STRUCTURE–ACTIVITY RELATIONSHIPS OF SYNTHESIZED PROCYANIDIN OLIGOMERS: DPPH RADICAL SCAVENGING ACTIVITY AND MAILLARD REACTION INHIBITORY ACTIVITY

Akiko Saito[1,2*] and Noriyuki Nakajima[2,3*]

[1]Present address: Antibiotics Laboratory, Chemical Biology Department, Advanced Science Institute, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan; E-mail: saiaki@riken.jp

[2] Biotechnology Research Center, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan

[3] Department of Biotechnology, Faculty of Engineering, Toyama Prefectural University, 5180, Japan; E-mail: nori@pu-toyama.ac.jp

Abstract – Procyanidins are known as condensed tannins and/or oligomeric flavonoids, and are present in many edible plants and show interesting various biological activities. We previously developed and reported a simple and versatile method of synthesizing procyanidin oligomers consisting of (−)-epicatechin and (+)-catechin. Here, we report the structure–activity relationships (SAR) between the DPPH (1,1-diphenyl-2-picrylhydrazyl) radical scavenging activity and Maillard reaction inhibitory activities of various procyanidin oligomers synthesized, including dimers, trimers, tetramers, pentamers, 3-*O*-substituted oligomers, and other artificial oligomers.

INTRODUCTION

Compounds with strong antioxidant and superior radical scavenging abilities are currently of great research interest. Furthermore, foods with ingredients that can degrade active oxygen and free radicals have recently received increased attention. The free radical scavenging activity was assessed by several methods such as, superoxide anions ($O_2\bullet^-$) generated by hypoxanthine (HPX)/xanthine oxidase (XOD),[1] 1,1-diphenyl-2-picrylhydrazyl (DPPH•),[2] •OH radicals, damaged DNA detection assay (3D assay).[3] It is widely believed that the polyphenols present in fruits and vegetables, as well as various health foods have a beneficial impact on health. Polyphenolic compounds are also increasingly studied because of their strong biological activities. For example, epigallocatechin-3-*O*-gallate (EGCG), a major polyphenol in

green tea, which belongs to the flavan-3-ol group, has notably been the focus of intense research interest for its protective effect against a variety of cancer types, such as lung, prostate, and breast.[4]

The nonenzymatic browning reaction by heating a mixture of sugar with protein or amino acid is known as the Maillard reaction, which occurs extensively in food and *in vivo*.[5] The relationship of the Maillard reaction to diabetic complications has been noted[6] in the field of sitology.[7] The aminoguanidine (AG),[4] aspirin,[8] and vitamin B_6,[9] have been reported as important Maillard reaction inhibitors. Particularly, quercetin[10] and components of tea extracts (catechin derivatives),[11] grouped as polyphenols, are also strong inhibitors.

Procyanidins are known as condensed tannins and/or oligomeric flavonoids[12,13] and present in various foods, such as, fruits, wine, tea, soybeans, *etc*. Separation of the individual structural analogs of procyanidins from the plants is very difficult because these compounds are presented as mixtures containing a number of structurally related compounds, and the isolation of compounds depend on the type of plant used. There are many studies on structure–activity relationships (SAR) of phenolic compounds,[14] however, research on SAR of procyanidins is less well understood. Development of a systematic technology for synthesizing procyanidins in pure form is useful and important. There have been numerous studies concerning the isolation, synthesis, and bioactivity of procyanidin oligomers.[15,16]

We previously developed a simple, versatile, stereoselective and size-controlled method of synthesizing procyanidin oligomers consisting of (–)-epicatechin (**1**) and (+)-catechin (**2**) (**Figure 1**). Our synthetic method is applicable to various (4–8) linked procyanidin oligomers, such as dimers and trimers, galloyl derivatives of dimers and trimers, and longer oligomers in good yields.[17] Here, we describe the SAR studies between DPPH radical scavenging activity and the Maillard reaction inhibitory activity[18] of previously synthesized procyanidin oligomers and their synthetic intermediates having an unnatural structure.
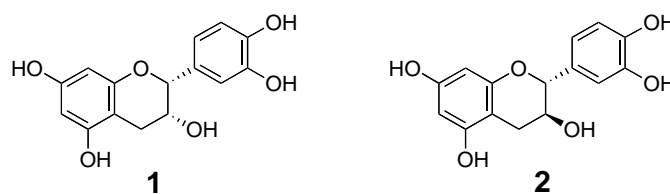

**Figure 1.** Structures of epicatechin (**1**) and catechin (**2**)

RESULTS AND DISCUSSION

DPPH radical scavenging activity and the Maillard reaction inhibitory activity

The SC_{50} values (concentration of 50% scavenging activity) of DPPH radical and the IC_{50} values (concentration of 50% inhibiting activity) of monomeric compounds exhibiting Maillard reaction

inhibitory activity are shown in **Table1**. The SC_{50} values of the DPPH radicals of (−)-epicatechin **1**, (+)-catechin **2**, and (−)-epigallocatechin **3** in natural products, were 2.2, 2.6 and 2.3 μM, respectively. Meanwhile, IC_{50} values of the inhibitory activity of the Maillard reaction of **1**, **2**, and **3** were 24, 5.0 and 12 μM, respectively. It appeared that the stereochemistry of the 3-hydroxy group affected Maillard reaction inhibitory activity, although radical scavenging activity was unaffected. Esters **4** and **7** showed SC_{50} values for the radical scavenging activity to be 4.0 and 4.3 μM, respectively. Compounds **5** and **6** in which the phenol groups are protected did not show DPPH radical scavenging activity at all. On the other hand, regarding the Maillard reaction inhibitory activity of compound **5**, in which the phenol groups are protected with acetyl groups, it had strong inhibitory activity equivalent to that of **1**. The results for monomeric compounds indicate that free phenol groups are necessary and that the stereochemistry of the 3-position does not affect DPPH radical scavenging activity. On the other hand, Maillard reaction inhibitory activities of catechin and its derivatives with 3β-hydroxy stereochemistry are higher than those of epicatechin compounds. In addition, acetylation compounds also have strong inhibitory activities against the Maillard reaction.

Table1. The DPPH radical scavenging and the Maillard reaction inhibitory activities of monomeric compounds
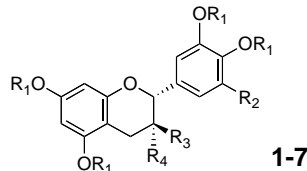
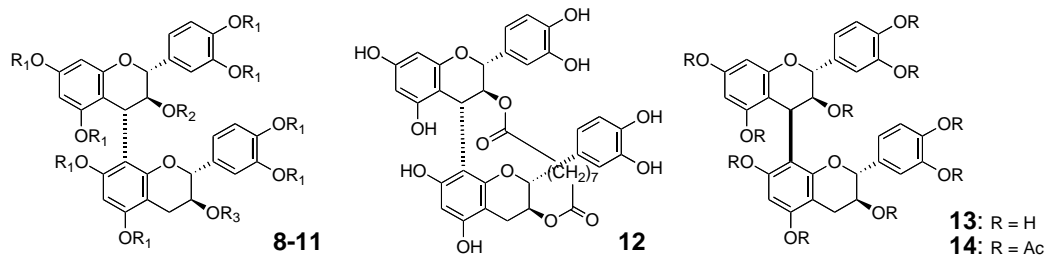| Entry	Structure	Compound	SC_{50} values (μM)	IC_{50} values (μM)
1	$R_1=R_2=R_3=H, R_4=OH$	**1** (epicatechin)	2.2	24
2	$R_1=R_2=R_4=H, R_3=OH$	**2** (catechin)	2.6	5.0
3	$R_1=R_3=H, R_2=R_4=OH$	**3** (epigallocatechin)	2.3	12
4	$R_1=R_2=R_4=H, R_3=OAc$	**4**	4.0	6.0
5	$R_1=Ac, R_2=R_4=H, R_3=OAc$	**5**	-[a]	5.0
6	$R_1=Me, R_2=R_4=H, R_3=OAc$	**6**	-[a]	-[a]
7	$R_1=R_2=R_4=H,$ $R_3=OC(O)CH_2CH_2CO_2H$	**7**	4.3	14

[a] No activity at 100 μM.

Table 2 shows the SC_{50} values of the DPPH radical scavenging activity and the IC_{50} values of the Maillard reaction inhibitory activity of synthetic various (+)-catechin dimers. (+)-Catechin-(4α-8)-(+)-catechin dimer **8**, known as procyanidin B3, and (+)-catechin-(4β-8)-(+)-catechin dimer **13** were isolated from various plants[9,10] and compounds **9-11** and

14 are their acetyl derivatives. Compound **12** is the diester derived from the synthetic intermediate of procyanidin B3 by intramolecular coupling (**Scheme 2**). The SC_{50} values of the radical scavenging activity of dimeric compounds **8**, **10**, **11**, **12**, and **13** were 1.3, 0.8, 1.8, 1.4 and 0.7 μM, respectively, and they were lower compared to that of catechin **2**, a monomeric compound. Interestingly, 3,4-*cis* isomer (**13**) of procyanidin B3 showed strong radical scavenging activity as compared with procyanidin B3 (**8**). This indicates that the radical scavenging activity of procyanidins is influenced not only by the number of phenolic groups but also by structure.[19] In the Maillard reaction inhibitory activity, the IC_{50} values of **8**–**14** were 3.0, 7.0, 1.0, 5.0, 7.3, 3.0 and 4.0 mM, respectively. Compounds **8**, **10**, **13**, and **14** inhibited more strongly than catechin **2**, but **9**, **11** and **12**, in which the 3- and 3"-positions are protected by ester groups, were not strong inhibitors in spite of double the number of phenol groups. 3-*O*-Acetate **10** was the strongest inhibitor of the Maillard reaction in the procyanidin B analogs.

It is well known that galloyl derivatives such as EGCG show strong biological activities. We have synthesized many gallate derivatives of procyanidins to enhance their activities. **Table 3** summarizes the results for the DPPH radical scavenging activity and the Maillard reaction inhibitory activities. The SC_{50} values of radical scavenging activity of gallate dimers **15-26** were 0.8, 0.7, 0.6, 0.8, 0.6, 0.6, 3.2, 1.1, 1.1, 0.9, 0.8 and 0.9 μM, respectively. Radical scavenging activity of the epicatechin−containing dimers, activity, the IC_{50} of **15**–**32** were in the level of 0.2 to 10 μM, respectively. It appears that the 3"-*O*-gallate dimers (**16**, **19**, **22**, and **25**), in which the bottom unit is substituted with the galloyl group, had relatively

Table 2. The DPPH radical scavenging and the Maillard reaction inhibitory activities of various (+)-catechin dimers

Entry	Structure	Compound	SC_{50} values (μM)	IC_{50} values (μM)
1	$R_1=R_2=R_3=H$	**8** (procyanidin B3)	1.3	3.0
2	$R_1=R_2=R_3=Ac$	**9**	-[a]	7.0
3	$R_1=R_3=H, R_2=Ac$	**10**	0.8	1.0
4	$R_1=H, R_2=R_3=Ac$	**11**	1.8	5.0
5	-	**12**	1.4	7.3
6	R = H	**13**	0.7	3.0
7	R = Ac	**14**	-[a]	4.0

[a]No activity at 100 μM.

weak inhibitory activity. The Maillard reaction inhibitory activity of the epicatechin–containing oligomers, **15–20** and **24–26**, increased in the following order, 3,3"-*O*-digallate > 3-*O*-gallate > 3"-*O*-gallate. On the other hand, the oligomers consisting of catechin showed different trends of 3-*O*-gallate > 3,3"-*O*-digallate >3"-*O*-gallate. In addition, the Maillard reaction inhibitory activity of these gallate-substituted oligomers was not correlated with the DPPH radical scavenging activity.

Table 4 summarizes the results for the DPPH radical scavenging and the Maillard reaction inhibitory activities of peracetyl 3-*O*-galloyl dimers. Peracetates **27-32** shows no DPPH radical scavenging activity at 100 μM. On the other hand, peracetates effectively inhibit the Maillard reaction, the IC_{50} values of Maillard reaction inhibitory activity were 1.2, 3.5, 3.5, 0.9, 4.2 and 0.6 μM, respectively. The Maillard reaction inhibitory activity of peracetate **27–32** was same level as free phenol dimers **15–26**.

The activities of longer oligomers and their peracetates were shown in **Table 5** and **Table 6**. SC_{50} values of radical scavenging activities of **33–43** were 0.7, 0.7, 1.2, 1.1, 0.9, 0.9, 0.8, 0.4, 0.6, 0.4, and 0.4 μM, respectively. Nearly all these compounds have strong radical scavenging activities. The galloyl trimers **40**

Table 3. The DPPH radical scavenging and the Maillard reaction inhibitory activities of 3-*O*-galloyl dimers
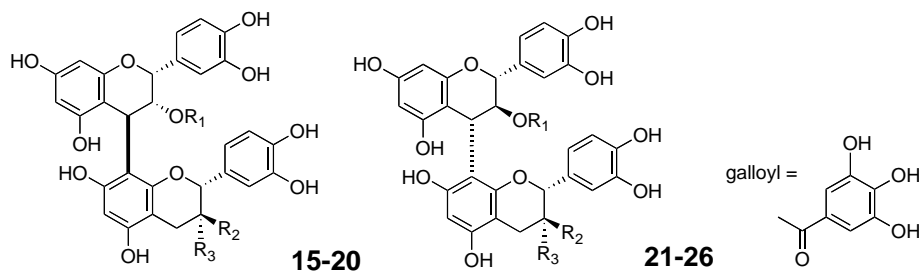
Entry	Structure	Compound	SC_{50} values (μM)	IC_{50} values (μM)
1	R₁=galloyl, R₂=OH, R₃=H	**15**	0.8	2.4
3	R₁=R₃=H, R₂=Ogalloyl	**16**	0.7	3.7
5	R₁=galloyl, R₂=Ogalloyl, R₃=H	**17**	0.6	0.4
7	R₁=galloyl, R₂=H, R₃=OH	**18**	0.8	0.9
9	R₁=R₂=H, R₃=Ogalloyl	**19**	0.6	5.5
11	R₁=galloyl, R₂=H, R₃=Ogalloyl	**20**	0.6	0.3
13	R₁=galloyl, R₂=OH, R₃=H	**21**	3.2	0.2
14	R₁=R₃=H, R₂=Ogalloyl	**22**	1.1	2.5
15	R₁=galloyl, R₂=Ogalloyl, R₃=H	**23**	1.1	1.0
16	R₁=galloyl, R₂=H, R₃=OH	**24**	0.9	3.0
17	R₁= R₂=H, R₃=Ogalloyl	**25**	0.8	10
18	R₁=galloyl, R₂=H, R₃=Ogalloyl	**26**	0.9	1.0

Table 4. The DPPH radical scavenging and the Maillard reaction inhibitory activities of peracetyl 3-*O*-galloyl dimers
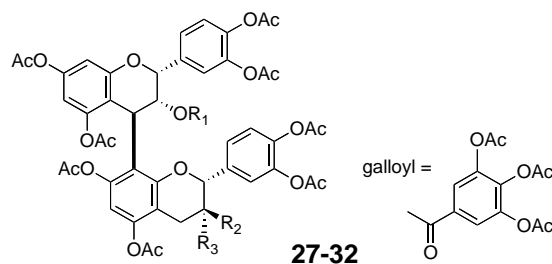
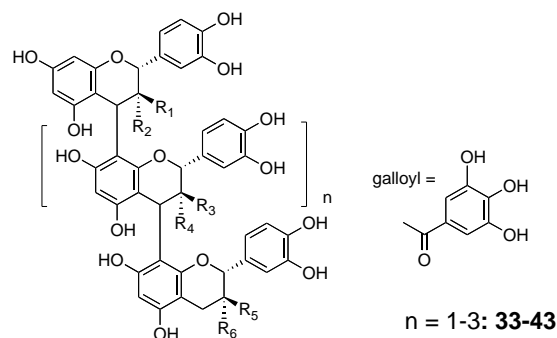| Entry	Structure	Compound	SC_{50} values (μM)	IC_{50} values (μM)
1	R_1=galloyl, R_2=OAc, R_3=H	**27**	- [a]	1.2
2	R_1=Ac, R_2=galloyl, R_3=H	**28**	- [a]	3.5
3	R_1=galloyl, R_2=Ogalloyl R_3=H	**29**	- [a]	3.5
4	R_1=galloyl, R_2=H, R_3=OAc	**30**	- [a]	0.9
5	R_1=Ac, R_2=H, R_3=Ogalloyl	**31**	- [a]	4.2
6	R_1=galloyl, R_2= H, R_3=Ogalloyl	**32**	- [a]	0.6

[a]No activity at 100 μM.

and **41**, tetramer **42**, and pentamer **43** was highly active.[21] These compounds also showed strong inhibitory activities against the Maillard reaction (IC_{50} values were 0.5, 0.4, and 0.7 μM, respectively), but the activity of the pentamer **58** was low (IC_{50} value was 3.9 μM). Unfortunately, it is not clear whether the number of hydroxyl groups and/or structure affect activity.

Table 6 summarizes the results for the DPPH radical scavenging activity and the Maillard reaction inhibitory activities of peracetates. Peracetates **44**-**48** shows the Maillard reaction inhibitory activity at 1.0, 1.5, 2.0, 1.4, and 1.9 μM, respectively but no DPPH radical scavenging activity at 100 μM. The Maillard reaction inhibitory activity of peracetate **44**–**48** was same level as phenol trimers **33**–**37**.

Collectively, among the 48 compounds we studied, **40** (SC_{50}: 0.4 μM, galloyl derivative of **33**), **42** (0.4 μM, tetramer), **43** (0.4 μM, pentamer), **41** (0.6 μM, galloyl-dimer, galloyl derivative of **34**), **17** (0.6 μM, galloyl-dimer), **19** (0.6 μM, galloyl-dimer), **20** (0.6 μM, galloyl-dimer), **13** (0.7 μM, dimer), **16** (0.7 μM, galloyl-dimer), **33** (0.7 μM, trimer), **34** (0.7 μM, trimer), **10** (0.8 μM, dimer), **15** (0.8 μM, galloyl-dimer), **18** (0.8 μM, galloyl-dimer), **25** (0.8 μM, galloyl-dimer), and **39** (0.8 μM, trimer) were effective as radical scavengers. Galloyl derivatives and tetramer and pentamer showed strong radical scavenging activity. On the other hand, it is interesting to note that no galloyl compound, such as catechin dimer analogs **10** and **13**, had relatively high activity, implying that radical scavenging activity is not proportional to the number of hydroxyl groups. Furthermore, reading the Maillard reaction inhibitory activity, **21** (IC_{50}: 0.2 μM, galloyl-dimer), **20** (0.3 μM), **17** (0.4 μM), **41** (0.4 μM), **40** (0.5 μM), **32** (0.6 μM, galloyl-dimer,

Table 5. The DPPH radical scavenging and Maillard reaction inhibitory activities of trimers, tetramer and pentamer


n = 1-3: **33-43**

Entry	Structure	n	Compound	SC_{50} (μM)	IC_{50} (μM)
1	$R_1=R_3=R_5=H$, $R_2=R_4=R_6=OH$	1	**33** (procyanidin C1)	0.7	0.8
2	$R_1=R_3=R_6=H$, $R_2=R_4=R_5=OH$	1	**34**	0.7	1.1
3	$R_1=R_4=R_5=H$, $R_2=R_3=R_6=OH$	1	**35**	1.2	1.4
4	$R_1=R_4=R_6=H$, $R_2=R_3=R_5=OH$	1	**36** (procyanidin C4)	1.1	1.9
5	$R_2=R_4=R_6=H$, $R_1=R_3=R_5=OH$	1	**37** (procyanidin C2)	0.9	0.8
6	$R_2=R_3=R_5=H$, $R_1=R_4=R_6=OH$	1	**38**	0.9	7.0
7	$R_2=R_3=R_6=H$, $R_1=R_4=R_5=OH$	1	**39**	0.8	3.0
8	$R_1=R_3=R_5=H$, $R_2=R_4=R_6=Ogalloyl$	1	**40**	0.4	0.5
9	$R_1=R_3=R_6=H$, $R_2=R_4=R_5=Ogalloyl$	1	**41**	0.6	0.4
10	$R_1=R_3=R_5=H$, $R_2=R_4=R_6=OH$	2	**42**	0.4	0.7
11	$R_1=R_3=R_5=H$, $R_2=R_4=R_6=OH$	3	**43**	0.4	3.9

peracetate of **20**), **42** (0.7 μM), **33** (0.8 μM, trimer), **37** (0.8 μM, trimer), **18** (0.9 μM), **30** (0.9 μM, galloyl-dimer, peracetate of **18**), **10** (1.0 μM), **23** (1.0 μM, galloyl-dimer), **26** (1.0 μM, galloyl-dimer) and **44** (1.0 μM, trimer, peracetate of **33**) were effective as inhibitors. Similar to radical scavenging activity, galloyl derivatives and longer oligomers have strong Maillard reaction inhibitory activity. Interestingly, **21**, which is a weak radical scavenger, shows strongest Maillard reaction inhibitory activity.

CONCLUSION

We studied the structure-activity relationships of synthesized procyanidins. In assays, galloyl derivatives and longer oligomers showed strong activities to the DPPH radical scavenging and the Maillard reaction inhibition. However, the SAR of procyanidins yielded complicated results in spite of simple *in vitro* assays such as assessing radical scavenging and Maillard reaction inhibitory activities. It is expected that the behavior of procyanidins *in vivo* is more complex. Further study is required to elucidate the SAR *in vitro* and *in vivo*. We are presently attempting to synthesize more various procyanidin analogs in order to examine their biological activities.

Table 6. The DPPH radical scavenging and Maillard reaction inhibitory activities of trimeric peracetates
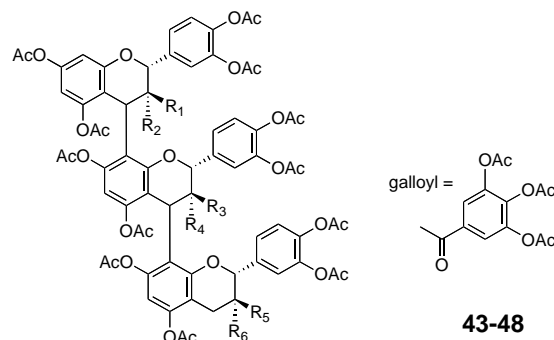
Entry	Structure	Compound	SC_{50} values (µM)	IC_{50} values (µM)
1	$R_1=R_3=R_5=H, R_2=R_4=R_6=OAc,$	**44**	$-^a$	1.0
2	$R_1=R_3=R_6=H, R_2=R_4=R_5=OAc$	**45**	$-^a$	1.5
3	$R_1=R_4=R_5=H, R_2=R_3=R_6=OAc$	**46**	$-^a$	2.0
4	$R_1=R_4=R_6=H, R_2=R_3=R_5=OAc$	**47**	$-^a$	1.4
5	$R_2=R_4=R_6=H, R_1=R_3=R_5=OAc$	**48**	$-^a$	1.9

^{a}No activity at 100 µM.

EXPERIMENTAL SECTION

DPPH radical scavenging activity[22-24]

All the samples were assayed after HPLC purification. DPPH radicals were measured as follows. A solution of DPPH radicals in EtOH (30 µM, 10 mL) was added to 1 mL of the test sample in DMSO and incubated at 30°C for 30 min. The scavenging activity was estimated by measuring the absorption of the reaction mixture at 517 nm using a microplate reader (Model 3550, BIO-RAD).

Maillard reaction inhibitory activity[18]

All the samples were assayed after HPLC purification. The evaluation of i*n vitro* glycation, the Maillard reaction was achieved by using a method described previously. In brief, 500 µL of reaction mixture was prepared containing 400 µg BSA (fraction V, Sigma Chemical Co. Ltd.), 200 mM glucose with or without 10 µL of the synthesized compound in 50 mM phosphate buffer pH 7.4, and the reaction mixture was heated to 60°C on a heat block for indicated hours. A negative control sample was kept at 4°C until measurement. After cooling, aliquots of 100 µL were transferred to new 1.5 mL plastic tubes and 10 µL of 100% w/v TCA was added to each tube. The supernatant containing glucose and the compound was removed after agitation and centrifugation (15,000 rpm, 4°C, 4 min.), and the precipitate of AGEs-BSA was dissolved with 400 µL of alkaline PBS to serve for screening. The changes in fluorescence intensity (ex. 370 nm, em. 440 nm) based on AGEs were monitored by using the spectrofluorometer RF-1500 (Shimadzu, Japan). Real inhibition activity was estimated by subtracting the quenching effect from the

apparent inhibitory activity, which was calculated by the above method. The quenching effect was measured by using the same sample dissolved with alkaline PBS after TCA treatment of the mixed solution of 2 µL of inhibitor solution and 100 µL of the control solution incubated for 30 hours.

REFERENCES AND NOTES

1. E. K. Hodgson and I. Fridovich, *Biochim. Biophys. Acta*, 1976, **430**, 182.

2. W. Brand-Williams, M. E. Cuvelier, and C. Berset, *Lebensm. Wiss. -Technol*., 1995, **28**, 25.

3. B. Salles, C. Provot, P. Calsou, and I. A. Hennebelle, *Anal. Biochem*., 1995, 231.

4. C. S. Yang, P. Maliakai, and X. Meng, *Annu. Rev. Pharmacol. Toxicol.*, 2002, **42**, 25.

5. A. Kanane and T. P. Labuza, *Prog. Clin. Biol. Res*., 1989, **304**, 301.

6. V. M. Monnier, R. R. Kohn, and A. Cerami, *Proc. Natl. Acad. Sci. U. S. A.,* 1984, **81**, 583.

7. M. Brownlee, H. Vlassara, A. Kooney, P. Ulrich, and A. Cerami, *Science*, 1986, **232**, 1629.

8. R. Huby and J. J. Harding, *Exp. Eye Res.*, 1988, **47**, 53.

9. A. A. Booth, R. G. Khalifah, and B. G. Hudson, *Biochem. Biophys. Res. Commun.*, 1996, **220**, 113.

10. Y. Morimitsu, K. Yoshida, S. Esaki, and A. Hirota, *Biochem. Biotechnol. Biochem.*, 1995, **59**, 2018.

11. T. Nakagawa, T. Yokozawa, K. Terasawa, S. Shu, and L. R. Juneja, *J. Agric. Food Chem.*, 2002, **50**, 2418.

12. J. B. Harborne, The Flavonoids: Advances in research from 1986. Chapman and Hall: London, 1993.

13. J. B. Harborne and H. Baxter, The Handbook of Natural Flavonoids, John Wiley & Sons, NY, (1999).

14. G. Cao, E. Sofic, and R. L. Prior, *Free Rad. Biol. Med.*, 1997, **22**, 749; W. Bors and C. Michel, *Free Rad. Biol. Med.*, 1999, **27**, 1413; M. Y. Moridani, G. Galati, and P. J. O'Brien, *Chemico-Biological Interactions*, 2002, **139**, 251.

15. K. Ohmori, N. Ushimaru, and K. Suzuki, *Proc. Natl. Acad. Sci. U. S. A.,* 2004, **101**, 12002; I. Tarascou, K. Barathieu, Y. Andre, I. Pianet, E. Dufourc, and E. Fouquet, *Eur. J. Org. Chem.,* 2006, 5367; P. K. Sharma, A. Kolchinski, H. A. Shea, J. J. Nair, Y. Gou, L. J., Jr. Romanczyk, and H. H. Schmitz, *Org. Process Res. Dev.,* 2007, **11**, 422; Y. Mohri, M. Sagehashi, T. Yamada, Y. Hattori, K. Morimura, T. Kamo, M. Hirota, and H. Makabe, *Tetrahedron Lett.,* 2007, **48**, 5891; K. Oyama, M. Kuwano, M. Ito, K. Yoshida, and T. Kondo, *Tetrahedron Lett.,* 2008, **49**, 3176; A. C. Matthew, S. L.

Bonnet, and J. H. van der Westhuizen, *Org. Lett.*, 2008, **10**, 3865; Y. Mohri, M. Sagehashi, T. Yamada, Y. Hattori, K. Morimura, T. Kamo, M. Hirota, and H. Makabe, *Heterocycles,* 2009, **79,** 549.

16. N. E. Es-Safi, S. Ghidouche, and P. H. Ducrot, *Molecules*, 2007, **12**, 2228.

17. A. Saito, N. Nakajima, A. Tanaka, and M. Ubukata, *Biosci. Biotechnol. Biochem.*, 2002, **66**, 1764; A. Saito, N. Nakajima, A. Tanaka, and M. Ubukata, *Tetrahedron,* 2002, **58**, 7829; A. Saito, N. Nakajima, A. Tanaka, and M. Ubukata, *Tetrahedron Lett.*, 2003, **44**, 5449; A. Saito, N. Nakajima, A. Tanaka, and M. Ubukata, *Heterocycles,* 2003, **61**, 287; A. Saito, N. Nakajima, N. Matsuura, A. Tanaka, and M. Ubukata, *Heterocycles*, 2004, **62**, 479; A. Saito, A. Tanaka, M. Ubukata, and N. Nakajima, *Synlett,* 2004, 1069; A. Saito, A. Tanaka, M. Ubukata, and N. Nakajima, *Synlett,* 2004, 2040; A. Saito, Y. Doi, N. Matsuura, A. Tanaka, M. Ubukata, and N. Nakajima, *Bioorganic & Medicinal Chemistry,* 2004, **12**, 4783; A. Saito, M. Emoto, A. Tanaka, Y. Doi, K. Shoji, Y. Mizushina, H. Ikawa, H. Yoshida, N. Matsuura, and N. Nakajima, *Tetrahedron*, 2004, **60**, 12043; A. Saito, Y. Mizushina, H. Ikawa, H. Yoshida, Y. Doi, A. Tanaka, and N. Nakajima, *Bioorganic & Medicinal Chemistry*, 2005, **13**, 2759; H. Sakuda, A. Saito, Y. Mizushina, H. Ikawa, H. Yoshida, A. Tanaka, and N. Nakajima, *Heterocycles*, 2006, **67**, 175.

18. N. Matsuura, T. Aradate, C. Sasaki, H. Kojima, J. Hasegawa, and M. Ubukata, *J. Health Sci.*, 2002, **48**, 520.

19. C. A. Rice-Evans, N. J. Miller, and G. Paganga, *Free Rad. Biol. Med.*, 1996, **20**, 933.

20. Previous studies obtained same results. N. Saint-Cricq de Gaulejac, C. Provost, and N. Vivas, *J. Agric. Food Chem.*, 1999, **47**, 425; M. Dueñas, T. Hernández, I. Estrella, and R. Rabanal, *Food Chemistry*, 2003, **82**, 373.

21. These data was also reported to the following paper. A. Saito, Y. Mizushina, A. Tanaka, and N. Nakajima, *Tetrahedron*, 2009, **65**, 7455.

22. M. S. Blois, *Nature,* 1958, **181**, 1199.

23. F. Nanjo, K. Goto, R. Seto, M. Suzuki, M. Sakai, and Y. Hara, *Free Radical Biol. Med.*, 1996, **21**, 895.

24. T. Hatano, H. Miyatake, M. Natsume, N. Osakabe, T. Takazawa, H. Ito, and T. Yoshida, *Phytochemistry*, 2002, **59**, 749.