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Structure-antioxidant Activity Relationships of Isoflavonoids*1

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ABSTRACT

The antioxidant activities of six isoflavonoids isolated from *Sophora japonica* wood and bark were examined by DPPH (1,1-diphenyl-2-picrylhydrazyl) free radical scavenging method. This study was focused on the relationship between antioxidant activity of isoflavonoids and their chemical structures. From the results of this study, it could be concluded that the hydroxyl groups that linked at ring B and ring A in isoflavonoids have importance in the antioxidant activity. Additionally, the absence of the 2,3-double bond on the isoflavonoid enhances its antioxidant activity.

Keywords : antioxidant activity, isoflavonoid, structure-activity relationships

1. INTRODUCTION

Flavonoids are phenolic substances which consist of two phenyl rings connected by a three-carbon bridge and exhibit biological activities like antiviral, antiinflammatory and antiallergic actions (Bohm, 1998). Antioxidant activity of flavonoids has been also studied because of their ability to reduce free radical formation and to scavenge free radicals.

Oxidation can be defined as the transfer of electrons from one atom to another and its occurrence in living organisms is known to cause damage to DNA, protein and lipids (Maxwell, 1995). This damage appears to be one of the major ageing factors of living organisms such as cancer, cardiovascular disease, immune system decline and brain dysfunction. Among antioxidants, derived from natural products, which protect living organisms from these damages are polyphenols such as flavonoids, tannins and lignans (Kandaswami & Middletone, 1994).

Among these polyphenols, flavonoids, which contain fifteen carbon atoms in their molecules and arrange in a C_6-C_3-C_6 configuration, have some significant antioxidant activity (Hanasaki *et al.*, 1994). The flavonoids containing a number of hydroxyl groups on ring A, B and C have antioxidant activity because of their electron donating ability. The glycosylation of flavonoids

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reduces their antioxidant activity compared with their aglycones. The ability of flavonoids to act as antioxidants and structure-antioxidant activity relationships has been extensively established (Rice-Evans *et al.*, 1996; Coa *et al.*, 1997).

However, the study on antioxidant activity of isoflavonoids and relationships between activity and chemical structure are less documented. Generally, isoflavonoids are based on a 3-phenylchroman skeleton, instead of a 2-phenylchroman skeleton and are almost restricted to the subfamily Papilionoidae of Leguminosae. As important secondary metabolic compounds, isoflavones have been reported to play essential roles in preventing certain types of cancers and in reducing the risk of cardiovascular diseases (Guo *et al.*, 2002). They are also known to reduce the activity of hemolysis and express estrogenic activities in animals.

In this study, we examined the antioxidant activity of isoflavonoids which were isolated from *S. japonica* and compared their activities with chemical structure of isoflavonoids in order to establish the relationship between isoflavonoids structure and their free radical scavenging activities.

2. MATERIALS and METHODS

2.1. Materials

Six isoflavonoids were used in antioxidant activity test. Among these compounds, irisolidone (1), biochanin A (2), formononetin (3) and dihydro-formononetin (4) were isolated from wood of *Sophora japonica* (Park *et al.*, 2001), and calycosin (5) and genistein (6) from its bark (Park *et al.*, 2002). These compounds were identified by spectroscopic methods including MS, ^{1}H-, ^{13}C- and 2D-NMR analysis.

2.2. Antioxidant Activity

2.2.1. Chemicals

DPPH (1,1-diphenyl-2-picrylhydrazyl) was purchased from Sigma Co. and other reagents were the guaranteed grade.

2.2.2. Antioxidant activity

The antioxidant activity was measured by slightly modified method of Blois (1958), as described below. MeOH solution (4 mℓ) of samples (100 ppm) was added to a solution of DPPH in MeOH (4.5×10^{-4} M, 1 mℓ) and the reaction mixture was shaken vigorously. After storing at room temperature for 30 min, the remaining amounts of DPPH were determined by UV/Vis spectrophotometer (8452A Diode Array Spectrophotometer, Hewlett Packard Co.) at 520 nm. The mixture of 4 mℓ MeOH with a solution of 1 mℓ DPPH was used as control. The antioxidant activity of each sample was expressed as percentage of a decrease in absorbance of DPPH against that of a control.

3. RESULTS and DISCUSSION

3.1. Structure of Isoflavonoids

The antioxidant activity of six isoflavonoids were examined by DPPH radical scavenging method. Fig. 1 and Table 1 show the chemical structures and characteristics of isoflavonoids which were used in this study. All isoflavonoids have a methoxyl group in ring B (C-4') except genistein. In ring A, one or two hydroxyl groups are attached to compounds. Irisolidone has one more methoxyl group at C-6 position comparing with biochanin A.

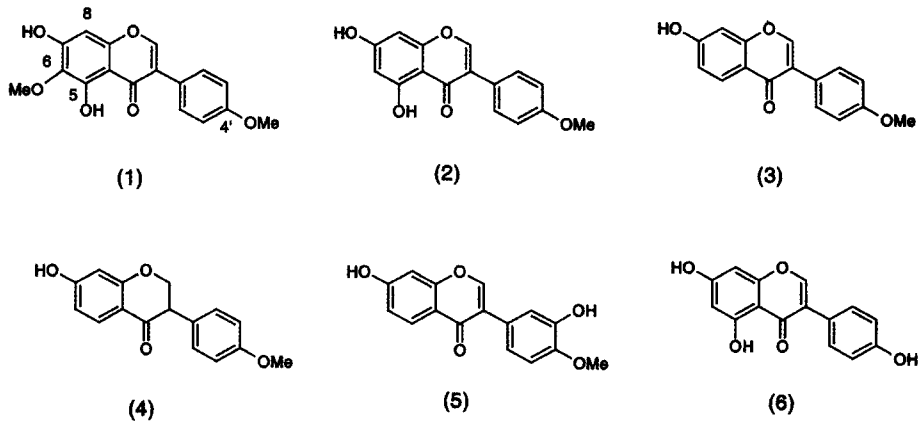


Fig. 1. Chemical structure of isoflavonoids used in this study.

Table 1. Isoflavonoids used in antioxidant activity test

Compounds	Family	Substituents				
		C-5	C-6	C-7	C-3'	C-4'
irisolidone (1)[a]	isoflavone	OH	OCH_3	OH	H	OCH_3
biochanin A (2)	isoflavone	OH	H	OH	H	OCH_3
formononetin (3)	isoflavone	H	H	OH	H	OCH_3
dihydroformononetin (4)	isoflavanone	H	H	OH	H	OCH_3
calycosin (5)	isoflavone	H	H	OH	OH	OCH_3
genistein (6)	isoflavone	OH	H	OH	H	OH

[a] Numbers in Fig. 1

3.2. Structure Relationships of Antioxidant Activity

3.2.1. Influence of B ring on antioxidant activity

The hydroxyl group on ring B of isoflavonoids generally increases their antioxidative activity. Genistein (6), which has hydroxyl groups on C-4' showed high radical scavenging activity (32.6%) whereas biochanin A (2) (a methoxyl group on C-4') exhibited low scavenging activity value (10.6%) on DPPH. Comparing the formononetin (3) with calycosin (5), the latter one with one hydroxyl group on ring B (C-3') had higher antioxidant activity (31.9%) than formononetin (5.8%). From these results, we could conclude that hydroxyl group increases the antioxidant activity of isoflavonoids. Comparing antioxidant potencies of genistein, daidzein and biochanin A, Pietta (2000) also determined that the absence of the hydroxyl group at C-4' position of isoflavonoids diminished their activities. Genistein was found to be a more potent antioxidant than daidzein and biochanin A.

3.2.2. Influence of A ring on antioxidant activity

To examine the influence of substituents of ring A on antioxidative activity of isoflavonoids, the activities of irisolidone (1), biochanin A (2)

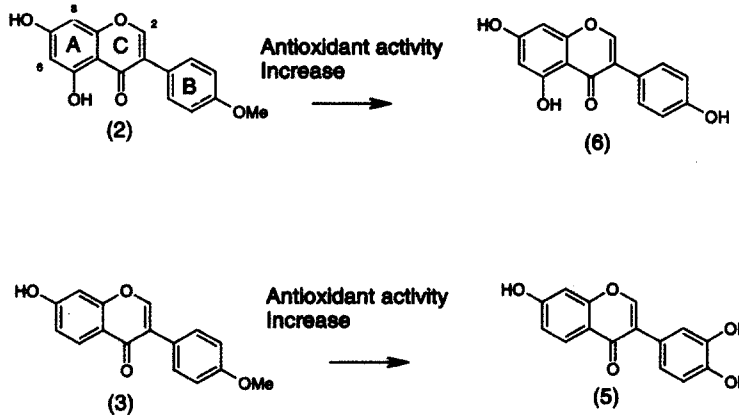


Fig. 2. The influence of hydroxyl group in the B ring on the antioxidant activity of the isoflavonoids.

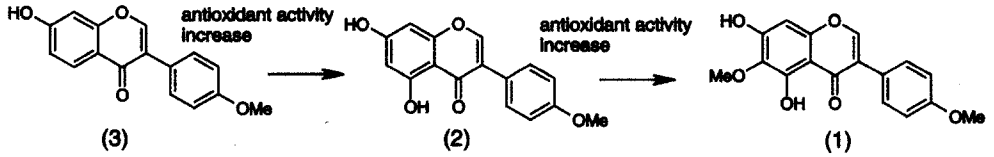
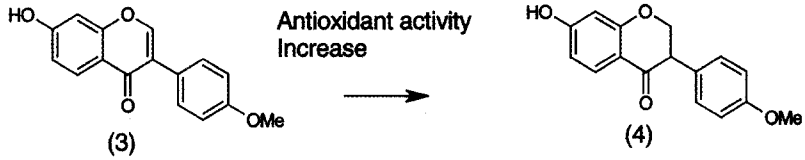


Fig. 3. The influence of substituents in the A ring on the antioxidant activity of the isoflavonoids.

and formononetin (3) were measured by DPPH method. As shown in Fig. 3, the order of the radical scavenging activity against DPPH radicals is irisolidone (12.6%) > biochanin A (10.6%) > formononetin (5.8%), under the same experimental condition. Among three compounds, irisolidone was the most effective antioxidant in the DPPH method. The antioxidant activity of biochanin A (2), with lacking the C-6 methoxyl group of irisolidone, was less effective than that of irisolidone. The antioxidant activity of formononetin (3), with lacking the C-7 position hydroxyl group of biochanin A, was also less effective than that of biochanin A. According to Cooper-Driver & Bhattacharya (1998), the antioxidant activity of flavonoids is based on the presence of hydroxyl group at C-5 or C-7. However, the present investigation shows that the presence of hydroxyl group and methoxyl group on ring A increases the antioxidant activity of isoflavonoids.

3.2.3. Influence of C ring on antioxidant activity

To elucidate the influence of ring C on antioxidant activity of isoflavonoids, formononetin (3) and dihydroformononetin (4) which do not have C-2 double bond on C ring, were used. From the results of Fig. 4, the activity of dihydroformononetin (7.7%) is higher than that of formononetin (5.8%). This result suggests that the absence of the C-2 double bond enhances antioxidant activity. These results are interesting because it is believed that the presence of C-2 double bond increases the antioxidant activities of flavonoids. According to the study of Arora *et al.* (1998), the higher antioxidant activity of dihydroformononetin may be a result of its non-planar structure that confers dihydroformononetin with a greater flexibility for conformational changes.



(3) (4)

Fig. 4. The influence of the absence of the C-2 double bond in the C ring on the antioxidant activity of the isoflavonoids.

4. CONCLUSIONS

In order to find out the structure-antioxidant activity relationship of isoflavonoids, six isoflavonoids which were isolated from *S. japonica* wood and bark, were used for DPPH free radical scavenging activity. From the results of this study, we can conclude that the number and position of hydroxyl groups was found to be an important determinant of antioxidant activity. Hydroxyl groups at the C-4' and C-3' on ring B and at the C-5 and C-7 position on ring A play an important role in the antioxidant activity of isoflavonoids. In addition, the absence of the C-2 double bond of isoflavonoids increase the antioxidant activity.

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