Pergamon

Bioorganic & Medicinal Chemistry Letters 10 (2000) 2709–2712

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Reaction of 2,2,5,7,8-Pentamethyl-6-chromanol, an α-Tocopherol Analogue, with NO in the Presence of Oxygen

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Received 6 August 2000; accepted 16 September 2000

Abstract—An α-tocopherol model compound, 2,2,5,7,8-pentamethyl-6-chromanol, reacted with nitric oxide (NO) in the presence of various amounts of oxygen to afford four major products. Distribution of the products was varied depending on the ratio of NO and O_2, and the preincubation time of NO and O_2.

α-Tocopherol (α-Toc) is a ubiquitous antioxidant in biological systems and protects biological molecules from the oxidation induced by various kinds of active oxygens.[1–4] Its action is derived from the quenching of active oxidants with one electron reduction and the radical chain reaction is terminated by this process.

Nitric oxide (NO) is one of the most important biological radical molecules and has been known to fill the role of mediator in many physiological phenomena.[5] In addition, NO brings about cytotoxic activity when it is generated in relatively high concentration, and reacts with molecular oxygen or superoxide to give dinitrogen trioxide (N_2O_3), nitrogen dioxide (NO_2), or peroxynitrite.[6,7] These higher nitrogen oxides (NO_x) are known to have high reactivity and oxidation activity in spite of the slight reactivity of NO itself.[8]

Since these active species derived from NO are supposed to give oxidative damages to the body, it is important to investigate their interaction with α-Toc, which is one of the major antioxidants in biological systems. In spite of its relevance and importance, there are few reports concerning the reaction of α-Toc and nitrogen oxides. Janzen et al. reported that α-Toc reacted with NO in the absence of oxygen to form tocopheroxyl radical which was detected by ESR and the radical signals were reversibly changed by the presence or the absence of excess NO.[9] The stable reaction products were, however, not reported in this paper. d'Ischia reported the reaction of α-Toc with NO in the presence of oxygen to give many oxidation products, one of which was a novel ring contraction product.[10] He also reported that α-tocoquinone was obtained in a low yield. However, the yield of the product was less than 10%, and the whole reaction process remained unclear.

In the course of our study of autooxidation of α-Toc,[11] we have been interested in the interaction of α-Toc and NO or other nitrogen oxides. It has been reported that NO and O_2 react in various manners depending upon the ratio of these two chemicals, and the reaction processes are quite complex[12] because diverse intermediates have different reactivities toward co-existing organic molecules. Thus we decided to investigate the reaction of α-Toc with nitrogen oxides by changing the ratio of NO and O_2, and in addition, changing the preincubation time of two gases to clarify the reaction mechanism. In order to simplify the analysis of the reaction mixture, a known α-Toc analogue, 2,2,5,7,8-pentamethyl-6-chromanol (PMC), was adopted as a first substrate. It was found that high yields of products were obtained by controlling the amount and ratio of NO and O_2, and that the products distribution was varied by the ratio and mixing time of two gases. This paper describes these results.

When the reaction was carried out using PMC **1** and an equimolar amount of NO in air in dichloroethane (DCE), 2-(3-hydroxy-3-methylbutyl)-3,5,6-trimethyl-1,4-benzoquinone (PMCquinone) (**2**) was obtained in 33% yield accompanied by many minor products. The decrease of

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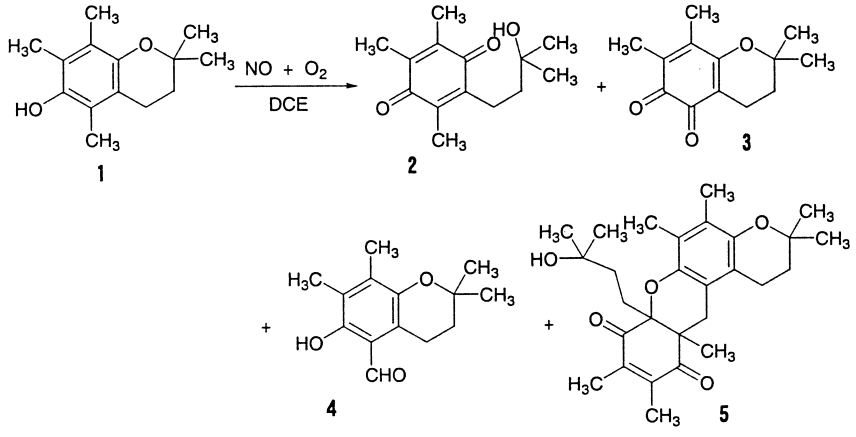
0960-894X/00/$ - see front matter
PII: S0960-894X(00)00563-1

O_2 amount made the reaction mixture simpler, and two major products were obtained whose structures were assigned as **2** and 2,2,7,8-tetramethylchroman-5,6-dione (PMCred) (**3**).[13] Among the other minor products, two compounds were identified as 5-formyl-2,2,7,8-tetramethyl-6-chromanol (**4**)[14] and 2,3-dihydro-3,3,5,6,9,10,11a-heptamethyl-7a-(3-hydroxy-3-methylbutyl)-1*H*-pyrano[2,3-*a*]xanthene-8(7a*H*),11(11a*H*)-dione (**5**)[15] (Scheme 1 and Table 1). All the reactions were carried out three times, and the reaction yields shown are mean values.

The reaction seldom proceeded by the mixing of PMC and 10 equiv of NO in the absence of O_2 (Table 1, entry 5), thus there seems to exist no interaction between PMC and NO. In the case of 1 equiv of NO (entry 1), however, about a half amount of PMC was consumed accompanied by formation of a small amount of **2**. The reason for these controversial phenomena was attributed to a slight contamination of oxygen in the experiment of entry 1, in which the inner pressure was lower than that of entry 5.[16] Product distribution varied when PMC and NO were allowed to stir for 2 h before the addition of O_2 (entry 2 versus entry 7). The results indicate that the non-productive interaction exists between PMC and NO in the absence of O_2, as suggested in the literature.[9] When 1 or 2 equiv of NO was used, PMC was consumed in the presence of 0.5 equiv of O_2 to give almost equimolar amounts of **2** and **3** (entry 9 and 10) and the yields became higher with lesser amount of NO (entry 10). In these cases, the timing of O_2 addition brought about a large effect on the products yields (entry 9 versus entry 8), which also suggests the direct interaction between NO and PMC in the absence of O_2. By decreasing the NO amount, it is necessary to make the reaction time longer, but the use of excess amount of O_2 resulted in the considerable consumption of PMC (entry 12). In this case, the minor products **4** and **5** were obtained more than in the cases under the former conditions. For the comparison of the reactivity, 1 equiv of NO_2 was used instead of NO and O_2, and the results were shown in entries 14–16. In a short reaction time (10 min), **2** was obtained in 41% yield without considerable formation of **3** (entry 15), and the yield of **3** gradually increased with the elongation of the reaction time. Although the reaction with NO_2 corresponds to the reaction with NO and 0.5 equiv of O_2 from the viewpoint of the stoichiometry, the results were different as shown in entries 14 and 10. Thus these also suggested that the formation of NO_2 was incomplete in the mixture of NO and 0.5 equiv of O_2.

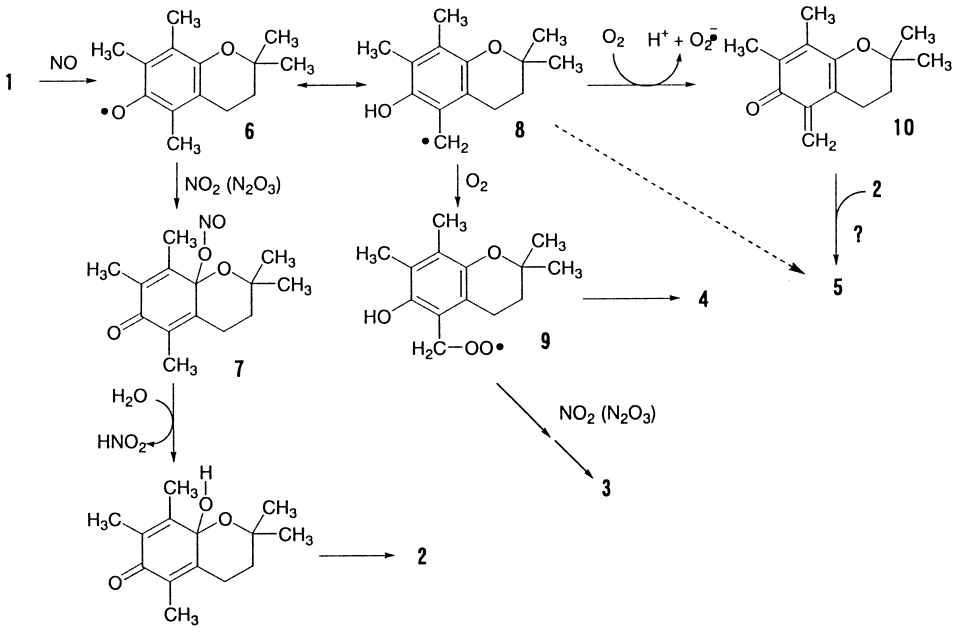


Scheme 1.

Table 1. The yie1ds[a] of four oxidation products of PMC by the reaction with NO in the presence of various amounts of oxygen

Entry	NO (equiv)	Added O_2 (equiv) 0 h	Added O_2 (equiv) 2 h	Reaction time (h)	Recovered PMC (%)	Yield of **2** (%)	Yield of **3** (%)	Yield of **4** (%)	Yield of **5** (%)
1	1	—	—	2	50.8 ± 3.1	7.2 ± 4.4	2.3 ± 0.7	2.4 ± 0.7	1.8 ± 0.4
2	1	—	1	2	0.0 ± 0.0	36.1 ± 7.6	53.8 ± 6.2	0.0 ± 0.0	2.5 ± 1.1
3	1	—	1	1	0.0 ± 0.0	31.3 ± 0.9	15.0 ± 6.5	2.8 ± 2.4	3.1 ± 1.7
4	1	—	1	0.2	22.6 ± 4.1	17.7 ± 2.5	3.7 ± 0.3	3.6 ± 0.4	3.2 ± 0.6
5	10	—	—	2	98.7 ± 3.7	1.9 ± 0.7	0.0 ± 0.6	0.0 ± 0.3	0.4 ± 0.2
6	10	—	1	0.2	0.0 ± 0.0	17.0 ± 2.6	16.0 ± 1.6	0.0 ± 0.0	0.0 ± 0.0
7	1	1	—	2	0.0 ± 0.0	17.2 ± 1.0	26.7 ± 1.2	0.0 ± 0.0	1.6 ± 0.6
8	2	0.5	—	2	5.0 ± 1.5	19.2 ± 6.3	15.3 ± 5.6	5.2 ± 1.0	5.1 ± 0.6
9	2	—	0.5	2	1.8 ± 1.4	38.3 ±10.1	35.7 ±12.0	7.2 ± 1.0	5.1 ± 2.1
10	1	—	0.5	2	0.0 ± 0.0	44.1 ± 6.6	45.8 ± 2.8	6.2 ± 3.6	2.9 ± 0.7
11	0.1	—	10	2	79.1 ± 2.5	6.3 ± 1.3	0.6 ± 0.4	0.0 ± 0.0	9.2 ± 0.8
12	0.1	—	10	24	28.9 ± 7.5	14.7 ± 2.0	2.2 ± 1.6	6.1 ± 0.9	16.7 ± 2.1
13	0.1	—	1	24	70.0 ± 2.4	5.8 ± 1.1	1.8 ± 0.4	1.4 ± 1.2	9.2 ± 1.7
14		NO_2 (1 equiv)		2	0.0 ± 0.0	51.1 ± 12.1	16.0 ± 9.6	2.5 ± 2.3	14.4 ± 1.3
15		NO_2 (1 equiv)		0.2	4.9 ± 7.0	40.9 ± 16.8	2.4 ± 0.6	0.0 ± 0.0	15.6 ± 0.8
16		NO_2 (1 equiv)		24	0.0 ± 0.0	22.6 ± 9.4	32.0 ± 14.1	0.0 ± 0.0	6.1 ± 5.5

[a]The yields were measured three times and represented with standard deviation.



Scheme 2.

Since the overall product yields are up to 90%, the results are thought to afford the rational background for the discussion of total reaction mechanism. Although there must be several pathways to give these products, one of the supposed reaction mechanisms is as shown in Scheme 2. It is well known that NO reacts with O_2 to form N_2O_3 or NO_2 according to the ratio of NO/O_2. Thus, based on the stoichiometry, the major reactive species in the reaction are regarded as NO_2 $(+N_2O_3)$ + little O_2 (entries 2–4, 7), N_2O_3 $(+NO)$ (entries 6, 8, 9), NO_2 (entry 10), and $NO_2 + O_2$ (entries 11–13), respectively, although these reactive species interconvert with each other in the reaction mixture. This interconversion is suggested by the fact that the result obtained from the use of NO_2 (entry 14) was different from that in entry 10. The reaction is supposed to commence with the hydrogen abstraction with NO, N_2O_3 or NO_2 to form phenoxy radical **6**. The data in Table 1 show that NO interacts with PMC without the aid of O_2, thus NO must have the reactivity toward PMC to give the phenoxy radical. In the presence of reactive NO_2 (or N_2O_3), **6** was supposed to be further oxidized by NO_2 (or N_2O_3) to form PMCquinone **2**. When active NO_x was decreased, this process must become slower, and oxygen can substitute for NO_x to oxidize **6**, and the reaction pathway is supposed to change into the formation of PMCred **3** or **4**. When the amount of NO_x was lowered further, the oxidation might proceed via the sole participation of oxygen after the initial formation of **6**. Since **5** was thought to be a product of Diels–Alder reaction of a quinonoid **10** and **2**,[15] the reaction was carried out in the presence of excess **2**, but the yield of **5** was not increased. Therefore, there must be an alternative pathway to the formation for **5** other than the one shown in Scheme 2. Even in the presence of 0.25 equiv of NO, PMC was consumed by excess O_2 and elongation of the reaction time (entries 11, 12). These data suggest there is a pathway where NO_2 might act in a catalytic manner for the oxidation. The similar results were reported by Kochi et al. that hydroquinone was oxidized by catalytic amounts of NO_2 in the presence of excess amount of oxygen.[17]

In this paper, we described the reaction of PMC and NO in the presence of various amounts of oxygen to form the products, four of which were identified and quantified. It is the first finding that the oxidized products were obtained in good yields by the restriction of the amounts of NO and oxygen. In addition, the product distribution was altered by the change of NO/O_2 ratio. Our preliminary experiments showed that the reaction with α-tocopherol gave analogous results presented in this paper, and these results will be reported in a near future.

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16. In our experiment, a septum, which is known to pass a small amount of gases through it, was used for a stopper of the reaction vessel. After the replacement of inner air with argon, the inner gases were monitored using GLC. In our reaction system using a reaction vessel with 50 mL of volume, it was shown that ca. 1 μL of oxygen was introduced to the inside per hour when the inner pressure was 1 atm. The increase of inner pressure should reduce this contamination process.
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