18–19 min, 100% B to 60% B. The flow rate was 1 cm^3 min^{-1}; detection wavelength 254 nm.

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