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C – Carbon: an Essential Element

Carbon as a Natural Element, Chemistry and Life

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Carbon as a Natural Element, Chemistry and Life

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José A.S. Cavaleiro got his B.Sc. degree at the University of Coimbra, Portugal and his Ph.D. degree at the Robert Robinson Laboratories, University of Liverpool, U.K. (supervision of Profs. George W. Kenner and Kevin M. Smith). His academic career as a staff member started at Coimbra University and later continued at Lourenço Marques (Mozambique) and Aveiro (Portugal) Universities. Since 1986 he has been Professor of Chemistry at the Aveiro University. He was the supervisor of B.Sc., M.Sc. and Ph.D. students as well as of post-doctoral researchers. He is the recipient of several prizes (e.g., Parke–Davis prize, Liverpool University, 1973; Ferreira da Silva prize, Portuguese Chemical Society, 2004; Spanish-Portuguese prize Madinaveitia–Lourenço, Royal Spanish Chemical Society, 2010). His research interests are centered on the synthesis, reactivity, and applications (medicinal, catalytic, and others) of porphyrins and related compounds; also he has been engaged on studies on natural compounds, mainly terpenoids and flavonoids. He has been acting as referee of many organic chemistry publication sources and also as an evaluation member of colleagues CVs on request from Universities, academies and societies abroad. He is the author of 540 scientific publications in major journals of organic chemistry.

ABSTRACT

Life would not be taking place or it would be totally different if carbon, the number 6 element of the Periodic Table, would not exist. Carbon compounds are present in vital functions. As an example, the detoxification of xenobiotics is played by the enzymes of the Cytochromes P450 group. The mimicking of such processes might lead to significant biological information. That is illustrated with mimicking studies on the oxidative transformation of six carbon compounds which are potential drugs.

1. INTRODUCTION

Carbon is an element known since prehistoric times. It is the element with atomic number 6 in the Periodic Table put forward by Dmitri Ivanovich Mendeleyev. It was in 1869 that the first version of the Table was proposed, thus bringing a desired order to the chaos which was taking place in the chemical grouping science involving the known elements. As Krebs stated, the Periodical Table proposed was "the most elegant organizational chart ever devised". Although dealing with 63 elements known at the

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time, the table already predicted the place for future elements which could be discovered, and that gracefully has happened [1,2].

Carbon is the 15th most abundant element in the crust of our planet and the 2nd one by mass in the human body after the oxygen. Carbon is a vital element to all kinds of life. Without carbon our life would be different if not impossible. However, the number of carbon atoms is not at all so abundant; in our body for each 200 atoms group of H,O,C the number of carbon atoms is just 19! Carbon is the one which is able to make links with several other different atoms and with itself. From simple to very complex and robust structures, with linear, branched and cyclic shapes are made having carbon as the significant atom connection element. Proteins, DNA / RNA, carbohydrates, fats, are examples of such biomolecules.

2. CARBON COMPOUNDS. NATURAL AND SYNTHETIC DERIVATIVES. VITAL FUNCTIONS.

Nature is a "fantastic chemist" in our everyday life with the biosynthesis, mode of action and catabolism of many carbon compounds; a wide range of them are responsible for the bioprocesses which rule the life on earth. Many other carbon compounds, related or not related with the natural ones, have been obtained by chemists in their studies about new synthetic methodologies and potential applications for the new compounds. In such way it can be stated that millions of carbon compounds do exist and that is due to the way played by Nature or to the synthetic studies carried out by chemists. In many cases chemists aim to understand Nature and such target implies to carry out studies on the mimicking of the natural processes. Once more such chemical/biochemical world is due to the fantastic properties of carbon as the element present in all those compounds.

Vital functions played by Nature rule the way life is happening on earth and almost all involve carbon molecules. One of them is the xenobiotics' detoxification. This is usually an oxidative process catalyzed by a class of metalloenzymes, known as the Cytochrome P450-dependent monooxygenases (CyP450). Such enzymes have protoporphyrin-IX [(**1**), Figure 1] in the form of Iron (III) complexes containing cysteine groups as axial ligands.

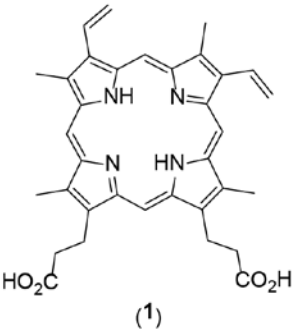


Figure 1
Structure of Protoporphyrin-IX (1)

It should be mentioned at this stage that protoporphyrin-IX is also a common precursor to the natural derivatives of such porphyrin which are involved in the respiratory and photosynthetic processes. In fact, respiration, photosynthesis and detoxification processes make a fantastic interconnection between the living species worlds.

The Cytochrome P450 enzymes can be found in almost all forms of life. A wide range of (regio, stereo) selective catalyzed monooxygenations by O_2 takes place. Living organisms on earth have had along many thousands (perhaps millions) of years an adaptation to their life conditions at each moment. That has included the constant metabolism of drugs and other xenobiotics in their environmental living space. It is Nature involving carbon compounds (CyP450) against other carbon derivatives, with an implicit target of better life.

The mechanism of the Cytochrome P450-catalyzed oxidative processes is shown in Figure 2, and the overall transformation can be represented by the following equation, where RH is the substrate and H_2D the nicotinamide adenine dinucleotide phosphate, [NAD(P)H], the reductant cofactor species.

$$RH + O_2 + H_2D \xrightarrow{P450} ROH + D + H_2O$$

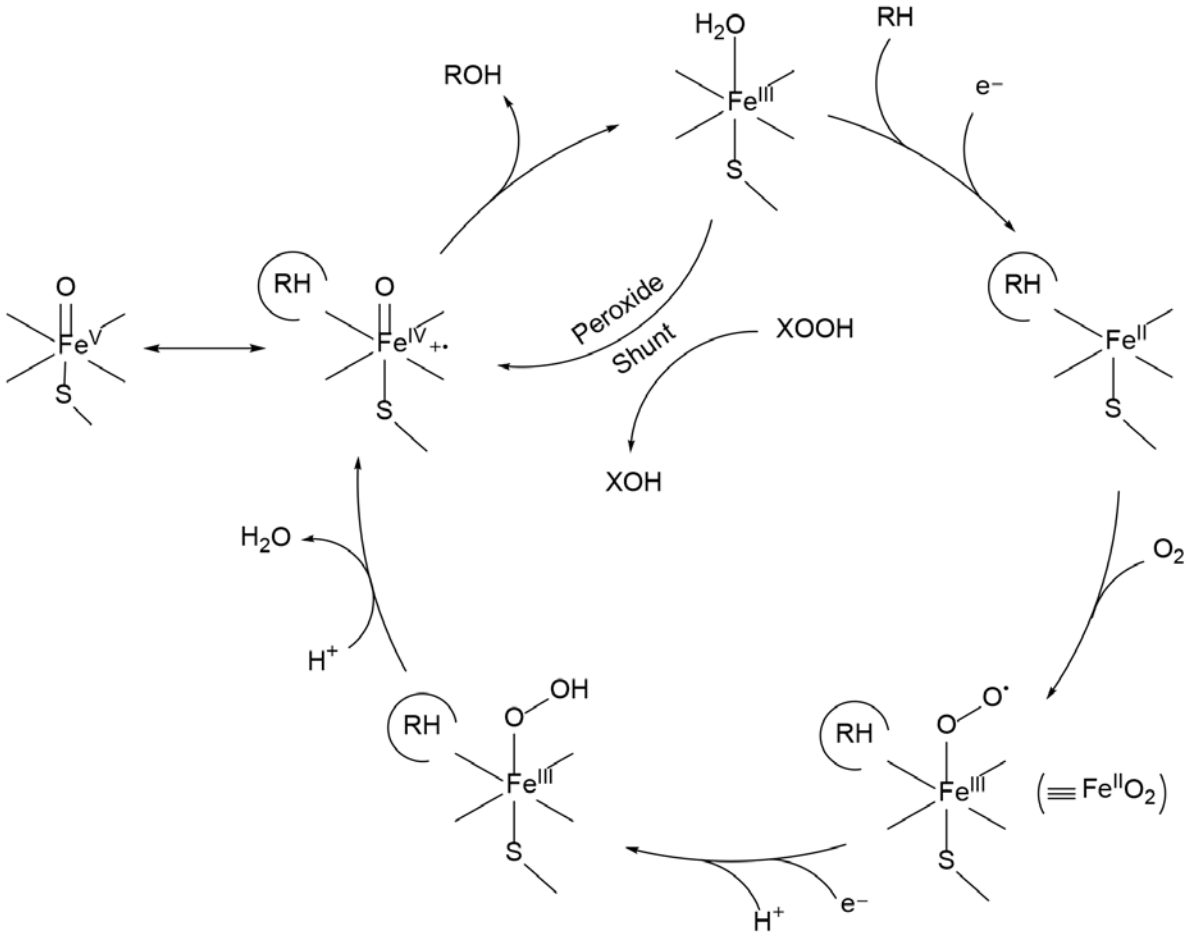
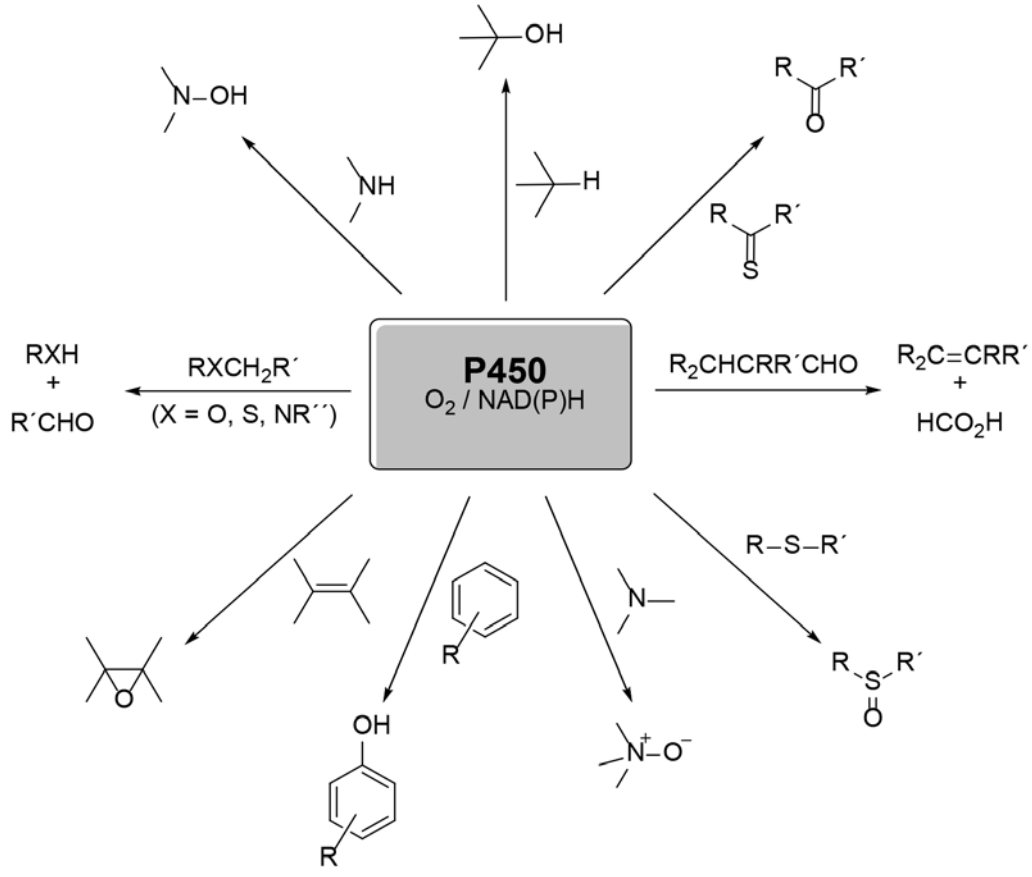


Figure 2
Possible CyP450 catalytic cycle

Presumably the substrate interacts with the hydrophobic CyP450 site (near the porphyrin iron center) and that is followed by a NAD(P)H electron transfer which is followed by the 0_2 binding. After that, a second electron transfer takes place leading to the formation of a hydroperoxoiron(III) species which by an heterolytic cleavage gives rise to the Iron(IV) oxoporphyrin radical cation. This is a powerful oxidant species and its interaction with the substrate gives rise to a significant variety of oxidized products [3,4].

Several types of natural oxidative transformations take place (Scheme 1). As significant examples it can be mentioned those involving epoxidations, hydroxylations, N-oxides, sulfoxides, C=O, etc.



Scheme 1
Examples of oxidation transformations catalyzed by P450 monooxygenases

An interesting feature of the CyP450 action was found in the 1970s. It was demonstrated that in the presence of oxygen donors (H_2O_2, RO_2H, periodate, iodosylbenzene) isolated liver CyP450 samples catalyzed the hydroxylation of hydrocarbons. Such transformation pointed to new synthetic procedures and applications; it is known as the peroxide shunt pathway (Fig. 2).

3. METALLOPORPHYRINS AND CYTOCHROME P450 MIMICKING PROCESSES

3.1. Metalloporphyrins as P450 mimicking catalysts

A significant amount of information can be found in the literature about the understanding of the Cytochrome P450 natural processes. The mimics of such processes allow to preview the action and metabolism of new drugs and also to apply such procedure in fine chemistry. It will be possible to transform a cheap substrate into another value-added one. The natural compounds' field is highly open to this possibility.

Metalloporphyrins have been used as catalysts in biomimetic studies. The most used macrocycles have been Fe, Mn, Cr, Ru complexes of *meso*-tetraphenylporphyrin derivatives. Pioneering studies on the epoxidation of alkenes and the hydroxylation of alkanes were reported by Groves and collaborators

using the Fe(III) complex of *meso*-tetraphenylporphyrin and PhIO as the oxygen donor. However, that porphyrin macrocycle is not very stable under the oxidizing reaction conditions. Certain derivatives of such macrocycle containing electron-withdrawing groups at the *meso*-phenyl groups or at the β-peripheral positions have been considered to be more robust porphyrins and have been widely used in further studies.

3.2. Aveiro studies using Hydrogen Peroxide

The Aveiro group has studied the oxidation of several acyclic and cyclic substrates, many of them being natural compounds (e.g., mono- and diterpenoids). The metalloporphyrins used have been Fe(III) and Mn(III) complexes of *meso*-tetraaryl-substituted porphyrin derivatives considered to be more robust than those from *meso*-tetraphenylporphyrin. The transformations have been studied mainly under homogeneous conditions, at room temperature, and the oxygen donor has been hydrogen peroxide, an environmentally safe oxidant [6,7].

This communication will consider the studies carried out with six compounds which have demonstrated significant medicinal applications and can be used as drugs. Such compounds are shown in (Figure 3). The here reported porphyrin catalyst used was the Mn(III) complex of *meso*--tetra-2,6-dichlorophenylporphyrin [Mn(TDCPP)Cl] since its use was common to the studies carried out with the six substrates.

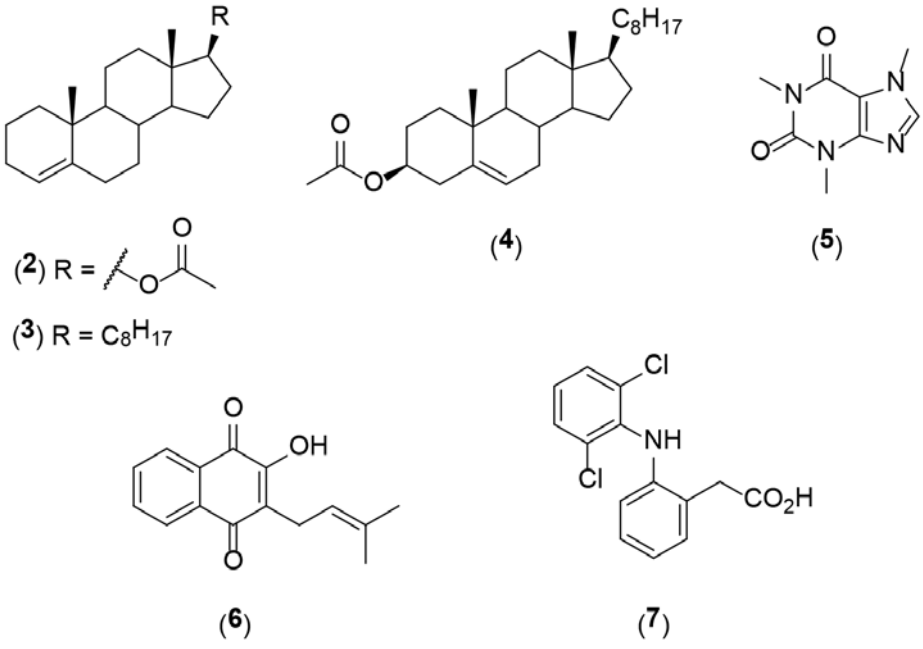


Figure 3
Structures of substrates used in this work

3.2.1 Steroids

Certain steroid derivatives have excellent medicinal applications, being used against several diseases and particularly in the treatment of breast cancer. Such compounds act by blocking the estrogen

biosynthesis thus giving rise to the tumors regression. Since the functionalization at positions 4 and 6 of the steroid backbone have been considered to be synthetic targets, three compounds were chosen for these stereoselective studies. Those substrates were 17β-acetoxy-4-androstene (**2**), 4-cholestene (**3**) and 3β-acetoxy-5-cholestene (**4**), having in mind that the goal targets were to study the epoxidation procedures of such Δ^4- and Δ^5-steroids.

It has been known since several decades that the direct epoxidation of such type of steroids with peroxy acids leads mainly to the formation of α-epoxides. But in our work different experimental conditions were being used. The oxygen donor was an aqueous solution of hydrogen peroxide and the catalyst and co-catalyst have been [Mn(TDCPP)Cl] and ammonium acetate. The use of the classical oxidant *m*-chloroperbenzoic acid (*m*-CPBA) was also carried out for comparative purposes.

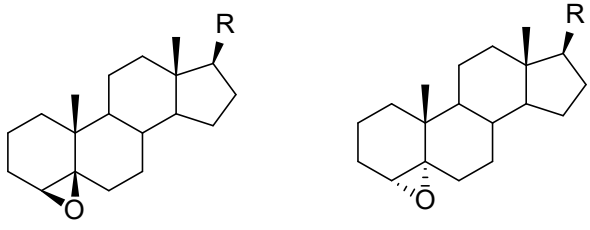
The oxidation of the two Δ^4-steroid substrates (**2**) and (**3**) is a selective epoxidation with a β/β+α ratio of 70% in a 1h reaction with 90% conversion. The main products are the two β (**2a**,**3a**) and the two α (**2b**,**3b**) epoxides (Figure 4). Other products at trace levels were obtained from allylic oxidation.

The oxidation of the Δ^5-steroid (**4**) under similar experimental conditions was 100% chemoselective for epoxidation. Conversion of 80% and β/β+α selectivity ratio of 90% were obtained for the two epoxides (**4a**, **4b**) (Figure 5).

The reactions of (**2**), or (**3**), or (**4**) with *m*-CPBA and in the absence of the porphyrin catalyst is chemoselective in the formation of the epoxides, but with epoxidation selectivity values β/β+α of 40% with 100% conversions in 1 h reactions time.

It is considered that the oxidation with H_2O_2 / Mn(TDCPP)Cl / NH_4OAc follows the CyP450 shunt pathway (Figure 2), with the formation of the high-valent oxo species, which might be the final oxidant species. Considering the substrates stereo-hindrance the oxidant species should approach the double bond from the *cis* side, and in such way the β-epoxides are preferentially formed.

It can be stated that Δ^4- and Δ^5-steroids can be successfully transformed into the corresponding epoxides. And with the new environmentally safe conditions the major product in each case is the β-epoxide derivative. These are then available for further reactions involving the epoxide moieties and a significant number of new steroids derivatives can be obtained.



(**2a**) R = OAc (**2b**) R = OAc

(**3a**) R = C_8H_{17} (**3b**) R = C_8H_{17}

Figure 4
β- and α-epoxides, respectively (**2a**),(**3a**) and (**2b**),(**3b**), obtained from androstene (**2**) / cholestene (**3**) and H_2O_2 / Mn(TDCPP)Cl / NH_4OAc

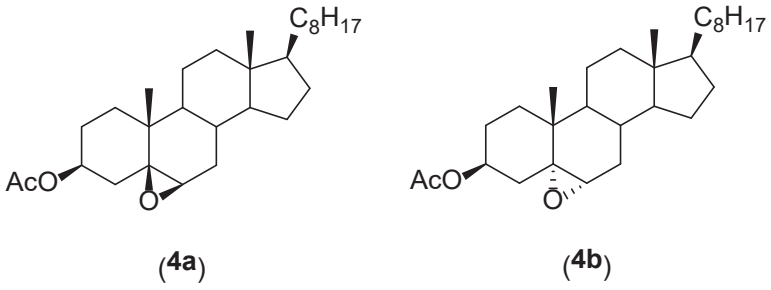


Figure 5
β- and α-epoxides obtained from cholestene (**4**) and H_2O_2 / Mn(TDCPP)Cl / NH_4OAc

3.2.2. Caffeine

Caffeine (**5**) is usually taken as beverages constituent or combined with analgesics. It can be considered as a legal drug and so its oxidative transformation has been studied. It has been shown that *in vivo* its oxidation involves the 3-*N*-demethylation. Several studies have been carried out in laboratories using ozone. The major product obtained has been dimethylparabanic acid (**5a**). We have carried out the oxidation studies of caffeine using H_2O_2 /Mn(TDCPP)Cl / NH_4OAc. The reaction took place with 90% conversion being (**5a**), (**5b**) and (**5c**) the most abundant products of which (**5c**), a new spiro-derivative was always the major one, Figure 6. It was also shown that the formation of **(5b)** was due to a secondary reaction of **(5a)** with ammonium acetate and the formation of **(5c)** could be explained by epoxidation at the double bond linking the two heterocyclic moieties of caffeine, followed by C–N bond cleavage, hydrolysis and lactonization. This is a new racemic spiro--derivative of caffeine [9].

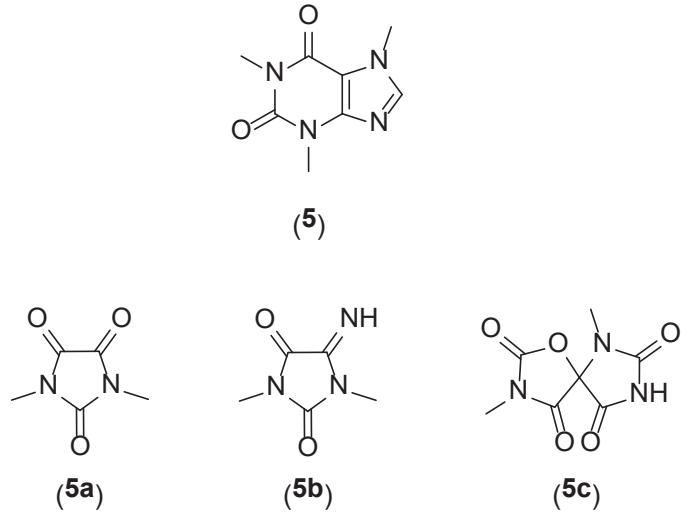


Figure 6
Structures of caffeine (**5**) and of its products obtained in the reaction with Mn(TDCPP)Cl/H_2O_2/NH_4OAc.

3.2.3. Lapachol

Lapachol **(6)** is a natural naphthoquinone present in the heartwood of several trees. It is known that lapachol as well some of its derivatives have demonstrated an important set of biological activities (anti-inflammatory, anti-tumor, antibacterial, fungicidal and others). Lapachol has been the subject of a wide range of synthetic studies involving structural modifications for the synthesis of eventually more active derivatives. The identification of its *in vivo* metabolites, mainly those coming from the CyP450 acting enzymes, is another target of great significance.

Several studies on the oxidation of lapachol have been reported on literature. But such procedures have had no environmental concern. In the present work the already described environmentally safer conditions [Mn(TDCPP)Cl, H_2O_2, NH_4OAc] were used. A comparative study using *m*-CPBA, the classical procedure, was also carried out [10].

The products obtained in one of the two mentioned procedures are different from those obtained in the other one. And the oxidation reaction times with *m*-CPBA are much longer than those with the porphyrin catalyst and H_2O_2. In the *m*-CPBA procedure the two already known *ortho*-naphthoquinones **(6a)** and **(6b)** were obtained; the other procedure involving the porphyrin catalyst and H_2O_2 gave rise to two new *para*-naphthoquinones **(6c)** and **(6d)** and to a new lactone **(6e)**, Figure 7.

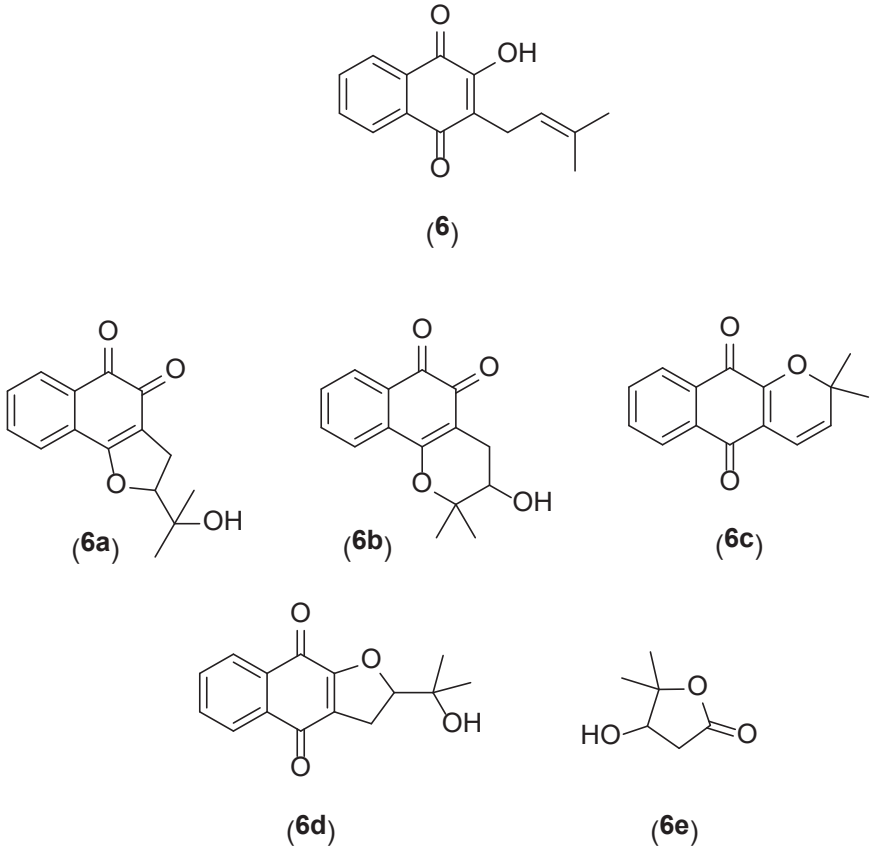
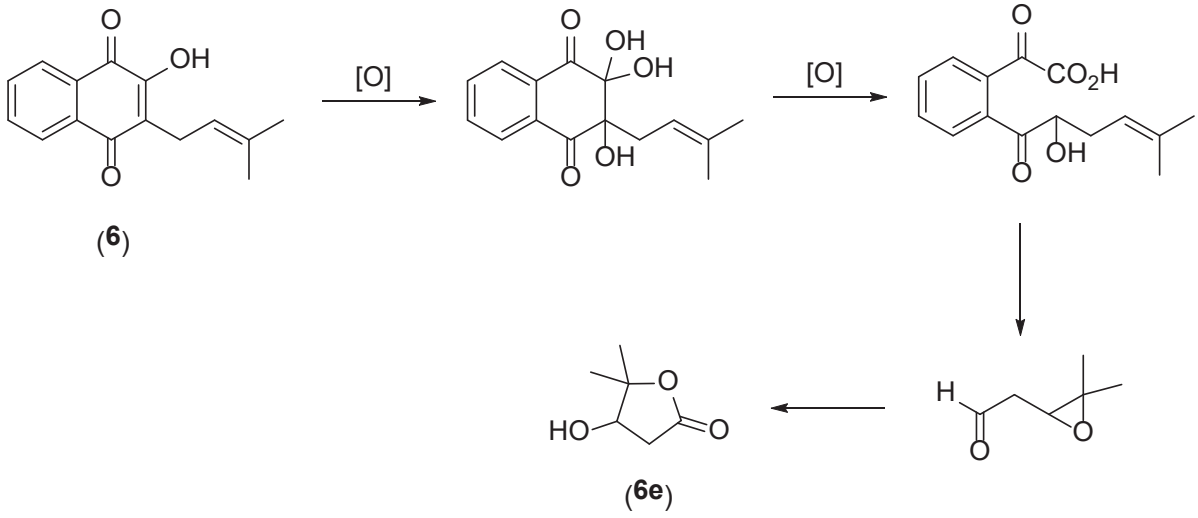


Figure 7
Structures of lapachol (**6**) and of the *m*-CPBA and Mn(TDCPP)Cl/H_2O_2/NH_4OAc oxidized products, respectively [(**6a**),(**6b**)] and [(**6c**),(**6d**),(**6e**)].

It is then clear that the system [Mn(TDCPP)Cl, H_2O_2, NH_4OAc] when applied to the lapachol oxidation gives rise to *para*-naphthoquinones and to a lactone. There is epoxidation not only at the lapachol side chain double bond but also at the double bond present in the quinone ring, which brings the possibility of the molecule cleavage. A possible mechanism for the formation of lactone **(6e)** is shown in Scheme 2.



Scheme 2
Possible formation of lactone (**6e**).

3.2.4. Diclofenac

Diclofenac **(7)** is an anti-inflammatory drug now having a frequent humans' use. Metabolites containing hydroxyl groups in the phenyl rings and other decarboxylated derivatives have been isolated from the CyP450 oxidative action. Literature data reveals that diclofenac and its derivatives have been isolated from environmental samples and oxidative methods have been considered for their removal. Biomimetic models might give an important contribution for such situation even with the possibility of showing *in vivo* unstable metabolites.

The oxidation of diclofenac was undertaken by using the environmentally safe conditions already reported for the previous compounds and involving the [Mn(TDCPP)Cl, H_2O_2, NH_4OAc] system. The products' mixture was not simple and seven compounds were obtained, chromatographically purified and identified by the usual spectroscopic techniques and for a few of them by using X-ray crystallography, (Figure 8). Mechanistic proposals for the *in vitro* formation of such products have been put forward. The oxidation process might involve oxidative decarboxylation, followed by formation of alcohol, ester and aldehyde derivatives [11].

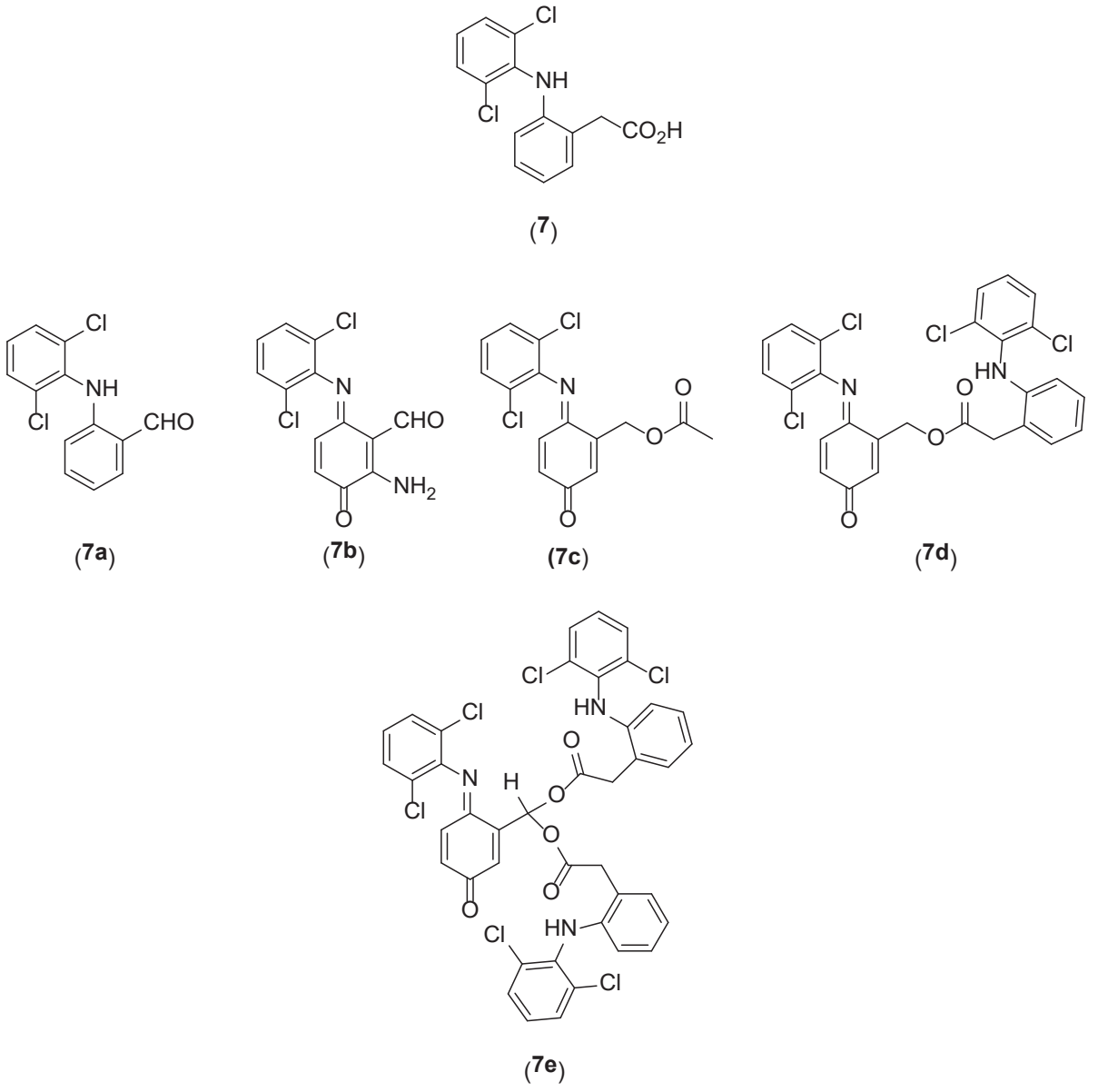


Figure 8
Structures of diclofenac (**7**) and derivatives (**7a**)-(**7e**) obtained.

FINAL REMARKS

Carbon compounds are present in vital functions. The mimicking of such transformations give rise to a better understanding of their action and to develop new chemical procedures leading to new useful carbon derivatives. A mimicking of the natural detoxification process was chemically applied to each substrate; new products can be considered as possible metabolites occurring from the natural use of each potential drug.

ACKNOWLEDGEMENTS

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