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Primidone oxidation catalyzed by metalloporphyrins and Jacobsen catalyst

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ABSTRACT

Primidone (PRM) oxidation by various oxidants such as iodosylbenzene (PhIO), *tert*-butyl hydroperoxide 70 wt.% (*t*-BOOH), 3-chloroperoxybenzoic acid (*m*-CPBA) and hydrogen peroxide 30 wt.%, mediated by either a salen complex or metalloporphyrins, was investigated. The catalytic systems led to phenylethylmalondiamide (PEMA) and phenobarbital (FENO), the same metabolites obtained *in vivo* with P450 enzymes, although three other products were also detected. Product formation was highly dependent on the oxidant, co-catalyst (imidazole), pH and dioxygen. These biomimetic chemical models have potential application in the synthesis of drug metabolites, which should provide samples for pharmacological tests. They can also be employed in studies that pursue the elucidation of *in vivo* drug metabolism.

1. Introduction

Metabolic processes aim at converting drugs into less biologically active intermediates. After being absorbed, xenobiotics spread through the organism, where they either exert some sort of action or become the target of metabolic mechanisms. These mechanisms can make drugs less harmful; however, they can also bioactivate xenobiotics, leading to toxicity. The biotransformation of drugs comprises two phases. Phase I promotes changes to the original shape of the molecule, as in the case of the addition of a functional group, which includes oxidation, hydrolysis and reduction reactions. Phase I enables the occurrence of Phase II, which consists of a conjugation step [1–3]. The objective of Phases I and II is to render the compound more polar, which should facilitate its elimination from the body.

The cytochrome P450 monoxygenases are among the various enzymatic systems responsible for metabolic processes. The P450 enzymes belong to a superfamily of hemeproteins that are ubiquitous in all living organisms, and they are involved in the metabolism of a wide variety of chemical compounds [4–7].

In the presence of oxygen donors, synthetic metalloporphyrins are known to mimic the various reactions of cytochrome P450 enzymes, such as the oxidation and oxygenation of various drugs and biologically active compounds. Some examples include carbamazepine [8–10], lidocaine, odapipam, aminopyrine [11], acetaminophen [12,13], chloroquine [14], among others [15,16]. Recent reviews by Bernadou and Meunier [17], and Mansuy [4] have compiled most of these studies.

Salen complexes have attracted researchers' attention as promising catalysts for the selective oxidation of organic molecules, which should lead to the preparation of novel compounds in both chemical and pharmaceutical industries [18–24]. These catalysts can also form complexes that mimic metalloporphyrin chemistry, so they can be employed to mimic the action of cytochrome P450 in drug metabolism [25,26]. Because reports on the use of salen complexes for drug oxidation are rare in the literature, this has been the focus of our research. In a previous work, we reported the salen-catalyzed selective oxidation of the antiseizure drug carbamazepine to its main *in vivo* metabolite, carbamazepine 10,11-epoxide [26].

Primidone (PRM, 5-ethylhexahydro-4,6-dioxo-5-phenylpyrimidine, Fig. 1) is an antiseizure drug that has been in use since the early 1950s. It is efficient for the treatment of partial and tonic–clonic seizures, although it also displays sedative action. PRM is converted into phenylethylmalondiamide (PEMA) and phenobarbital (FENO) by cytochrome P450 (CPY2C9/19) [27].

PEMA and FENO have pharmacological actions similar to those of the original compound, so these active metabolites maintain the therapeutic effect of PRM even after it has been metabolized. The synthesis of PEMA and FENO is of great importance because in many cases FENO is employed as a substitute for PRM [28,29]. Toxicity has been attributed to the metabolites generated from

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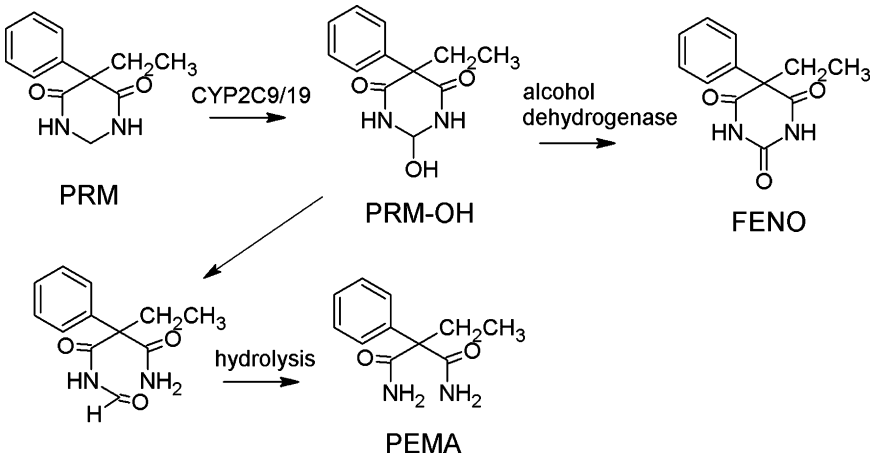

Fig. 1. *In vivo* metabolism of primidone. PRM: primidone; PRM-OH: 2-hydroxyprimidone; FENO: phenobarbital; PEMA: phenylethylmalondiamide.

PRM. Studies on FENO have demonstrated its carcinogenic potential in rats [30], and the mitogenic activity of PEMA has been demonstrated in Salmonella [31].

This work reports on the oxidation of PRM in homogeneous medium by various oxidants, namely iodosylbenzene (PhIO), hydrogen peroxide, *tert*-butyl hydroperoxide (*t*-BOOH) and 3-chloroperoxybenzoic acid (*m*-CPBA), catalyzed by either a metalloporphyrin or the salen complex known as Jacobsen catalyst. These studies employing P450 models may help understand the mechanisms involved in xenobiotics degradation since they provide information about reaction selectivity and aid the characterization of potentially reactive and toxic metabolites.

2. Experimental

2.1. Materials and equipment

PRM and FENO were purchased from Sigma–Aldrich Chemical Co. The Jacobsen catalyst Mn(salen) was purchased from Acros Oganics. The porphyrins 5,10,15,20-tetrakis-(pentafluorophenyl) porphyrin, H_2(TFPP), and 5,10,15,20-tetrakis(4-carboxyphenyl) porphyrin, H_2(TCPP), were acquired from Mid-Century. Iron and manganese insertions into these free base-porphyrins were carried out using the method of Adler et al. [32]. Fig. 2 shows the structures of the metalloporphyrins and the salen complex used

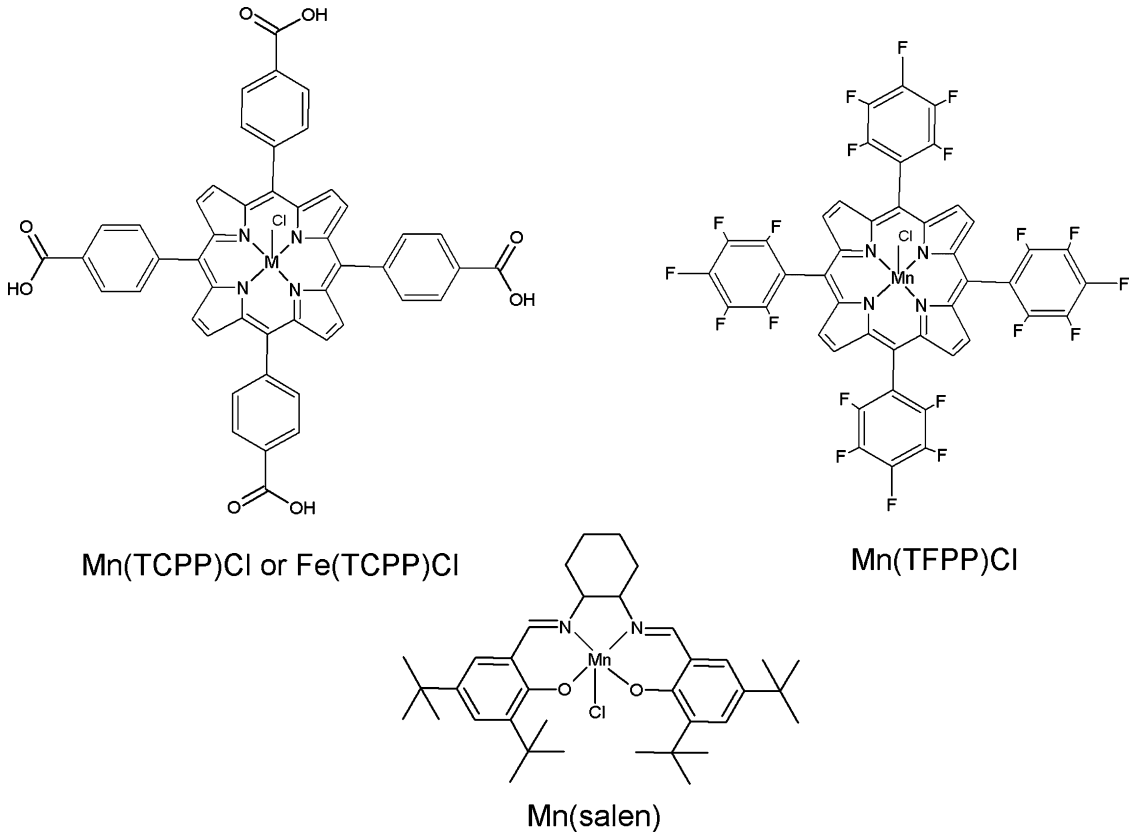

Fig. 2. Metallopophyrins and the salen complex used in this study (M = Fe^{III} or Mn^{III}). M(TCPP)Cl: 5,10,15,20-tetrakis(4-carboxyphenyl)porphyrin metal (III) chloride (metal: Fe or Mn); Mn(TFPP)Cl: 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin manganese (III) chloride; Mn(salen): Jacobsen catalyst or (R,R)-(−)-N,N-bis(3,5-di-*tert*-butylsalicylidene)-1,2-cyclohexanediamine manganese(III) chloride.

in this study. *tert*-Butyl hydroperoxide, 70 wt.% solution in water, and 3-chloroperoxybenzoic acid were acquired from Acros Oganics. Hydrogen peroxide (30% in water) was supplied by Fluka and stored at 5 °C, and it was periodically titrated to confirm its purity. PhIO was obtained through iodosylbenzenediacetate hydrolysis [33], and its purity was measured by iodometric assay [33]. Acetonitrile (ACN) HPLC grade was purchased from Mallinckrodt. Water used in the experiments was purified by a Milli-Q, Millipore System. Imidazole was acquired from Acros Oganics.

The HPLC analyses were performed on a SHIMADZU liquid chromatograph equipped with an LC-10AS solvent pump, an SPD-M 10A VP spectrophotometric detector coupled to a CTO-10A VP column oven, and an SCL-10A VP system controller. Separation of PRM and the oxidation products was carried out in a Lichrospher 100RP-18 column. GC–MS was performed using a QP2010 mass spectrometer (Shimadzu) fitted with a GC17A gas chromatograph (Shimadzu). The ionization voltage was 70 eV. Gas chromatography was conducted in the temperature-programming mode, using a DB-5MS column (30 m × 0.25 mm × 0.25 μm). Reaction products were identified by comparison of their retention times with known reference compounds, and by comparing their mass spectra to fragmentation patterns obtained from the NIST spectral library stored in the computer software (version 1.10 beta, Shimadzu) of the mass spectrometer.

2.2. Oxidation reactions

Reactions were carried out in a 3 mL vial containing a screw cap. Briefly, PRM (4.6 mg, 2.1×10^{-5} mol) and the oxidant (PhIO, *m*-CPBA, *t*-BOOH or H_2O_2, 2.4×10^{-5} mol) in acetonitrile (2 mL) were added to a vial containing the Jacobsen catalyst or the metalloporphyrins (6.0×10^{-7} mol) in acetonitrile (non-ionic catalyst) or aqueous medium (anionic catalyst). Reactions were carried out for 6 h, under magnetic stirring at room temperature, at a catalyst/oxidant/drug molar ratio of 1:40:35, which was the standard condition in our studies. Another catalyst/oxidant/drug molar ratio was also used, namely 1:150:30 (excess oxidant conditions). Imidazole was added to some reactions, at a catalyst/axial ligand molar ratio of 1:10.

The pH effect was investigated in buffered aqueous solution and acetonitrile 1:1 (2 mL). Reactions at pH 2 were performed in phosphate buffer (H_3PO_4/NaH_2PO_4, 0.1 mol L^{-1}); at pH 4 in acetate buffer (0.1 mol L^{-1}); at pH 6-8 in phosphate buffer (NaH_2PO_4/Na_2HPO_4, 0.1 mol L^{-1}); at pH 10 in ammonium buffer (NH_4Cl/NH_4OH). The pH of the reaction solution was adjusted by adding either HCl (0.5 mol L^{-1}) or NaOH (0.5 mol L^{-1}) solutions whenever necessary.

At the end of the reaction, magnetic stirring was interrupted, and an aliquot of the reaction mixture (50 μL) was withdrawn. After Mn(salen) or metalloporphyrin extraction, this aliquot was analyzed by high-performance liquid chromatography (HPLC). Mn(salen) and metalloporphyrin extraction was carried out by addition of hexane (500 μL) and a mobile phase (500 μL). The mixture was vortex-mixed and centrifuged; the aqueous phase (mobile phase) was then injected into the chromatographic system. This clean-up procedure did not remove unreacted PRM or the oxidation products.

The oxidation products were identified by comparing their retention times with those of authentic PEMA and FENO standards. Yields are based on the added drug and were determined by a calibration curve. Other minor products were identified by mass spectrometry.

Control reactions were carried out in the absence of catalyst, under the same conditions as the catalytic runs. No products were detected.

3. Results and discussion

Primidone oxidation reactions were carried out in the presence of Mn(salen) or the metalloporphyrins Mn(TFPP)Cl, Mn(TCPP)Cl and Fe(TCPP)Cl (Fig. 2), which are commercially available second-generation metalloporphyrins well-established in the literature as good catalysts for hydrocarbon and drug oxidation [4,8–10,17]. The reactions were carried out in ACN, taking the solubility of the drug and its metabolites in this solvent into account. Solubility was also a determining factor for the choice of reaction conditions, once the studied substrate was a solid with limited solubility in the volume employed in the reactions (1.5 mL).

PhIO was firstly used as an oxygen donor because it is considered as a standard and simple oxidant which contains only one oxygen atom and is well-adapted for the selective and clean formation of metal-oxo intermediates [4].

The oxidation of PRM by PhIO led to the preferential formation of FENO (19%); only traces of PEMA and other unknown products were detected. FENO formation occurs via oxidation of the methylene group situated between two nitrogen atoms. The oxidation of this group takes place via an intermediate secondary alcohol *in vivo* [34]. This same intermediate can be postulated for the studied system, which would favor the cleavage of the pyrimidinic ring, with oxidative decarboxylation of the carbon located between the nitrogen atoms, thus generating PEMA in the same way as in the *in vivo* reactions (Fig. 1) [34].

m-CPBA, H_2O_2 and *t*-BOOH were also used as oxidants. These compounds may undergo heterolytic cleavage of the non-symmetrical O–O bond upon coordination to the salen or metalloporphyrin central metal ion, leading to the formation of the active species Mn^{V}(O)salen or Mn^{IV}(O)porp$^{\bullet+}$. The situation can be also more complex because Mn(salen), like many transition metal complexes, can promote the homolytic cleavage of the O–O bond, leading to the formation of a less reactive intermediate, Mn^{IV}(OH)salen, as well as RO$^{\bullet}$ radicals, thus favoring the occurrence of radicalar mechanisms [4]. Since these intermediate species have different reactivities, the product yields obtained with these peroxides can account for the main mechanism the PRM oxidation goes through.

Table 1 shows the results obtained in the oxidation of PRM by *m*-CPBA, *t*-BOOH or H_2O_2 catalyzed by the Jacobsen catalyst, after 6 h of reaction.

Contrary to what had been observed when PhIO was the oxidant, *m*-CPBA, *t*-BOOH and H_2O_2 generated PEMA in yields as high as 31%, as well as small quantities of FENO (Table 1). Besides FENO and PEMA, three other unknown products were also detected in the PRM oxidation by these peroxides, which were designated PD 1, PD 2 and PD 3, according to their order of elution, being PD1 the second most abundant (Table 1). The unknown products were isolated and analyzed by mass spectrometry (GC–MS). The mass

Table 1
Results obtained for primidone oxidation reactions catalyzed by Mn(salen) with different oxidants (*m*-CPBA, *t*-BOOH, H_2O_2).

Entry	Product	*R* (%)		
		m-CPBA	*t*-BOOH	H_2O_2
1	PEMA	31	23	27
2	FENO	2	9	5
3	PD 1	12	14	14
4	PD 2	Trace	Trace	Trace
5	PD 3	Trace	Trace	Trace

Catalyst/substrate/oxidant molar ratio = 1:40:35; blank reactions (absence of catalyst): no products detected; reaction time = 6 h; yield *R* (%) based on added substrate; PD 1, PD 2 and PD 3 are unknown products and the yields for these products were calculated by estimating the average response factor for known products.

Table 2
Main fragments of the products PD 1 (PBA) and PD 2 (BL) generated during the oxidation of primidone as determined by GC–MS.

Compound	Molecular ion	Fragments (*m*/*z*)
PD 1 (PBA) (2-phenylbutyramide)	163	163 (17%), 148 (7%), 135 (24%), 119 (20%), 91 (100%), 78 (12%), 65 (10%)
PD 2 (BL) (α-phenyl-γ-butyrolactone)	162	162 (100%), 133 (10%), 117 (69%), 107 (98%), 91 (33%), 77 (72%), 56 (100%), 51 (52%)

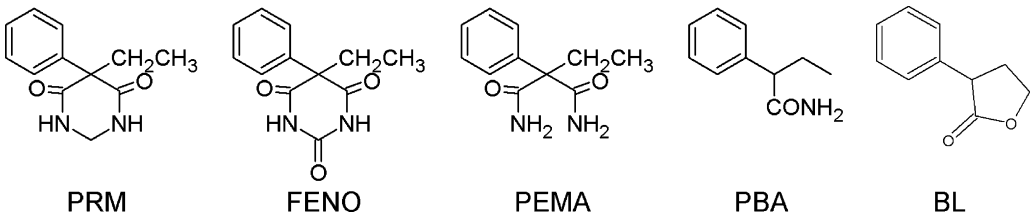

Fig. 3. Chemical structure of primidone and the metabolites identified in this work. PRM: primidone; FENO: Phenobarbital; PEMA: phenylethylmalondiamide; PBA: 2-phenylbutyramide; BL: α-phenyl-γ-butyrolactone.

spectrum of PD1 displayed an *m*/*z* signal at 163 and a fragmentation pattern corresponding to 2-phenylbutyramide (PBA) (Table 2 and Fig. 3). This product had already been identified by Foltz et al. as a minor PRM metabolite [35]. PD 2 displayed a peak at *m*/*z* 162 in the mass spectrum and a fragmentation pattern coincident with that of α-phenyl-γ-butyrolactone (BL) (Table 2 and Fig. 3), which had also been detected in patients with toxicity to PRM [36,37]. It was not possible to identify PD 3, since it was formed in amounts lower than the detection limit of the mass spectrometer.

To investigate whether any of these products are formed through radicalar mechanisms, the catalytic activity of the Mn(salen)/*t*-BOOH system was evaluated in the absence of O_2 (under argon atmosphere). Systems containing low dioxygen concentration should lead to a decrease in the amount of products generated from parallel radicalar reactions and an increase in the yields of products formed via the metal-oxo intermediate. *t*-BOOH was chosen as the standard oxidant for these studies because it tends to undergo homolytic cleavage of the peroxide O–O bond when it coordinates to the salen metal center. This homolytic cleavage is favored by the *t*-butyl groups, which donate electrons via an inductive effect, thus generating radicalar products [23].

There were no differences in the total yields obtained in reactions carried out in air or argon atmospheres. However, the product distribution depicted in Fig. 4 shows there was an increase in the yields of PEMA and a decrease in the amount of PBA for reactions in absence of O_2. These results indicate that PBA is formed via a radicalar mechanism, initiated by the homolytic cleavage of the peroxide O–O bond and propagated by the O_2 present in the reaction medium.

Table 3
Total product yield obtained for primidone oxidation reactions catalyzed by Mn(salen) in the standard conditions (1) and in excess oxidant (2).

Entry	Oxidant	*R* (%)[a]
1	*t*-BOOH[b]	45
2	*t*-BOOH[c]	58

[a] Yield *R* (%) based on added substrate.
[b] Catalyst/substrate/oxidant molar ratio = 1:40:35.
[c] Catalyst/substrate/oxidant molar ratio = 1:150:30; blank reactions (absence of catalyst): no products detected; reaction time = 6 h.

Table 3 depicts the total yields (*R* (%)) obtained in the PRM reactions developed in the presence of excess of *t*-BOOH (catalyst/oxidant/substrate molar ratio = 1:150:30). Fig. 5 shows the relative product distribution, as percentage.

Excess *t*-BOOH resulted not only in higher total yields, as expected, but also in changes in the relative product distribution, as shown in Fig. 5. Excess *t*-BOOH caused an increase in PBA yields and a decrease in PEMA yields. This indicates that there may be additional oxidation of PEMA to PBA, favored by excess oxidant.

The influence of nitrogen bases was also evaluated in PRM oxidation. Nitrogen ligands such as imidazole (Im) act as cocatalysts in systems involving Mn(salen) complexes, leading to a considerable increase in reaction speed, catalytic yields and selectivity [38]. The use of nitrogen ligands has three advantages: (i) they can coordinate to the Mn ion of the Mn(salen) complex in the position *trans*- to the metal-oxo bond, which stabilizes the active intermediate catalytic species Mn^{V}(O)salen [39] responsible for efficient, stereoselective oxidations; (ii) they prevent the fast reaction between Mn^{V}(O)salen

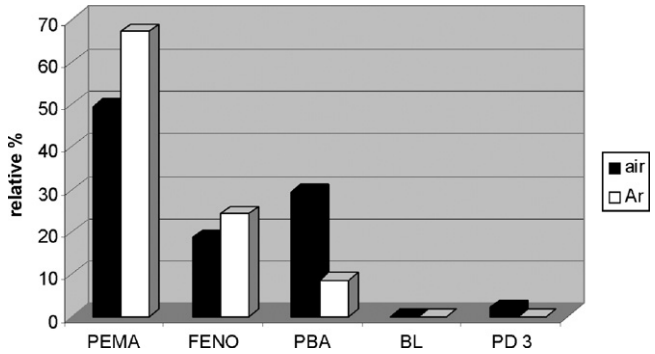

Fig. 4. Product distribution, as percentage, obtained in the Mn(salen)-catalyzed PRM oxidation by *t*-BOOH in ACN in air atmosphere and in the absence of dioxygen (argon atmosphere). PD 3 is an unknown product.

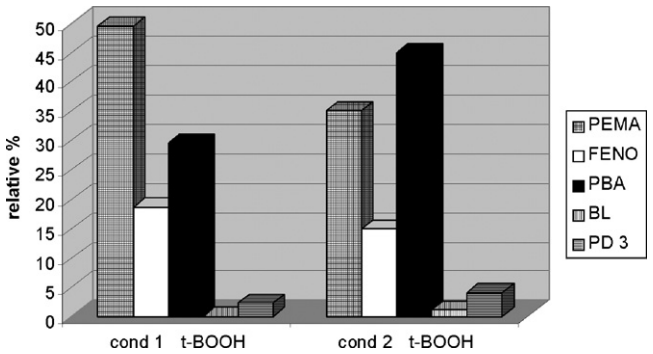

Fig. 5. Product distribution, as percentage, obtained in the Mn(salen)-catalyzed PRM oxidation by *t*-BOOH in ACN, in the standard conditions (cond 1) and in excess oxidant (cond 2). PD 3 is an unknown product.

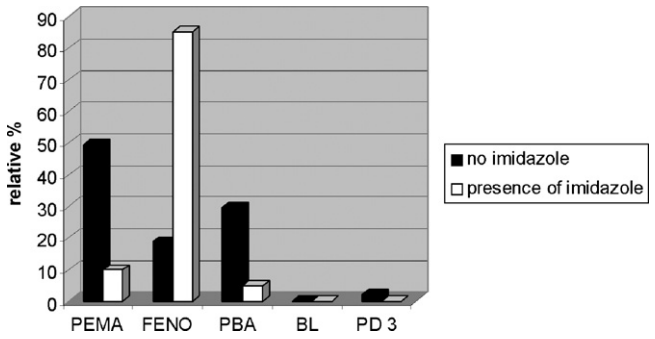

Fig. 6. Product distribution, as percentage, obtained in the Mn(salen)-catalyzed PRM oxidation by *t*-BOOH in ACN in the standard conditions and in the presence of imidazole. PD 3 is an unknown product.

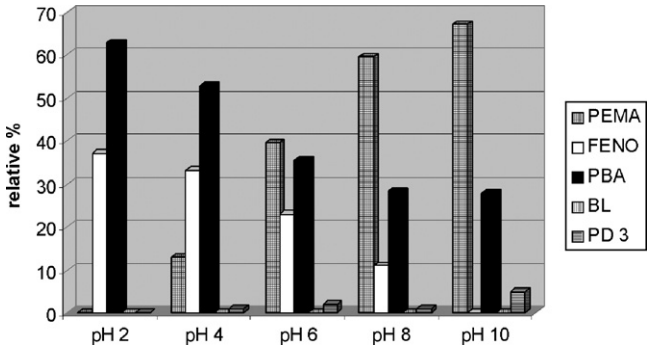

Fig. 7. Product distribution, as percentage, obtained in the Mn(salen)-catalyzed PRM oxidation by *t*-BOOH in buffered aqueous solution/ACN 1:1 at different pH values. PD 3 is an unknown product.

and Mn(salen), which would result in the formation of the intermediate Mn^{IV}(O)salen, responsible for little efficient and poorly selective radicalar reactions [26,40]; (iii) they act as acid–base catalysts, favoring the heterolytic cleavage of the peroxide bond, thus leading to the desired intermediate Mn^{V}(O)salen [26,40].

Fig. 6 shows the graph for relative product distribution, as percentage, from the Mn(salen)-catalyzed PRM oxidation in both the presence and absence of imidazole, using *t*-BOOH as the oxygen donor.

The presence of imidazole did not lead to any significant changes in the total yields of oxidized products from PRM (R = 45% and 48%, in the absence and in the presence of imidazole, respectively). In terms of product distribution (Fig. 6), though, the catalytic results obtained in the presence of imidazole provided important information on reaction mechanism.

Imidazole favored formation of the metabolite FENO, with concomitant decrease in the amounts of PEMA and PBA. The results reveal that PEMA and FENO are formed via distinct and competitive mechanisms, which involve different catalytically active species. The metabolite FENO is probably formed via the Mn^{V}(O)salen species, which is stabilized in the presence of the nitrogen ligand. As for PEMA, it may be formed via the intermediate Mn^{IV}(O)salen, generated from the reduction of the intermediate Mn^{V}(O)salen in the absence of imidazole, as discussed previously.

Because PEMA and FENO are generated from the same intermediate in the *in vivo* metabolism (Fig. 1), and because literature reports state that the preparation of these compounds involves different pH conditions [34], the pH influence on product formation was investigated for the studied system. Fig. 7 gives evidence of the strong dependence of product distribution on pH. PBA was preferentially formed in acid media (pH 2 and 4), thus indicating that this product might be generated from the hydrolysis or decomposition of the PRM-OH intermediate in acidic conditions. FENO was obtained in larger quantities compared with PEMA, once acid media favors additional oxidation of the PRM-OH intermediate. As the pH increased, PEMA became the main reaction product, thus confirming that the alkaline hydrolysis of the PRM-OH intermediate is an important mechanism for PEMA formation.

In terms of total yields, basic media favored higher PRM conversion (65% in pH 10 against 47% in pH 2).

All these variable effects were also investigated using PhIO as oxidant. However, no differences in total product yield or product selectivity were observed, and FENO was the main product in all conditions.

Control reactions carried out to investigate the Mn(salen)-catalyzed PEMA oxidation generated PBA as product. Another control reaction using Mn(salen) as catalyst and FENO as substrate led to BL as product. Therefore, PEMA is not generated from FENO degradation, and PEMA formation must compete with FENO production, as suggested previously.

The results obtained in the present study led to the proposal of a reaction scheme (Fig. 8) for the Mn(salen)-catalyzed PRM oxidation, on the basis of the following observations:

(i) PEMA and PBA are not formed when PhIO is employed as oxidant. (ii) In the case of the Mn(salen)/*t*-BOOH system, excess oxidant leads to increased PBA production, with concomitant decrease in the amounts of generated PEMA (Fig. 5). (iii) The presence of imidazole enhances selectivity toward FENO, together with a decrease in the amounts of PEMA and PBA (Fig. 6). (iv) Reactions in the absence of O_2 result in increased PEMA and FENO production, with a reduction in PBA formation (Fig. 4). (v) Control reactions using PEMA as substrate lead to PBA as product. (vi) Control reactions using FENO as substrate produce BL; no PEMA is detected in this case.

Taking observations (i) and (vi) into account, one can conclude that the metabolites PEMA and FENO are formed via distinct, competitive mechanisms. PhIO leads to the preferential formation of the active intermediate Mn^{V}(O)salen [41], while peroxides and peracids may result in two intermediates, depending on the type of O–O bond cleavage taking place (hemolytic or heterolytic)

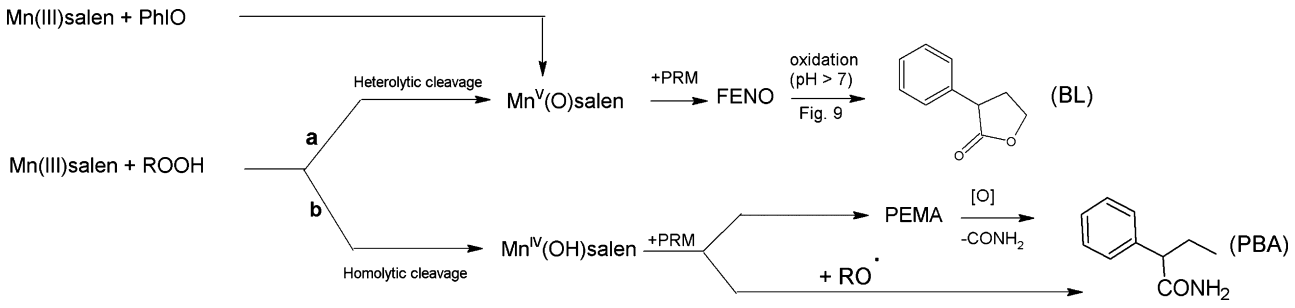

Fig. 8. Scheme for Mn(salen)-catalyzed primidone oxidation.

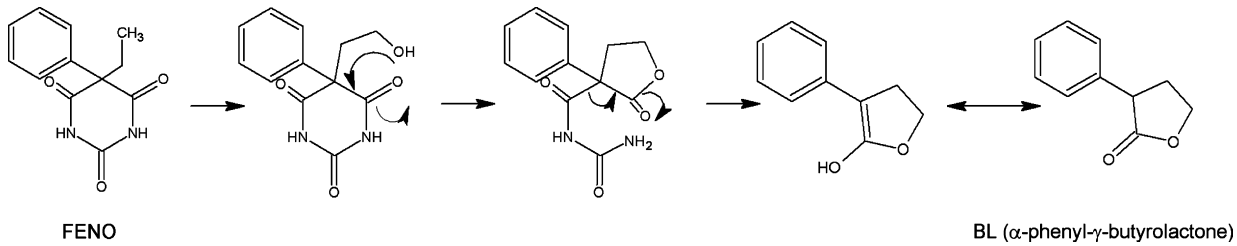

Fig. 9. Mechanism of the formation of BL (α-phenyl-γ-butyrolactone), adapted from Al-Tarakji-Khalfhl et al. [44].

[26,42,43]. Evaluation of the variables affecting the system were carried out employing *t*-BOOH as oxidant, which mainly undergoes homolytic cleavage because of the presence of the electron-donating *t*-butyl groups (heterolytic cleavage takes place to a lesser extent). On the basis of observations (ii) and (v), one can conclude that PBA is formed via additional PEMA oxidation, once excess oxidant favors this reaction, with increased selectivity toward PBA. On the basis of observations (iii) and (iv), it is possible to propose that FENO is generated via the active species Mn^{V}(O)salen, since formation of this intermediate is favored in the presence of imidazole and under inert atmosphere [39,40]. On the other hand, PBA is produced via a radicalar mechanism, as a result of the homolytic cleavage of the O–O bond, thus leading to RO• radicals, a reaction that is propagated by O_2 present in the reaction medium.

Formation of the active species Mn^{V}(O)salen depends on the heterolytic cleavage of the metal-oxo bond [42,43]. This species reacts with PRM, transferring its oxygen atom to the substrate, thus generating FENO (Fig. 8a). This product may undergo β-hydroxylation of the ethyl chain, followed by alcoholysis of the pyrimidinetrione ring. This should produce BL (Fig. 9), as observed by Al-Tarakji-Khalfhl et al. in natural systems [44].

Homolytic cleavage of the peroxide O–O bond should result in Mn^{IV}(OH)salen species and RO• radicals, as well as other radicals. These radicals, together with the dioxygen present in the reaction medium produce PEMA. The latter product could undergo additional oxidation, to generate PBA (Fig. 8b).

Metalloporphyrins were also employed as catalysts for PRM oxidation, for comparison purposes. Results depicted in Fig. 10 are given in terms of total product yield and selectivity; results shown in Fig. 11 concern product distribution.

Mn(TFPP)Cl was the most efficient catalyst, since it led to 79% total product yield being 40% of FENO. The anionic metalloporphyrins Mn(TCPP)Cl and Fe(TCPP)Cl led to relatively low total product yield, 39% and 24%, respectively. This is probably due to the lower solubility of PRM in aqueous medium, where these anionic metallocomplexes are soluble.

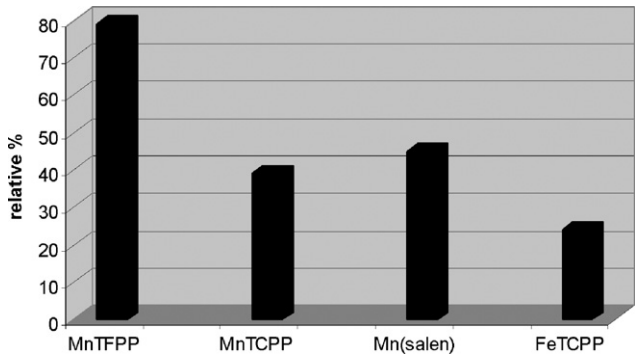

Fig. 10. Total yield of the metalloporphyrin-catalyzed PRM oxidation reactions compared with the Mn(salen)-catalyzed reaction.

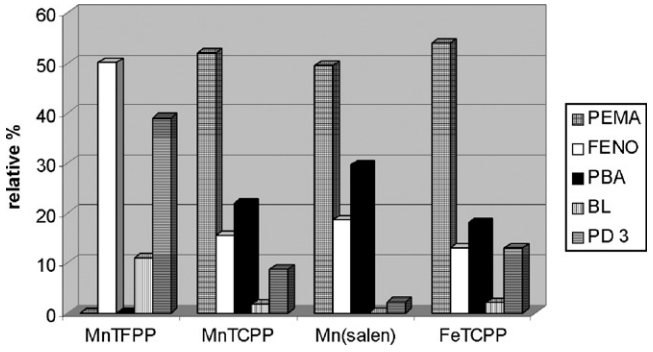

Fig. 11. Product distribution, as percentage, obtained with different catalysts in PRM oxidation by *t*-BOOH. Solvent = ACN for MnTFPP and Mn(salen); solvent = aqueous medium/ACN 1:1 for MnTCPP and FeTCPP.

Except for Mn(TFPP)Cl, all the metallocomplexes studied in this work have a similar activity profile, with preferential PEMA formation; FENO, PBA and PD 3 were formed in lower amounts. Mn(TFPP)Cl also stood out among the other catalysts in terms of selectivity: it generated FENO as the main product, while PEMA and PBA were not detected.

With respect to the metal ion, manganeseporphyrins were more efficient than the corresponding ironporphyrins, as reported for other systems [39]. The Jacobsen catalyst was less efficient than Mn(TFPP)Cl; however, the salen complex is cheap and easily prepared, besides being more selective toward PEMA, which is the main PRM metabolite in natural systems.

4. Conclusion

This work has demonstrated the ability of the salen complex and metalloporphyrins to mimic the action of P450 in primidone oxidation, with formation of two of the metabolites found in the *in vivo* system: PEMA and FENO. The formation of these products is highly dependent on the oxidant, co-catalyst (Im), pH and dioxygen. To the best of our knowledge, this is the first report on the *in vitro* oxidative metabolism of primidone using metalloporphyrins and the salen complex as catalyst. Our studies have also revealed the potential application of these biomimetic chemical models in the synthesis of drug metabolites. This should provide samples for pharmacological tests, as well as aid studies that pursue the elucidation of *in vivo* drug metabolism, thus overcoming the difficulty in working with enzymes *in vitro*.

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