REACTION MECHANISMS OF CATECHINS ON DPPH RADICAL

Masayuki Suzuki[1], Masao Mori[1] and Fumio Nanjo[1]
1 Food Research Laboratories, Mitsui Norin Co, LTD., 223-1 Miyabara, Fujieda, 426-0133, Japan

Catechins , are the main constituents of tea and protect from many diseases. One of their main protective roles is the antioxidative effect against many kind of radicals. 1,2) We have previously reported the radical scavenging activity of catechins.1,3) In these report, the numbers of radical scavenging of catechins and related compounds at the initial stage of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging reaction were measured as stoichiometric factors, for the purpose of comparing the structure and radical scavenging activity. These stoichiometric factors were found to depend on the structure of their B-ring and existence or lack of the 3-*O*-gallate group. The stoichiometric factor 2 was shown by the catechol moiety, and 5 was shown by the pyrogallol moiety. However, after the rapid reaction of catechins and related compounds with DPPH radicals in the early stage, a slow reaction was observed to proceed.

In this report, we examined the mechanisms of radical scavenging reaction of 4-methyl catechol(4-MC) and (+)-catechin(+C) on the DPPH radicals for the purpose of elucidating the complex radical scavenging reaction of catechins.

Radical scavenging mechanism of 4-MC

Isolation and identification of reaction products formed from 4-MC after the reaction of the compound with an equal molar of the DPPH radicals in EtOH were performed. Two reaction products were obtained; One of which was relatively stable while the other was instable. FAB-MS and NMR analyses showed that the stable product is 4-methyl-5-ethoxy-*ortho*-benzoquinone (**3**). The unstable product formed as a sole reaction product when the reaction was carried out in CH_3CN. 4-MC and compound **3** were produced respectively from the instable compound by reduction with ascorbic acid and by the addition of EtOH. NMR experiments using the reaction mixture in combination with the above result clearly indicated that the instable product is 4-methyl-

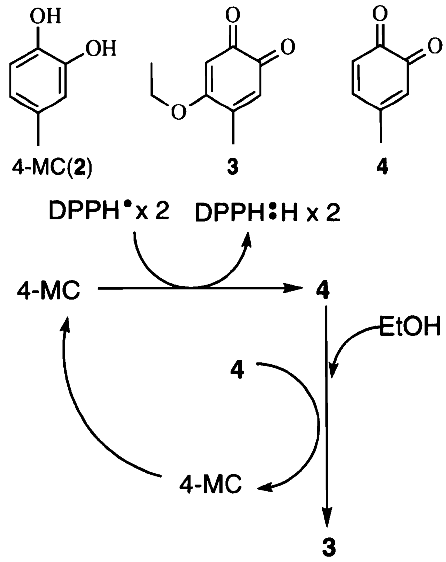
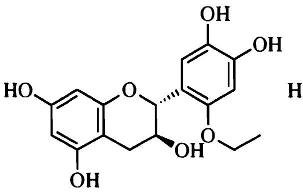


Fig. 1 Reaction mechanisms of 4-Methyl catechol with DPPH radicals.

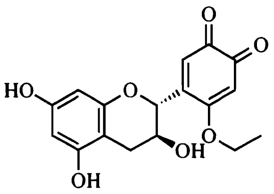
ortho-benzoquinone (**4**). The time course analysis of the formation of compounds **3** and **4** was performed by HPLC. Formation of the reaction intermediate (**4**) began to take place along with the rapid decrease of 4-MC immediately after initiating the reaction. Subsequent reaction in which the formation of **3** and the regeneration of 4-MC took place was observed along with the decrease of **4**. On the basis of above result, radical scavenging mechanisms of 4-MC on the DPPH radicals in EtOH were proposed as shown in Fig. 1.

Radical scavenging mechanism of +C

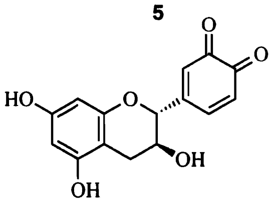
+C was reacted with an equal molar of DPPH radicals in EtOH under dark conditions. The reaction product **5** could not be isolated because of its instability. The structure of **5** was estimated from the structure of **6** which was produced from **5** by reduction of ascorbic acid. The *ortho*-bezoquinone form of +C (**7**) was identified as the first product of the scavenging reaction by the same experiments as used in the identification of **4**. On the other hand, when the reaction was conducted under irradiation of fluorescent or day light, compound **8**[6] was formed through **7**, while **5** was not formed.



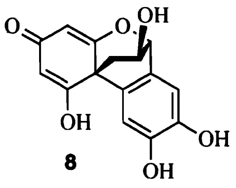
5



6



7



8

+C and 4-MC were found to have firstly a rapid scavenging activity followed by a slow scavenging ability. On the basis of the results obtained in this study the rapid scavenging activity was thought to be maintained until the catechol moiety in +C and 4-MC was converted to *ortho*-benzoquinone form. This reaction consumed 2 molecules of DPPH radicals which agreed with the stoichiometric factors of +C and 4-MC. Although the DPPH radical may be scavenged rapidly by 4-MC and +C which were regenerated in the process of formation of **3** and **5** from compounds **4** and **6** respectively, the apparent slow scavenging activity observed may be due to the rate-limiting reactions of **4** and **6** with EtOH.

1. Nanjo, F. ; Goto, K; Seto, R; Suzuki, M; Sakai, M.; Hara, Y. *Free Radical Biology & Medicine*, **1996**, *21*, 895
2. Nakayama, M.; Hirose, Y. *FFI Journal of Japan*, **1994**, *161*, 4
3. Nanjo, F.; Mori, M.; Goto, K.; Hara, Y. *Biosci Biotechnol Biochem.* **1999,** *63*, 1621
4. Hirose, Y.; Yamaoka, M.; Akashi, H.; Nozaki, H.; Fujita, T.; Nakayama, M. *Chemistry Letters* **1992**, 2361