

Antioxidant Activity of Hydroxyflavonoids

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The antioxidant activity of 28 natural and synthetic hydroxyflavonoids was estimated through the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, superoxide scavenging activities and lipid peroxidation inhibition. The result showed the hydroxylation pattern had close relationship with the appearance of activities.

Key words — hydroxyflavonoid, antioxidant activity, structure-activity relationship

INTRODUCTION

Flavonoids are most abundantly distributed as polyphenols in plant kingdom and their structures are diversified by oxidation, alkylation, and glycosylation *etc.*¹⁾ Many flavonoids have been isolated from medicinal plants, fruits and vegetables plants as biological active constituent. Antioxidant activity is prominent among their various biological activities. Although many antioxidant activities of the naturally occurring flavonoids have examined, there are few reports on mechanism of antioxidation or the structure-activity relationship. In the present paper, we report the systematic antioxidant activi-

ties [1,1-diphenyl-2-picrylhydrazyl (DPPH) radical scavenging, the superoxide scavenging and the lipid peroxidation inhibition] used 28 natural and synthetic flavonoids. In addition, the structure-activity relationship of hydroxyflavonoids is mentioned.

MATERIALS AND METHODS

Materials — Among the estimated flavonoids, **1**, **5–9**, **16–22**, **25** and **27** were commercially purchased or previously isolated compounds from plant materials.

Synthesis of Flavonoids — 4'-Benzyloxy-2'-hydroxy-, 4-benzyloxy-2'-hydroxy- and 3,4-dibenzyloxy-2'-hydroxychalcones were converted to the corresponding flavones by 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) oxidation in dry dioxane,²⁾ following by debenylation (Pd/C-H₂) to lead to **2–4**. 2'-Hydroxy-, 4'-benzyloxy-2'-hydroxy-, 2-benzyloxy-2'-hydroxy-, 3-benzyloxy-2'-hydroxy- and 3,4-dibenzyloxy-2'-hydroxy-chalcones were oxidized by the Algar-Flynn-Olyamada (AFO) method³⁾ to prepare **10** and the corresponding benzylated flavonols (7-benzyloxy-, 4'-benzyloxy-, 2'-benzyloxy-, 3'-benzyloxy- and 3',4'-dibenzyloxy flavonols). The resultants were debenzylated by same manners described above to lead to **11–14** and **23**. On the other hand, 3',4'-dibenzyloxyflavonol was methylated in CH₃I/K₂CO₃ after debenylation to give **24**. Flavone **15** was obtained by methylation of **12**. The structures of synthetic flavones were confirmed by the analysis of EIMS and ¹H NMR spectral data. The numbering system of flavone and chalcone are shown in Fig. 1.

Antioxidant Activity — DPPH radical scavenging,^{4,5)} superoxide dismutase (SOD) scavenging activities (NBT method),⁶⁾ and inhibition of lipid peroxidation (TBA method)⁷⁾ were evaluated according to the literatures.

RESULTS AND DISCUSSION

Among the estimated flavonoids (Table 1), unsubstituted flavone (**1**) and three monohydroxylated flavones (**2**, **3** and **10**) were inactive in any screening systems. Chrysin (**5**) which is introduced a hydroxyl group at C-5 in **2** showed a week activity in superoxide scavenging activity. An additional hydroxylation at C-4' of **5** (**6**: apigenin) showed slight increment in activities. However, naringenin (**9**) hy-

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drogenated at C-2 and C-3 (so-called flavanone), its activities completely disappeared. These results indicated that the presence of double bond at C-2 and C-3 was essential for the activity.⁸⁾ Although **6** showed the weak activities, a dramatic increment of the activities was observed in luteolin (**7**) with 3',4'-dihydroxyl groups. A synthetic flavone (**4**) with 3',4'-dihydroxyl groups also showed strong activities simi-

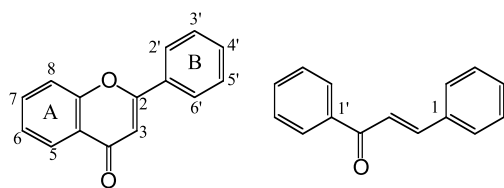


Fig. 1. Structures of Flavone and Chalcone

lar to **7**. From these results, it could be proposed that only 3',4'-dihydroxyl groups was needed for the appearance of activities. The activities of **24** and **25** could explain this proposal.

Further hydroxylation of **2** at C-3 (**11**) caused a slight increment of the activities, but **2** and **10** showed no activities. Drastic change was observed by the introduction of hydroxyl group at C-5 of **11** (galangin: **16**).

Among 3-hydroxyflavones (flavonols) with a hydroxyl group on B-ring (**12–14**), **12** showed stronger activities compared with **3** and **10**. There were no activities seen in **15** which is a methylated product of **12**. Although **13** showed weak scavenging activities, further hydroxylation at C-6' (**28**) caused an increment of the scavenging activity of DPPH radical.

Table 1. Antioxidant Activities (μM) of Flavonoids

| compound | DPPH | SOD | LPO |
|--|------|------|------|
| 1 flavone | a) | a) | a) |
| 2 7-hydroxyflavone | a) | a) | a) |
| 3 4'-hydroxyflavone | a) | a) | a) |
| 4 3',4'-dihydroxyflavone | 2.1 | 9.4 | 47 |
| 5 5,7-dihydroxyflavone (chrysin) | a) | 165 | a) |
| 6 5,7,4'-trihydroxyflavone (apigenin) | a) | 44 | 133 |
| 7 5,7,3',4'-tetrahydroxyflavone (luteolin) | 1.8 | 6.6 | 16 |
| 8 5,6,7-trihydroxyflavone (baicalein) | 5.1 | ND | 13 |
| 9 5,7,4'-trihydroxyflavanone (naringenin) | a) | a) | a) |
| 10 3-hydroxyflavone (flavonol) | a) | a) | a) |
| 11 3,7-dihydroxyflavone | 65 | 193 | a) |
| 12 3,4'-dihydroxyflavone | 4.4 | a) | 37 |
| 13 2',3-dihydroxyflavone | 70 | 138 | 106 |
| 14 3,3'-dihydroxyflavone | a) | a) | a) |
| 15 3,4'-dimethoxyflavone | a) | a) | a) |
| 16 3,5,7-trihydroxyflavone (galangin) | 11 | 63 | 56 |
| 17 3,5,7,4'-tetrahydroxyflavone (kaempferol) | 7.1 | b) | 86 |
| 18 kaempferol 4'-O-methylether | 13 | 12 | 23 |
| 19 3,5,7,3',4'-pentahydroxyflavone (quercetin) | 2.2 | b) | 15 |
| 20 3,5,7,3',4',5'-hexahydroxyflavone (myricetin) | 1.6 | b) | 33 |
| 21 3,5,7,2',4'-hydroxyflavone (morin) | 6.9 | 53 | 73 |
| 22 3,7,3',4'-tetrahydroxyflavone (fisetin) | 1.1 | 8 | 20 |
| 23 3,3',4'-trihydroxyflavone | 2.1 | 33 | 26 |
| 24 3-methoxy-3',4'-dihydroxyflavone | 1.4 | 46 | 21 |
| 25 quercetin 3-O-glucoside | 9.2 | 7.9 | 19 |
| 26 quercetin 4'-O-glucoside | 2.1 | 17 | 33 |
| 27 kaempferol 3-O-glucoside | a) | a) | a) |
| 28 3,2',6'-trihydroxyflavone | 9.5 | ND | ND |
| α -tocopherol | 5.2 | 2100 | 1000 |

a) > 200 mM. DPPH: scavenging activity of 1,1-diphenyl-2-picrylhydrazyl. SOD: scavenging activity of superoxide (NBT method). LPO: inhibitory activity of lipid peroxidation (TBA method). b) reacted with reagent before addition of enzyme. ND: not determined.

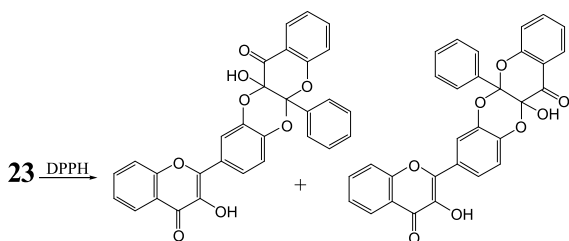


Fig. 2. Dimerization of 3,3',4'-Hydroxyflavone by DPPH Radical

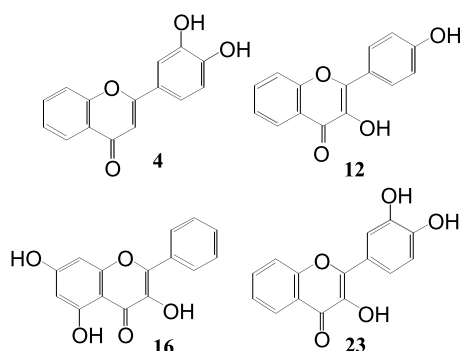


Fig. 3. Typification of Flavones Possessing Antioxidant Activities

Naturally occurring flavonol aglycones (**17**, **19**–**22**) showed strongly DPPH scavenging activity. Among them, **19**, **20** and **22** have 3',4'-dihydroxyl groups on B-ring. A synthetic flavonol (**23**) which has 3',4'-dihydroxyl groups similar to **19**–**22** also showed the strong activities. In the previous paper,⁹⁾ we described that quercetin (**19**) rapidly react with DPPH radical to afford two dimers. The dimerization was initiated by pulling off of hydrogen at C3-OH by DPPH radical. It could then be suggested that **20**, **22** and **23** scavenged DPPH radical by same manners as quercetin (Fig. 2). The flavonols **17** (kaempferol) and **21** (morin) had the active partial structures such as 3,5,7-(OH)₃ and 3,4'-(OH)₂ mentioned above. The activities observed in **17** and **21** could be explained as same as a **16** (galangin) type and/or a 3,4'-(OH)₂ flavone type. The activities of **18** and **26** were explained by the fact that the flavonols have a same partial structure as galangin. A flavone with 5,6,7-trioxygenated groups on A-ring (**8**: baicallein) showed a stronger activities compared

with **5**. The activities of **8** could be explained by the existence of a catechol or pyrogallol unit in A-ring.

From the results obtained above, hydroxyflavones possessing antioxidant activities except for **8** and **28** could be classified to four types based on the partial structures as drawn in Fig. 3. The present results will be useful to evaluate the antioxidant activities of hydroxyflavones. Although Burda *et al.*⁸⁾ had discussed the structure-activity relationship of the antioxidant activity of flavonoids, this paper provides further detailed information of antioxidant activity of flavonoids.

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