ORGANIC LETTERS

2011
Vol. 13, No. 18
4846–4849

Chemistry of *trans*-Resveratrol with Singlet Oxygen: [2 + 2] Addition, [4 + 2] Addition, and Formation of the Phytoalexin Moracin M

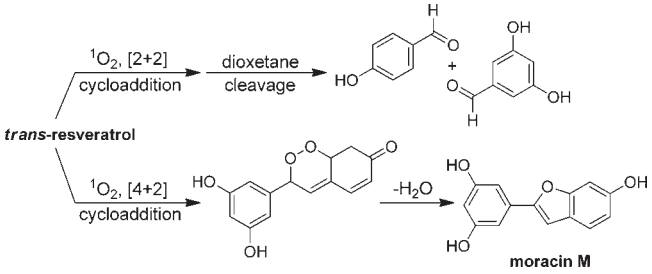
Jeff A. Celaje, Dong Zhang, Angela M. Guerrero, and Matthias Selke*

Department of Chemistry and Biochemistry, California State University, Los Angeles, Los Angeles, California 90032, United States

mselke@calstatela.edu

Received July 15, 2011

ABSTRACT



Resveratrol (1) reacts with singlet oxygen by two major pathways: A [2 + 2] cycloaddition forming a transient dioxetane that cleaves into the corresponding aldehydes and a [4 + 2] cycloaddition forming an endoperoxide that, upon heating, undergoes a rearrangement to moracin M. The rate constant by which singlet oxygen is removed by 1 (k_T) was determined by time-resolved infrared luminescence spectroscopy to be 1.5 × 10^6 M^{-1} sec^{-1} in CD_3OD, smaller than previously reported values. Chemical reaction accounts for ca. 25% of k_T.

A variety of beneficial health effects[1] (cardiovascular modulating effects,[1,2] antiaging effects,[1,3] and even antitumor effects[1,4]) have been attributed to the phenolic antioxidant *trans*-resveratrol (**1**, *trans*-5-(para-hydroxystyryl)-resorcinol), which is found in numerous plants, including *Vitis vinifera* (wine grapes). It is believed that so-called reactive oxygen species react with **1**, leading to their removal and formation of several *trans*-resveratrol oligomers.[5] These oligomers may also have a wide range of health benefits, including cyclooxygenase inhibitory activity.[5] It is therefore not surprising that in recent years a very large amount of research has been undertaken to elucidate the nature of the antioxidative properties of *trans*-resveratrol.

There have been several recent reports that *trans*-resveratrol is a selective quencher of singlet oxygen and thus may protect tissues and cells from photooxidative damage.[6] This includes inhibition of photooxidation of the fluorophore A2E, a pyridinium bisretinoid that acts as a singlet

(1) (a) Sinclair, D. A.; Baur, J. A. *Nat. Rev. Drug Discovery* **2006**, *5*, 493–506. (b) Das, M.; Das, D. K. *Mol. Aspects Med.* **2010**, *31*, 503–512.

(2) (a) Baur, J. A.; Pearson, K. J.; Price, N. L.; Jamieson, H. A.; Lerin, C.; Kalra, A.; Prabhu, V. V.; Allard, J. S.; Lopez-Lluch, G.; Lewis, K.; Pistell, P. J.; Poosala, S.; Becker, K. G.; Boss, O.; Gwinn, D.; Wang, M.; Ramaswamy, S.; Fishbein, K. W.; Spencer, R. G.; Lakatta, E. G.; Le Couteur, D.; Shaw, R. J.; Navas, P.; Puigserver, P.; Ingram, D. K.; de Cabo, R.; Sinclair, D. A. *Nature* **2006**, *444*, 337–342. (b) Soleas, G. J.; Diamandis, E. P.; Goldberg, D. M. *Clin. Biochem.* **1997**, *30*, 91–113. (c) Lee, S. K.; Zhang, W.; Sanderson, B. J. S. *J. Agric. Food Chem.* **2008**, *56*, 7572–7577.

(3) (a) Howitz, K. T.; Bitterman, K. J.; Cohen, H. Y.; Lamming, D. W.; Lavu, S.; Wood, J. G.; Zipkin, R. E.; Chung, P.; Kisielewski, A.; Zhang, L.-L.; Scherer, B.; Sinclair, D. A. *Nature* **2003**, *425*, 191–196. (b) Cohen, H. Y.; Miller, C.; Bitterman, K. J.; Wall, N. R.; Hekking, B.; Kessler, B.; Howitz, K. T.; Gorospe, M.; de Cabo, R.; Sinclair, D. A. *Science* **2004**, *305*, 390–392. (c) Westerheide, S. D.; Anckar, J.; Stevens, S. M., Jr.; Sistonen, L.; Morimoto, R. I. *Science* **2009**, *323*, 1063–1066.

(4) (a) Jang, M.; Cai, L.; Udeani, G. O.; Slowing, K. V.; Thomas, C. F.; Beecher, C. W. W.; Fong, H. H. S.; Farnsworth, N. R.; Kinghorn, A. D.; Mehta, R. G.; Moon, R. C.; Pezzuto, J. M. *Science* **1997**, *275*, 218–220. (b) Lin, J.-N.; Lin, V. C.-H.; Rau, K.-M.; Shieh, P.-C.; Kuo, D.-H.; Shieh, J.-C.; Chen, W.-J.; Tsai, S.-C.; Way, T.-D. *J. Agric. Food Chem.* **2010**, *58*, 1584–1592.

(5) Cichewicz, R. H.; Kouzi, S. A. In *Studies in Natural Products Chemistry*; Rahman, A. U., Ed.; Elsevier Science: New York, 2002; Vol. 26, pp 506−579.

(6) (a) Sparro, J. R.; Vollmer-Snarr, H. R.; Zhou, J.; Jang, Y. P.; Jockush, S.; Itagaki, Y.; Nakanishi, K. *J. Biol. Chem.* **2003**, *278*, 18207–18213. (b) Rimando, A. M.; Cuendet, M.; Desmarchelier, C.; Mehta, R. G.; Pezzuto, J. M.; Duke, S. O. *J. Agric. Food Chem.* **2002**, *50*, 3453–3457.

10.1021/ol201922u
Published on Web 08/22/2011

oxygen sensitizer.[6a] The self-sensitized photooxidation of A2E leads to macular degeneration, which is inhibited by *trans*-resveratrol.[6a] Pan et al.[7] reported a very large rate constant for the total singlet oxygen removal (k_T; this rate constant is the sum of all processes by which singlet oxygen is removed, namely physical quenching and chemical reaction) by **1** in an aqueous system, that is, $k_T = 3.92 \times 10^9\ M^{-1}\,sec^{-1}$, while Jung et al.[8] measured a k_T value of $2.55 \times 10^7\ M^{-1}\,sec^{-1}$ in methanol. The latter paper also suggested that there is a small amount (ca. 5% of k_T) of chemical reaction of **1** with singlet oxygen, although no products were reported. On the other hand, Pan et al. suggested, based on GC-MS analyses, that the resorcinol ring is oxidized by singlet oxygen to its corresponding endoperoxide, followed by rearrangement to quinones.[7] However, no NMR analysis of the products was performed, and reactivity of the electron-rich *trans* double bond was not considered by any of the authors. Given the very large discrepancy of the reported rate constants for singlet oxygen removal (over 2 orders of magnitude), the resulting uncertainty over the rate constant ratio of chemical reaction vs physical quenching, and the lack of conclusive assignment of any reaction products, a detailed study of the chemistry of singlet oxygen with *trans*-resveratrol is overdue. We now report that *trans*-resveratrol reacts with singlet oxygen via two entirely different pathways, both of which involve the *trans* double bond, namely [2 + 2] addition and [4 + 2] addition. The latter pathway leads to an endoperoxide that undergoes a surprising rearrangement to the phytoalexin moracin M.

We have determined the total rate by which singlet oxygen is removed by **1** using time-resolved near-infrared (NIR) spectroscopy (Figure 1). This was accomplished by flash generation of singlet oxygen followed by monitoring its near-infrared luminescence decay in a variety of solvents. Rate constants for singlet oxygen removal are summarized in Table 1. Our values in CD_3CN and CD_3OD are somewhat smaller than those obtained by Jung et al.[8] (about 1 order of magnitude) in methanol and over 3 orders of magnitude smaller than those reported by Pan et al.[7] Neither one of these groups used time-resolved methods, which are considered far more accurate than any other methodology for the determination of singlet oxygen quenching rate constants. There is no significant variation of our rate constants in protic vs aprotic solvents (i.e., methanol vs acetonitrile), indicating that hydrogen bonding by solvent molecules to the hydroxy groups of **1** does not affect the value of k_T and cannot account for the differences between our k_T values and those previously reported in the literature. At high pH in D_2O, the value of k_T for **1** increases considerably (more than 2 orders of magnitude). Likewise, in a 3:2 CD_3OD/D_2O mixture (where ionization of the phenol groups is higher) the k_T value is larger (by a factor of 6) than in neat CD_3OD. These increases are similar to what has been observed for singlet oxygen removal by other phenolic antioxidants under basic conditions.[9]

Table 1. Rate Constants of Singlet Oxygen Removal by *trans*-Resveratrol

solvent	temp (°C)	k_r (**1**) $\times 10^{-5}$ ($M^{-1}secs^{-1}$)[a]	k_T (**1**) $\times 10^{-6}$ ($M^{-1}secs^{-1}$)[b]
CD_3CN	22		1.6 ± 0.1
	0	3.0 ± 0.2	1.2 ± 0.2
CD_3OD	22		1.5 ± 0.1
CD_3OD/D_2O [c]	22		9.2 ± 0.2
D_2O, pH = 10 [d]	22		370 ± 20

[a] k_r = rate constant for chemical reaction of **1** with singlet oxygen; average of three runs, error is one standard deviation. Sensitizer: methylene blue, cutoff filter at 493 nm. [b] Average of three runs, error is one standard deviation. Sensitizer: methylene blue, λ_{exc} = 532 nm. Rate constants were determined from pseudofirst order decay of the singlet oxygen luminescence. [c] 3:2 ratio of CD_3OD/D_2O. [d] 0.05 M Na_2CO_3/0.05 M $NaHCO_3$ buffer in D_2O.

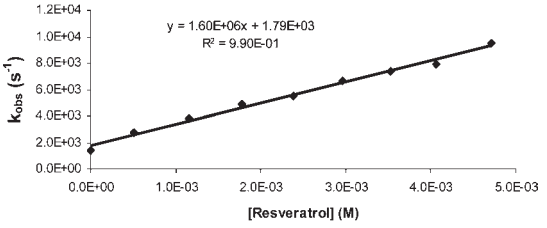


Figure 1. Singlet oxygen removal by *trans*-resveratrol in CD_3CN.

In contrast to previous reports,[7,8] we have found that *trans*-resveratrol reacts with singlet oxygen leading to a rather complex product mixture. Careful NMR analyses indicated that there are three major products. For all cases, the *trans* double bond of **1** must be involved in the reaction, since the signals for the *trans* vinyl protons in the ^1H NMR completely disappear when the progress of the reaction is directly monitored by carrying out the photooxidation in an NMR tube. Thus, the previous suggestion—based solely on mass spectral analyses—that singlet oxygen adds across the resorcinol ring cannot be correct. Indeed, we did not find any quinone products. Two products were separated from the crude product mixture and purified by flash column chromatography on silica gel. NMR analyses, including comparison with authentic samples, clearly demonstrated that these products were 4-hydroxybenzaldehyde (**2**) and 3,5-dihydroxybenzaldehyde (**3**). Their formation can easily be rationalized by [2 + 2] addition of singlet oxygen to the electron-rich *trans* double bond of **1** followed by cleavage of the resulting dioxetane (Scheme 1).[10] The [2 + 2]

(7) Jiang, L.-Y.; He, S.; Jiang, K.-Z.; Sun, C.-R.; Pan, Y.-J. *J. Agric. Food Chem.* **2010**, *58*, 9020–9027.

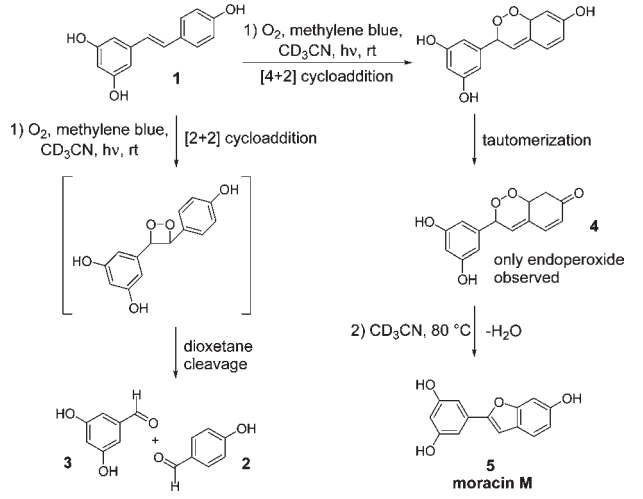
(8) Jung, M. Y.; Choi, D. S. *J. Agric. Food Chem.* **2010**, *58*, 11888–11895.

(9) Bisby, R. H.; Morgan, C. G.; Hamblett, I.; Gorman, A. A. *J. Phys. Chem. A* **1999**, *103*, 7457–7459.

(10) (a) Richardson, W. H.; Batinica, G.; Janota-Perret, K.; Miller, T.; Shen, D. *J. Org. Chem.* **1991**, *56*, 6140–6144. (b) Ohkubo, K.; Nanjo, T.; Fukuzumi, S. *Org. Lett.* **2005**, *7*, 4265–4268.

addition channel accounts for nearly 40% of the products for the reaction of **1** with singlet oxygen. Detailed NMR analyses revealed that the major product was an endoperoxide formed by a [4 + 2] addition of singlet oxygen to the *trans* double bond and the adjacent C=C double bond of the para-phenol ring of **1**, followed by tautomerization of the primary cycloaddition product to the corresponding ketone **4**. All attempts to isolate endoperoxide **4** as a pure compound by column chromatography led to its decomposition.

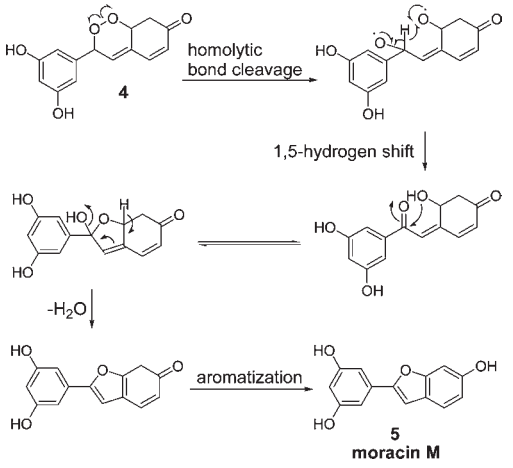
Scheme 1. Reactivity Pathways Leading to the Observed Product Mixture of the Reaction of Singlet Oxygen and *trans*-Resveratrol



Endoperoxide **4** accounts for nearly 60% of the products for the reaction of **1** with singlet oxygen. In addition to the three products mentioned above, we also noted a very small amount (< 5%) of an as-of-yet unidentified compound that is a secondary photoproduct formed upon prolonged irradiation of product **4**. While formation of **4** was unexpected, a similar [4 + 2] cycloaddition of singlet oxygen and *trans*-4-propenyl anisole has been reported by Greer et al.[11] However, since the anisole studied by Greer et al. did not possess the para-hydroxy group present in compound **1**, their primary product did not undergo the tautomerization that led to formation of **4** from the [4 + 2] adduct of resveratrol.

Endoperoxide **4** is stable at room temperature, but upon warming, it undergoes further rearrangement.[12] Detailed NMR analyses (including ^1H, ^{13}C, and COSY) indicate that the product of this rearrangement is an arylbenzofuran derivative, namely the known phytoalexin moracin M (**5**); this assignment was confirmed by comparison with the NMR data published in the literature.[13] Moracin M has been isolated from *Morus alba* L. (Moraceae) in China; the root bark of *Morus alba* L. is extensively used in traditional Chinese medicine.[13b] Moracin M has been reported to have antimicrobial activity, and is also believed to be a precursor to other arylbenzofurans with potent antioxidant activity.[14] The formation of **5** from the [4 + 2] addition product from the reaction of **1** with singlet oxygen represents a very simple and convenient synthesis of moracin M.[15] A possible mechanism for the formation of **5** from **4** is depicted in Scheme 2. The rearrangement should initially involve homolytic cleavage of the O–O bond of **4** followed by a 1,5-hydrogen shift to a γ-hydroxy enone. Cyclization to a hemiketal followed by loss of water concomitant with aromatization would then lead to moracin M.

Scheme 2. Proposed Mechanism of Moracin M Formation



A substrate may either react with singlet oxygen and/or physically remove it without chemical change (i.e., physical quenching of singlet oxygen). Previous reports have claimed that physical quenching of singlet oxygen may be an important aspect of the biological function of **1**.[7,8] To evaluate whether there is significant physical quenching by **1**, we have conducted competition experiments with several singlet oxygen acceptors that remove singlet oxygen only by chemical reaction. We have found that chemical reaction of **1** with singlet oxygen is rather slow, and consequently many singlet oxygen coacceptors commonly used for this type of experiment (for example, 9,10-dimethylanthracene) were unsuitable as they were completely consumed before an appreciable amount (> 5%) of **1** was photooxidized. We eventually found that 1,4-

(11) Greer, A.; Vassilikogiannakis, G.; Lee, K.-C.; Koffas, T. S.; Nahm, K.; Foote, C. S. *J. Org. Chem.* **2000**, *65*, 6876–6878.

(12) Our rearrangement leading to Moracin M involves a six-membered endoperoxide, in contrast with the bicyclic furan-derived endoperoxide rearrangements studied by Vassilikogiannakis et al.: Montagnon, T.; Tofi, M.; Vassilikogiannakis, G. *Acc. Chem. Res.* **2008**, *41*, 1001–1011.

(13) (a) Fukai, T.; Hano, Y.; Hirakura, K.; Nomura, T.; Uzawa, J. *Chem. Pharm. Bull.* **1980**, *32*, 808–811. (b) Piao, S.-J.; Qiu, F.; Chen, L.-X.; Pan, Y.; Dou, D.-Q. *Helv. Chim. Acta* **2009**, *92*, 579–587.

(14) Kapche, G. D. W. F.; Fozing, C. D.; Donfack, J. H.; Fotso, G. W.; Amadou, D.; Tchana, A. N.; Bezabih, M.; Moundipa, P. F.; Ngadjui, B. T.; Abegaz, B. M. *Phytochemistry* **2009**, *70*, 216–221.

(15) A transition metal-directed total synthesis of **5** has previously been reported: Widdowson, D. A.; Mann, I. S. *Tetrahedron* **1991**, *47*, 7981–7990.

dimethylnaphthalene (1,4-DMN) was a suitable coacceptor, and measured the rate constant for its reaction with singlet oxygen in CD_3CN to be $2.9 \times 10^5\ M^{-1}\,sec^{-1}$ at room temperature and $2.7 \times 10^5\ M^{-1}\,sec^{-1}$ at 0 °C. For this reference compound, all of the singlet oxygen removal is due to endoperoxide formation, that is, $k_T = k_r$.[16] The endoperoxide formed from reaction of singlet oxygen and 1,4-DMN slowly reverts back to 1,4-DMN (ca. 50% over 5 h) at room temperature[17] but is indefinitely stable at 0 °C. We therefore conducted the competition experiments between **1** and 1,4-DMN at 0 °C. Using the methodology developed by Higgins et al.,[18] we obtained a value for the chemical rate constant k_r for the reaction of singlet oxygen with *trans*-resveratrol of $3.0 \times 10^5\ M^{-1}\,sec^{-1}$. Since the value of k_T for **1** in CD_3CN at 0 °C is $1.2 \times 10^6\ M^{-1}\,sec^{-1}$, k_r is approximately 25% of k_T. Hence, physical quenching of singlet oxygen by **1** accounts for about 75% of the total removal of singlet oxygen.

The total rate constant for singlet oxygen removal by **1** is considerably smaller than previously reported, and is in fact somewhat smaller than those of other phenolic antioxidants such as vitamin E.[19] However, other resorcinol derivatives that do not possess the aromatic ether moiety of tocopherols have k_T values very similar to the ones reported in this paper, that is, in the 10^6–$10^7\ M^{-1}\,sec^{-1}$ range.[20,21] Given the rather small value of k_T by **1**, especially compared with tocopherols, we do not think that singlet oxygen scavenging is the main pathway from which the health benefits of **1** originate. On the other hand, our data demonstrates that chemical reaction of singlet oxygen with **1** is a significant pathway in the interaction of **1** with singlet oxygen. The reaction of **1** with singlet oxygen does not lead to formation of quinones, but instead involves an intriguing [4 + 2] cycloaddition channel leading to an endoperoxide that can rearrange to the phytoalexin moracin M. Further experiments to elucidate the chemistry of singlet oxygen with glycosylated and oligomeric derivatives of **1** are in progress.

Acknowledgment. We gratefully acknowledge support from the NIH-NIGMS 5SC1GM084776. J.A.C. is also acknowledging support from the NIH-RISE program (GM6133), and A.M.G. is acknowledging support from the NIH MARC program (GM08228). We thank Timothy Dong (UCLA), Dr. Errol Mathias (CSULA), and Dr. Ali Jabalameli (CSULA) for valuable experimental assistance.

Supporting Information Available. Experimental procedures, full spectroscopic data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

(16) Gollnick, K.; Griesbeck, A. G. *Tetrahedron Lett.* **1984**, *25*, 725–728.

(17) Adam, W.; Prein, M. *Acc. Chem. Res.* **1996**, *29*, 275–283.

(18) Higgins, R.; Foote, C. S.; Cheng, H. *Adv. Chem. Ser.* **1968**, *77*, 102–117.

(19) (a) Clough, R. L.; Yee, B. G.; Foote, C. S. *J. Am. Chem. Soc.* **1979**, *101*, 683–686. (b) Foote, C. S.; Ching, T.-Y.; Geller, G. G. *Photochem. Photobiol.* **1974**, *20*, 511–513. (c) Fahrenholtz, S. R.; Doleiden, F. H.; Trozollo, A. M.; Lamola, A. A. *Photochem. Photobiol.* **1974**, *20*, 505–509.

(20) Mukai, K.; Daifuku, K.; Okabe, K.; Tanigaki, T.; Inoue, K. *J. Org. Chem.* **1991**, *56*, 4188–4192.

(21) Mártire, D. O.; Braslavsky, S. E.; García, N. A. *J. Photochem. Photobiol., A* **1991**, *61*, 113–124.