Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Medicinal Chemistry 38 (2003) 925–928
EUROPEAN JOURNAL OF MEDICINAL CHEMISTRY
www.elsevier.com/locate/ejmech

Mini review

Comparison of the efficiency of various methods for the synthesis of models of metabolites: example of 4a-methylhexahydronaphtalenones

François Estour, Sabine Ménager, Bernardin Akagah, Olivier Lafont *

Laboratoire de pharmacochimie, département de chimie organique pharmaceutique, faculté de médecine et de pharmacie de Rouen, 22, boulevard Gambetta, 76183 Rouen Cedex 1, France

Received 22 September 2003; received in revised form 29 September 2003; accepted 30 September 2003

Abstract

In order to examine the biotransformations of xenobiotics, it is essential to realize studies of metabolism of drugs in living animals. It is generally difficult to extract quantitatively the metabolites from biological media or excreta. Alternative methods have then been developed. Application of such techniques to 4a-methylhexahydronaphthalenones, which constitute starting material for the stereospecific synthesis of terpenoids or steroids, is particularly demonstrative. By biosynthetical ways, it was not possible to access with good yields to all the metabolites obtained in vivo. A novel methodology, based on the use of a manganoporphyrin catalyst, allowed to synthezise large amounts of several models of metabolites corresponding to those which had been isolated in living rats. Only one of the metabolites obtained in vivo could not be synthezised by this biomimetic system. This proved that alternative methods are precious to obtain models with good yields, but need to be validated by controls in living animals.

Keywords: Regioselectivity; Stereoselectivity; Living animals; Microsomes; Fungi; Metalloporphyrins

1. Introduction

The major difficulty in the studies of metabolism is to obtain sufficient amounts of a large variety of models of oxidized metabolites in order to determine their biological (pharmacological or toxicological) effects. This problem is complicated by the fact that the metabolism of a drug most often leads to chiral compounds. It is then generally difficult to stereospecifically synthezise compounds which contain several chiral centers. 4a-Methyl-hexahydronaphtalenones (Scheme 1) constitute a noteworthy illustration of this difficulty. These so-called 'octalones' are starting materials for the stereospecific synthesis of more complicated substituted enones which are essential intermediates for the total synthesis of terpenoids or steroids [1–4].

These derivatives contain several methylene groups which are sensible to oxidation reactions (Scheme 2). The CH_2 in 8 is in allylic position. It is considered as activated because radical oxidation intermediates can be stabilized by resonance. Positions 7 and 6 are also sensible to oxidation, but more slightly. The conjugated double bond may also undergo an epoxidation reaction.

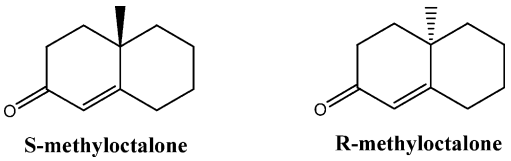
To obtain oxidized products of this synthon, different methods using living animals [5], microsomes [6] or microorganisms [7–9] have been tested. Recently, a novel preparative methodology based on the use of a manganoporphyrin catalysts was developed [10]. The purpose of this mini review is to discuss and to compare respective interests of these biological, biosynthetical and chemical methods.

2. Biological and biosynthetical pathways

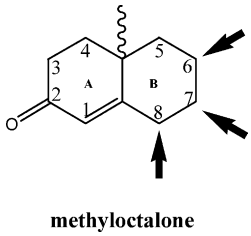
In order to identify a wider variety of metabolites, the study of metabolism in living animals constituted the reference pathway.

* Corresponding author.
E-mail address: olivier.lafont@univ-rouen.fr (O. Lafont).

0223-5234/03/$ - see front matter
doi:10.1016/S0223-5234(03)00186-7



Scheme 1. Enantiomers of methyloctalone.

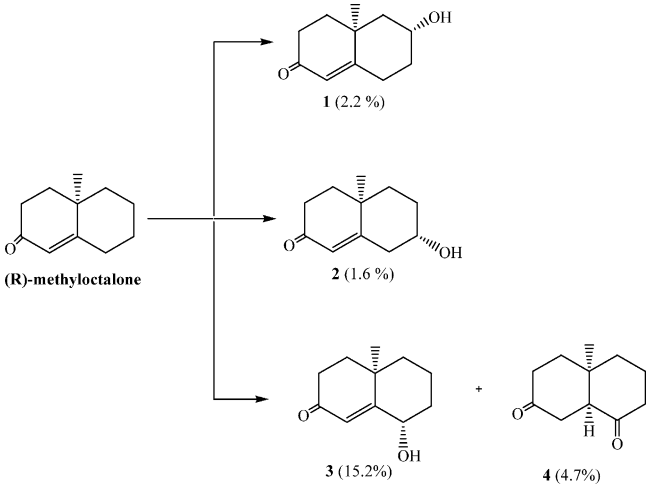


Scheme 2. Positions sensible to oxidation.

2.1. Metabolism in living animals

Direct oxidation of 4a-methylhexahydronaphthalenone (*R*) and (*S*) enantiomers in living rats has been examined by investigating the urinary metabolites [5]. It was possible to isolate and identify several oxidized products (Schemes 3 and 4).

For both enantiomers of starting compound, three positions of the B-ring proved to be sensible to oxidation. At positions 6 and 7, *cis*-hydroxylation occurred for each enantiomer, but hydroxy compounds (respectively **1**,**2** and **5**,**6**) were obtained with low yields. Concerning the position 8, the reactivity was significantly different between (*R*) and (*S*) enantiomers. In the case of (*R*)-compound, *cis*-hydroxylation was observed, and yielded the major oxidized product **3** (4a*R*,8*S*). The diketone **4** resulting from a rearrangement of compound **3** was also isolated, but with a weaker yield. In the case of (*S*)-enantiomer, the 8-hydroxylation took place in the *trans* position to give compound **7** (4a*S*,8*S*), but the yield was lower than for its optical isomer. Traces of saturated and unsaturated diketo compounds **8** and **9** were also obtained. Diketone **9** was formed by a subsequent oxidation of compound **7**.



Scheme 3. (*R*)-4a-Methyloctalone: oxidation metabolites in living rats.

2.2. Comparison with regio- and stereoselectivity in microsomal oxidation

In order to validate alternative methods, bioconversion of 4a-(*S*)-methylhexahydro–naphthalenone by rat microsomes have been investigated [6]. In this case, only the C-8 allylic position of the B-ring was sensible to oxidation. The two diastereomeric 8-hydroxylated metabolites **10** (4a*S*,8*R*) and **7** (4a*S*,8*S*) were obtained in the *cis*/*trans* ratio of 14/1 (Scheme 5).

The major oxidized product was then the *cis* isomer **10**, which had not been obtained in living rats, while the minor one, the *trans* isomer **7**, had also been isolated from living rats; Oxidation yields were not indicated by the authors, only the ratio was given. Moreover, this method did not allow the work with large amounts of starting material. Microsomal oxidation was then not efficient to access to the metabolites obtained in vivo, but yielded a new compound **10**.

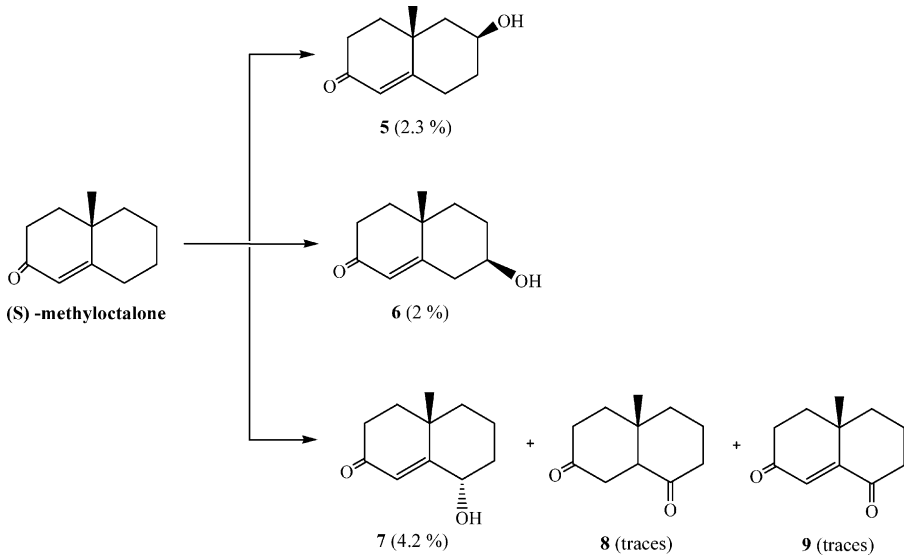
2.3. Comparison with oxidation by Rhizopus arrhizus

Oxidation by *R. arrhizus* led to a similar oxidation reaction [7]. The only difference was a *cis*/*trans* ratio of 13/1 instead of 14/1.

However, this kind of biological oxidation allowed to work with sufficient amounts of substrate (in the scale of gram), but with intermediate yields (about 20% of oxidation yield).

2.4. Comparison with oxidation by various fungal strains

With several fungal strains (*Absidia glauca*, *Beauvaria bassiana*, *Cunninghamella echinulata*, *Curvalaria lunata*, *Cylindrocarpon radicola*, *Mucor aromaticus*, *Mucor jansenii*, *Mucor plumbeus*, *Mucor racemosus*, *Mucor rouxii*), oxidation of both enantiomers of 4a-methylhexahydronaphthalenone led to C-6 and C-8 hydroxylated compounds and no oxidation was observed in position 7 [8,9]. This bioconversion was efficient in some cases. For example, in the case of incubation of (*R*) or (*S*)-methyloctalone with *M. plumbeus*, more than 70% of the starting product were metabolized. In the case of enantiomer S, for example, oxidation which occurred at position 6, yielded product **5** (4a*S*,6*S*). This *cis* compound was identical to the product obtained in the same position in living rats. When oxidation occurred at position 8, it was the *cis* diastereomer **10** (4a*S*,8*R*) which was isolated, but no *trans* isomer **7** (4a*S*, 8*S*) was detected (Scheme 6). The stereospecificity for position 8



Scheme 4. (*S*) 4a-Methyloctalone: oxidation metabolites in living rats.

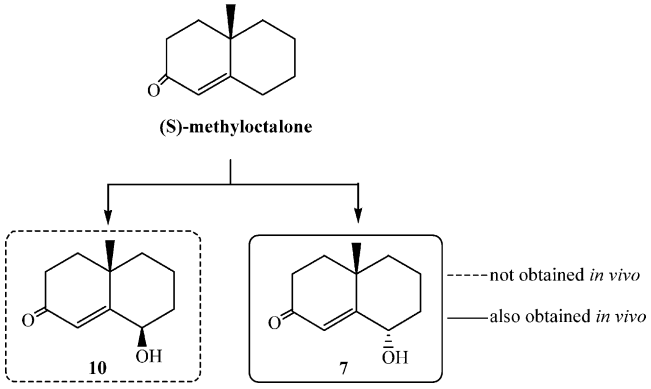
was then opposite to what occurred in living rats. This process was too different from metabolism in animals to be a very efficient method. It could only be used to prepare compound **5**.

3. Oxidation by a manganoporphyrin catalyst

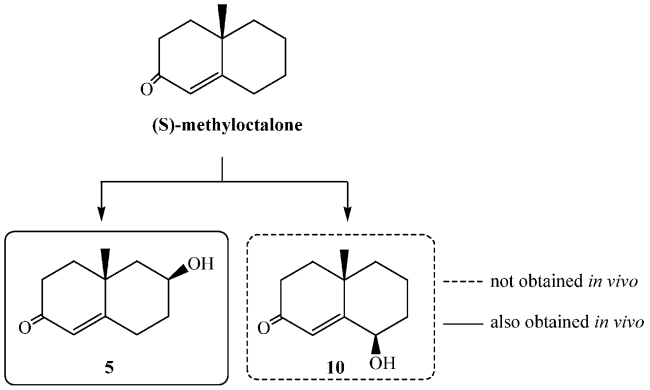
For both 4a-methyloctalone enantiomers, chemical synthesis in the presence of porphyrin catalysts led, as expected, to a similar behaviour. The technique used was realized according to a preparative method with Mn(TDCPP)Cl [TDCPP stands for tetrakis-(2,6-dichlorophenyl)porphyrin] in the presence of imidazole and hydrogen peroxide [10–12]. All enantiomeric models of metabolites were isolated in the same range of yields, but in order to compare the results with those obtained in living animals, only the case of (*S*) optical isomer is discussed here.

As it was the case in living rats, all three positions 6, 7 and 8 were oxidized. For each position 6 and 7, only one diastereomer was detected and isolated. In position 6 compound **11** (4a*S*,6*R*) resulting from a 6-*trans* hydroxylation was the only diastereomer isolated. It was the corresponding *cis* isomer **5** which had been extracted from biological media. Opposite to that, oxidation in position 7 led to the same isomer **6** (4a*S*,7*R*) which was also formed in living animals (Scheme 7).

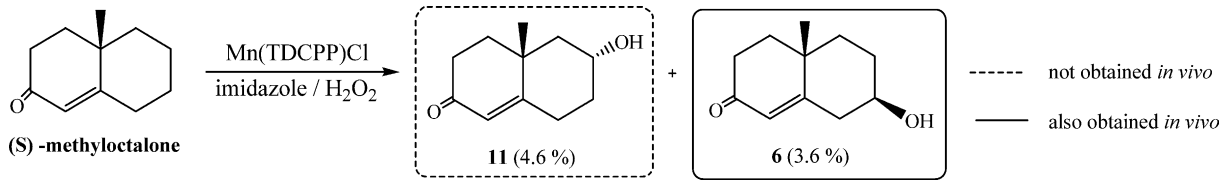
Allylic oxidation in position 8 allowed to isolate three oxidized products. Both *cis* **12** (4a*S*,8*R*) and *trans* **7** (4a*S*,8*S*) diastereomers were obtained by hydroxylation of the carbon C-8. Compound **7** had already been obtained in vivo but compound **12** had not been isolated in vivo. The diketo compound **9** resulting from a subsequent oxidation of **7** was also isolated (Scheme 8).



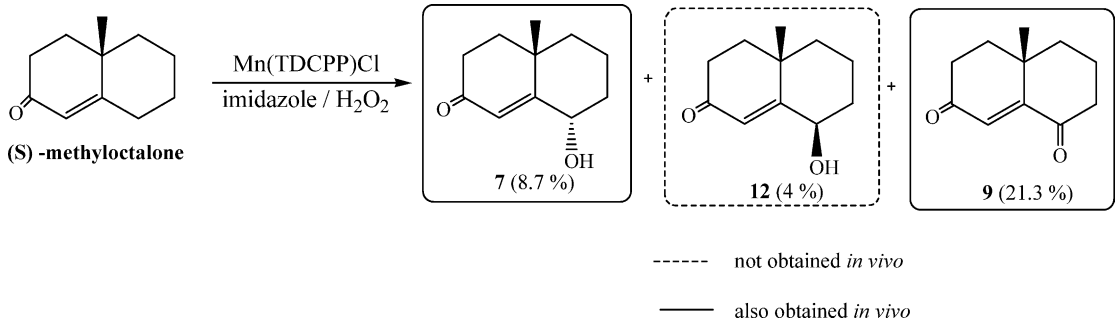
Scheme 5. Oxidation of (*S*)-4a-methyloctalone by rat microsomes and by *Rhizopus arrhizus*.



Scheme 6. Oxidation of (*S*)-4a-methyloctalone by *Mucor plumbeus*.



Scheme 7. 6- and 7-Hydroxylation of (*S*)-4a-methyloctalone by manganoporphyrin catalyst.



Scheme 8. Allylic oxidation of (*S*)-4a-methyloctalone by a manganoporphyrin catalyst.

By this chemical method, three metabolites **6**, **7** and **9** obtained in vivo were synthezised in good yields. Only in the case of oxidation in position 6, this chemical method did not allow to access to the same diastereomer **5** as in living animals. Hopefully, it was this compound which could be obtained by the incubation with fungal strains. Compound **11** was not a model of metabolite.

4. Conclusion

Metabolic studies of a drug in living animals constitute the reference method to identify the biotransformation of xenobiotics. Nevertheless, the main inconvenience of this approach is the quantitative extraction of metabolites from biological media or excreta before their purification by established preparative chromatographic systems.

In order to obtain sufficient amounts of models of metabolites for pharmacological or toxicological studies, alternative methods had to be developed. In the case of methyloctalone, biosynthetic methods did not allow to obtain biological oxidized compounds in good yields. A novel preparative approach based on the use of a manganoporphyrin system allowed a one-step synthesis of three metabolites in good yields obtained in living rats.

All these results confirmed that studies in living animals are essential to test the validity of the use of alternative methods ex vivo and in vitro, but that chemical methods using metalloporphyrin catalyst are precious to obtain large amounts of models of metabolites necessary to test their biological activity.

References

[1] M. Pfau, G. Revial, A. Guingant, J. d'Angelo, J. Am. Chem. Soc. 107 (1986) 273–274.
[2] G. Revial, Tetrahedron Lett. 30 (1989) 4121–4124.
[3] T. Volpe, G. Revial, M. Pfau, J. d'Angelo, Tetrahedron Lett. 28 (1987) 2367–2370.
[4] J. d'Angelo, G. Revial, T. Volpe, M. Pfau, Tetrahedron Lett. 29 (1988) 4427–4430.
[5] P. Vérité, S. Ménager, C. Cavé, D. André, J. d'Angelo, G. Revial, C. Combet Farnoux, O. Lafont, Bioorg. Med. Chem. Lett. 7 (1997) 587–592.
[6] T. Toda, N. Oshino, Tetrahedron Lett. 25 (1984) 91–94.
[7] H.L. Holland, B.J. Auret, Can. J. Chem. 53 (1975) 2041–2044.
[8] A. Hammoumi, G. Revial, J. d'Angelo, J.P. Girault, R. Azerad, Tetrahedron Lett. 32 (1991) 651–654.
[9] A. Hammoumi, J.P. Girault, R. Azerad, Tetrahedron Asymmetry 4 (1993) 1295–1306.
[10] J. Segrestaa, P. Vérité, F. Estour, S. Ménager, O. Lafont, Chem. Pharm. Bull. 50 (2002) 744–748.
[11] B. Akagah, F. Estour, P. Vérité, P. Lameiras, M. Nour, C. Cavé, S. Ménager, O. Lafont, Unpublished results.
[12] B. Akagah, F. Estour, J. Segrestaa, C. Cavé, S. Ménager, O. Lafont, Actual. Chim. Ther. 29 (2003) 117–120.