Pergamon

SCIENCE DIRECT®

Tetrahedron Letters 44 (2003) 4823–4825

TETRAHEDRON LETTERS

Conversion of naringenin and hesperetin by heterogeneous catalytic Baeyer–Villiger reaction into lactones exhibiting apoptotic activity

Roberta Bernini,[a,*] Enrico Mincione,[a] Manuela Cortese,[a] Raffaele Saladino,[a] Giampiero Gualandi[b] and Maria Cristina Belfiore[b]

[a]*Organic Chemistry, Dipartimento ABAC, Università degli Studi della Tuscia, Via S. Camillo De Lellis, 01100 Viterbo, Italy*
[b]*Genetic of Microorganisms, Dipartimento ABAC, Università degli Studi della Tuscia, Via S. Camillo De Lellis, 01100 Viterbo, Italy*

Received 10 April 2003; revised 29 April 2003; accepted 3 May 2003

Abstract—Naringenin and hesperetin were converted into the corresponding lactones by hydrogen peroxide activated by poly(4-vinylpiridine)-supported methyltrioxorhenium in *t*-butanol, an environmentally friendly catalytic system. The products showed a marked apoptotic activity in the genetic tests.

Among naturally occurring flavonoids, naringenin and hesperetin are very common in some edible fruits and vegetables as aglycons and glycosides. Naringenin is most abundant in grapefruit,[1] hesperetin in oranges, tangerines and lemons.[2] They occupy an important position in perfumery, cosmetic and in pharmaceutical formulations and hypocholesterolemic,[3] antiestrogenic,[4] hypolipidemic,[5] antihypertensive,[6] antiflammatory[7] activities have been reported.

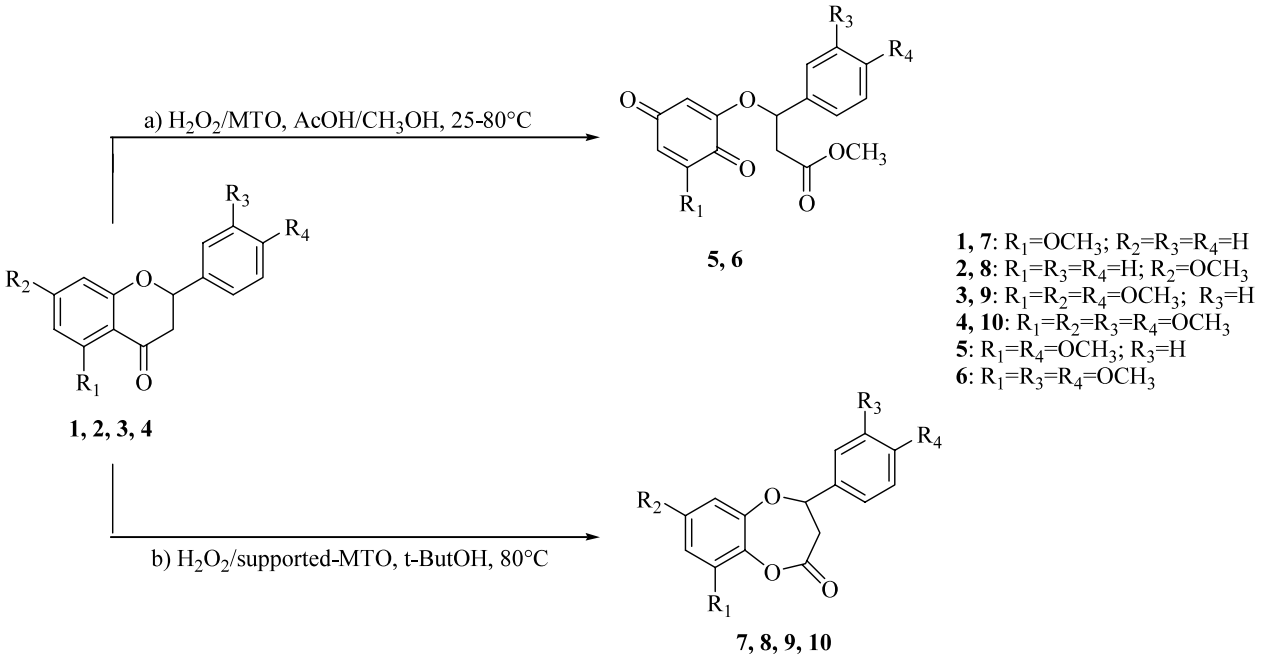
In a previous paper, we reported the Baeyer–Villiger rearrangement of some flavanones derivatives by the Methyltrioxorhenium (MTO)/Hydrogen Peroxide (H_2O_2) system in homogeneous acidic conditions to get the corresponding lactones.[8] However, the methylated naringenin **3** and hesperetin **4** in these conditions reacted to give the quinones **5** and **6** as the only reaction products (Scheme 1, pathway a) as a consequence of the opening of the formed lactones to the corresponding phenols further oxidized to quinones by MTO. With the aim to develop an efficient and useful method to prepare these lactones which are reported to have many biological activities,[9] now we set up the conditions to operate the Baeyer–Villiger reaction in heterogeneous catalytic conditions, as well in a neutral solvent, using the MTO supported on poly(4-vinylpyridine) cross-linked with divinylbenzene[10] (Scheme 1, pathway b). We chose this matrix because pyridine beside being an acid buffer is also a good ligand of MTO.[11] Different reaction conditions as well as supports were experimented. As reported in Table 1, high conversions and satisfactory yields of lactones **9** and **10** were obtained without quinone formation. The same 5-methoxyflavanone **1** and 7-methoxyflavanones **2** gave the corresponding lactones **7** and **8**. The demethylation previously observed in homogeneous catalysis did not occur now.[8] Shorter reaction times, higher conversions of the substrates (90 and 88%) and better yields of **9** and **10** (80 and 72%) were observed with MTO supported on the poly(4-vinylpyridine) 25% cross-linked (PVP-25%/MTO, Table 1: entries 3 and 8), evidencing the influence of the morphologic characteristics of the polymeric support on the efficiency of the catalyst.

In a typical experiment, to a suspension of 150 mg of the appropriate resin, prepared as reported,[10] in 5.0 ml of *t*-butanol, warmed at 80°C, were added the flavanone (0.3 mmol) and hydrogen peroxide H_2O_2 (50% water solution). The reaction was monitored by thin layer chromatography. At the end of the experiment, the suspension was filtered. The recovered catalyst was washed with ethyl acetate and after drying under high vacuum it was efficiently used for three further runs of oxidation. After the removal of the

Keywords: naringenin; hesperetin; Baeyer–Villiger reaction; heterogeneous catalysis.

* Corresponding author. Fax:+39(0)761357230; e-mail: berninir@unitus.it

0040-4039/03/$ - see front matter
doi:10.1016/S0040-4039(03)01139-0



Scheme 1. Oxidation of the methylated flavanones **3** and **4** to the corresponding quinones **5** and **6** by homogeneous catalytic system (pathway a) and conversion of **1**–**4** into the corresponding lactones by the heterogeneous MTO/H_2O_2 catalytic system (pathway b).

Table 1. Conversion of methylated naringenin **3** and hesperetin **4** by supported-MTO/H_2O_2 in *t*-butanol at 80°C; conversions and yields were calculated after chromatographic separation of the compounds

Entry	Substrate	Catalyst	Lactone	H_2O_2 (equiv.)	Time (h)	Conversion (%)	Yield (%)
1	**3**	–	**9**	10	24	–	–
2[a]	**3**	PVP-2%/MTO	**9**	10	12	80	71
3[b]	**3**	PVP-25%/MTO	**9**	10	8	90	80
4[c]	**3**	PVPN-2%/MTO	**9**	6	6	82	46
5[d]	**3**	PVPN-25%/MTO	**9**	6	6	98	42
6	**4**	–	**10**	10	24	–	–
7[a]	**4**	PVP-2%/MTO	**10**	10	8	76	58
8[b]	**4**	PVP-25%/MTO	**10**	8	6	90	72
9[c]	**4**	PVPN-2%/MTO	**10**	10	8	78	47
10[d]	**4**	PVPN-25%/MTO	**10**	6	6	98	35

[a] PVP-2%/MTO: poly(4-vinylpyridine) 2% cross-linked with divinylbenzene.
[b] PVP-25%/MTO: poly(4-vinylpyridine) 25% cross-linked with divinylbenzene.
[c] PVPN-2%/MTO: poly(4-vinylpyridine-*N*-oxide) 2% cross-linked with divinylbenzene.
[d] PVPN-25%/MTO: poly(4-vinylpyridine-*N*-oxide) 25% cross-linked with divinylbenzene.

solvent, the mixture reaction was purified by flash-chromatography. The lactones were characterized by ^1H and ^{13}C NMR, IR spectroscopy and mass-spectroscopy (EI) analyses.

We then submitted the natural naringenin and hesperetin, their relative methylated compounds **3** and **4**, and the corresponding lactones **9** and **10** to a toxicological examination in order to evaluate a possible apoptogenic activity. These assays were operated on a E2 human lymphoma cell line which is very resistant to apoptosis.[13] Both the natural naringenin and hesperetin did not induce significantly apoptosis on cells line, but the naringenin was shown be more apoptogenic than the hesperetin (Fig. 1).[†] Methylation of the hydroxyl group increased the apoptogenic efficiency, again showing the methylated form of naringenin **3** to be more apoptotic than the corresponding methylated form of hesperetin **4**. A further increase of the apoptosis was obtained with the lactone derivatives, the lactone **9** being the most active. Noteworthy, at the doses tested, these compounds show also a selective toxicity because they don't cause cellular necrosis.[‡] These results increase the interest for further researches on a possible role of these compounds in the tumoral therapies.

To the best of our knowledge, no references are reported on the utilization of this heterogeneous catalytic system in the Baeyer–Villiger reaction. Further

[†] All compounds were tested in the same range of μmolar concentrations.

[‡] Trypan blue test (data not shown).

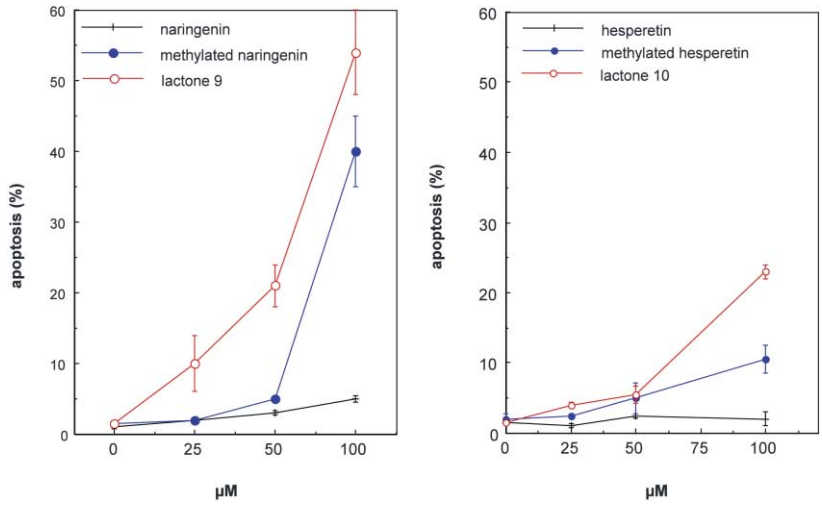


Figure 1. Apoptotic induction and estimation. Cells were seeded at conc. 3×10^5/ml and the compounds were added for 18 h. DMSO never excedeed 0.5% v/v. For morphological analysis of apoptosis $2–6\times10^5$ cells were fixed in 4% (v/v) paraformeldehyde and stained with a solution (0.2 μg/ml) of 4,6-diamino-2-phenylindole (DAPI). Apoptosis was quantified by scoring cells with condensed and fragmented nuclei, according to Ghibelli;[12] >500 cells in random fields were scored using fluorescence microscopy.

work is therefore in progress to extend this efficient and environmentally friendly method to other classes of organic compounds with particular regard to polyfunctionalized and structurally complex natural compounds.

Acknowledgements

We are grateful to Consorzio INCA and Italian 5% MURST-CNR 'Progetto Reflui Oleari' for their financial support.

References

1. Ping, C. H.; Saville, D. J.; Coville, P. T.; Wanwimolruk, S. *Pharmaceut. Acta Helvet.* **2000**, *74*, 379–385.
2. Montanari, A.; Chen, J.; Widmer, W. *Flavonoids in Living Systems*; Manthey and Buslig, Plenum Press: New York, 1998.
3. (a) Lee, M. K.; Moon, S. s.; Lee, S. E.; Bok, S. H.; Jeong, T. S.; Park, Y. B.; Choi, M. S. *Bioorg. Med. Chem.* **2003**, *11*, 393–398; (b) Wilcox, L. J.; Borradaile, N. M.; De Dreu, L. E.; Huff, M. W. *J. Lip. Res.* **2001**, *42*, 725–734; (c) Bok, S. H.; Lee, S. H.; Park, Y. B.; Bae, K. H.; Son, K. h.; Jeong, T. S.; Choi, M. S. *J. Nutr.* **1999**, *129*, 1182; (d) Lee, S. H.; Park, Y. B.; Bae, K. H.; Bok, S. H.; Kwon, Y. K.; Choi, M. S. *Ann. Nutr. Metab.* **1999**, *43*, 173–180; (e) Borradaile, N. M.; Carroli, K. K.; Kurowska, E. M. *Lipids* **1999**, *34*, 591–594.
4. Ruh, M. F.; Zacharewski, T.; Connor, K.; Howell, J.; Chen, I.; Safe, S. *Biochem. Pharm.* **1995**, *50*, 1485–1493.
5. (a) Bok, S. H.; Shin, Y. W.; Bae, K. H.; Jeong, T. S.; Kwon, Y. K.; Park, Y. B.; Choi, M. S. *Nutrition Res.* **2000**, *20*, 1007–10014; (b) Monforte, M. T.; Trovato, A.; Kirijavainen, S.; Forestieri, A. M.; Galati, E. M.; Lo Curto, R. B. *Il Farmaco* **1995**, *50*, 595–599.
6. Galati, E. M.; Trovato, A.; Kirjavanein, S.; Forestieri, A. M.; Rossitto, A.; Monforte, M. T. *Il Farmaco* **1996**, *51*, 219–221.
7. Galati, E. M.; Monforte, M. T.; Kirijavanein, S.; Forestieri, A. M.; Trovato, A.; Tripodo, M. M. *Il Farmaco* **1994**, *49*, 709–714.
8. Bernini, R.; Mincione, E.; Cortese, M.; Aliotta, G.; Oliva, A.; Saladino, R. *Tetrahedron Lett.* **2001**, *42*, 5401–5404.
9. Daya, S.; Gelebe, A. C.; Kaye, P. T. *Med. Sci. Res.* **1996**, *24*, 589–592.
10. Saladino, R.; Neri, V.; Pelliccia, A. R.; Caminiti, R.; Sadun, C. *J. Org. Chem.* **2002**, *67*, 1323–1332.
11. Rudolph, J.; Reddy, K. L.; Chiang, J. P.; Sharpless, K. B. *J. Am. Chem. Soc.* **1997**, *119*, 6189–6190.
12. Ghibelli, L.; Maresca, V.; Coppola, S.; Gualandi, G. *FEBS Lett.* **1995**, *337*, 9–14.
13. Gualandi, G.; Giselico, L.; Carloni, E.; Palitti, F.; Mosesso, P.; Alfonsi, A. *Mutagenesis* **2001**, *16*, 203–208.