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Structure-activity relationship of natural flavonoids in hydroxyl radical-scavenging effects

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KEY WORDS flavonoids; hydroxyl radical; structure-activity relationship; chemiluminescence

ABSTRACT

AIM: To study the relationship between the structure and hydroxyl radical ($^{\cdot}OH$)-scavenging activity of twelve natural flavonoids. **METHODS**: The hydroxyl radical-generating chemiluminescence system with ascorbate-$CuSO_4$-yeast-H_2O_2 was used to determine the hydroxyl radical-scavenging activity of twelve natural flavonoids. **RESULTS**: Guercetin, heliosin, hyperoside, kaempferol, baicalin, corylifolin, lysionotin, matteucinol, corylifolinin, and genistein could effectively scavenge $^{\cdot}OH$ and inhibit the chemiluminescence of the system. The IC_{50} values (95 % confidence limits) of the flavonoids were 12.1 (9.9 - 14.5) g/L, 15.8 (14.0 - 19.2) g/L, 19.5 (16.8 - 27.4) g/L, 20.1 (13.6 - 29.0) g/L, 34.6 (28.4 - 43.4) g/L, 66.8 (63.2 - 74.4) g/L, 187 (147 - 235) g/L, 211 (165 - 284) g/L, 262 (190 - 346) g/L, and 708 (498 - 994) g/L, respectively; whereas nobilelin and corylifolin-Ac could not scavenge $^{\cdot}OH$. **CONCLUSION**: (1) Phenolic hydroxyls in flavonoids were the main active groups capable of scavenging $^{\cdot}OH$; (2) Hydroxyl groups in ring B and A were important $^{\cdot}OH$-scavenging active groups; (3) The ortho-dihydroxyl groups in ring A and/or B could greatly enhance the $^{\cdot}OH$-scavenging activity of the rings; (4) Comparing the IC_{50} values of guercetin, heliosin, hyperoside, baicalin, lysionotin, and matteucinol, it was suggested that the hydroxyl groups on 3′,4′ position of ring B possessed high $^{\cdot}OH$-scavenging activity and the scavenging activity of hydroxyl groups in ring B was higher than that of hydroxyl groups in ring A. The hydroxyl group or glucoside on 3 position of ring C of the above mentioned 6 flavonoids was also related to the $^{\cdot}OH$-scavenging ability. (5) The structural types of flavonoids themselves could influence their $^{\cdot}OH$-scavenging activity.

INTRODUCTION

Flavonoids exist widely in the plant kingdom. They are important components of many Chinese traditional medicines. Flavonoids have been paid much attention due to their diverse pharmacological effects such as anticancer, anti-oxidation, anti-aging, antibacterial, and antiviral activities[1-4]. Although in recent years many researches had been focused on the free radical scavenging activity of flavonoids, the investigations on the structure-activity relationship of flavonoids were limited. Furthermore, these few investigations were conducted using mainly synthetic flavonoids in addition to some commonly used natural compounds. The close structure-activity relationship of eleven natural flavonoids of high purity in superoxide anion ($O_2^{\cdot-}$)-scavenging effects had been revealed by authors using a sensitive chemiluminescence system[5]. To further elucidate the structural mechanism of flavonoids in free radical-scavenging activity and to provide new experimental data for the future exploitation and utilization of flavonoids as health-care nutrients or medicaments, our present study aimed at the structure-activity relationship of twelve natural flavonoids (including the above mentioned eleven compounds) in $^{\cdot}OH$-scavenging effects, by using a highly sensitive chemiluminescence system with ascorbate-$CuSO_4$-yeast-H_2O_2 as $^{\cdot}OH$- generator[6].

MATERIALS AND METHODS

Materials Quercetin of 99 % purity was purchased from Fluka Co; heliosin, hyperoside, kaempferol,

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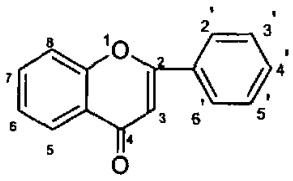
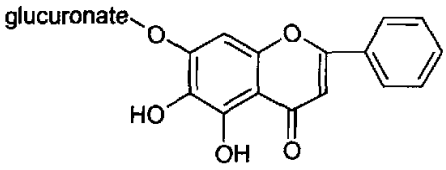
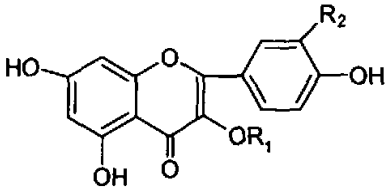


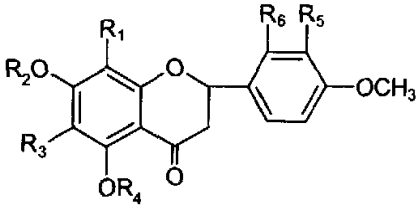
Core of flavonoids



Baicalin

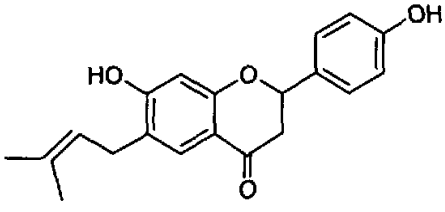


Quercetin: R_1=H, R_2=OH
Heliosin: R_1=Digalactose, R_2=OH
Hyperoside: R_1=galactose, R_2=OH
Kaempferol: R_1=R_2=H

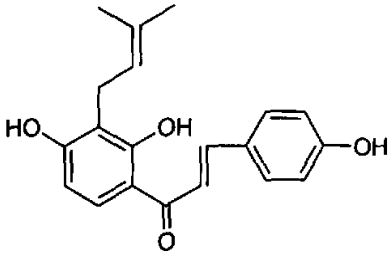


Lysinonotin: R_1=R_6=OCH_3, R_2=R_3=R_4=R_5=H

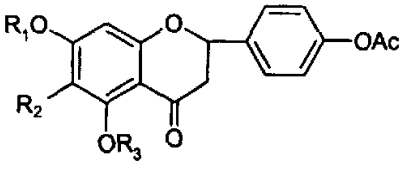
Nobilelin: R_2=R_4=CH_3, R_3=R_5=OCH_3, R_1=R_6=H



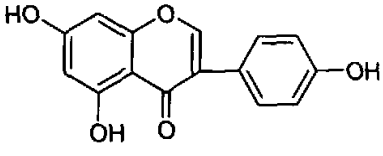
Corylifolin



Corylifolinin



Matteucinol: R_1=R_3=H, R_2=CH_3
Corylifolin-Ac: R_1=R_3=Ac, R_2=H



Genistein

Chemical structure of twelve natural flavonoids.

corylifolin, lysionotin, matteucinol, corylifolinin, genistein, nobilelin, and corylifolin-Ac were isolated and purified by Professor ZHU Da-Yuan and of over 98 % purity; baicalin of 97 % purity was obtained from Shanghai No 1 Chinese Traditional Medicine Factory. All other chemicals were Chinese products and of analytical grade. All solutions were prepared using deionized water of high purity.

Methods According to the previous report[6], a highly sensitive chemiluminescence system with ascorbate-$CuSO_4$-yeast-H_2O_2 as ·OH-generator was used to generate ·OH and determine the ·OH-inhibiting rates of different flavonoids.

Statistical analysis All data of chemilumines-

cence intensity and half inhibition concentration (IC_{50}) were presented as $\bar{x} \pm s$ and x (95 % confidence limits), respectively. They were compared with t-test.

RESULTS

It was shown that quercetin, heliosin, hyperoside, kaempferol, baicalin, corylifolin, lysionotin, matteucinol, corylifolinin, and genistein could effectively scavenge ·OH and inhibit the chemiluminescence of the system. Their scavenging rates were dose-dependent. The ·OH-scavenging ability of the first five flavonoids (their IC_{50} values were lower than 35 g/L) was prominently stronger than that of the last five flavonoids (their IC_{50} values were higher than 66 g/L). It was also shown that nobilelin and corylifolin-Ac had no inhibiting effects on the chemiluminescence of the system and thus were thought to be unable to scavenge ·OH (Tab 1).

DISCUSSION

Our present study showed that all the ten natural flavonoids with phenolic hydroxyl group could scavenge ·OH, whereas nobilelin with methoxy only and corylifolin-Ac with OAc groups only had no ·OH-scavenging effect. These results revealed that the phenolic hydroxyl was a main active group that scavenges ·OH.

The molecules of baicalin, corylifolin, lysionotin, and matteucinol all carry two phenolic hydroxyl groups, but their ·OH-scavenging activities is found to be remarkably different from each other. In addition, the ·OH-scavenging activities of these flavonoids is proved to be higher than that of genistein with three phenolic hydroxyl groups. These facts suggest that the ·OH-scavenging activities may be closely related to the position rather than the numbers of phenolic hydroxyl groups.

It is found that baicalin with an ortho-dihydroxyl group only in ring A (on 5, 6 position) and lysionotin and matteucinol with a meta-dihydroxyl group only in ring A (on 5,7 position) all possess, to certain extent, a ·OH-scavenging ability and the scavenging ability of baicalin is much stronger than that of lysionotin and matteucinol. These observations imply that hydroxyl groups in ring A are important ·OH-scavenging active groups, and the scavenging activity of ortho-dihydroxyl group is much higher than that of meta-dihydroxyl group.

It is shown that quercetin, heliosin, and hyperoside, which all carry a meta-dihydroxyl group in ring A (on 5, 7 position) and an ortho-dihydroxyl group in ring B (on 3′, 4′ position), can effectively scavenge ·OH. It is also found that the scavenging activity of quercetin, heliosin, and hyperoside is much higher than that of lysionotin and matteucinol. The main structural difference between these two groups of flavonoids lies in that there is no ortho-dihydroxyl group (on 3′, 4′ position) in ring B of the later two compounds. These facts indicate that hydroxyl groups, especially ortho-dihydroxyl groups (on 3′, 4′ position), in ring B are the most important active groups.

It has been pointed out that electron-withdrawing property of the chromonoid in ring C can increase O-H bond energy of hydroxyl groups in ring A and make H difficult to dissociate, whereas ring B that weakly conjugated with ring C is less affected by this property[7]. That is the reason why the scavenging activity of hydroxyl groups in ring B is higher than that in ring A. According to the previous report[8], ortho-phenolic dihydroxyl group forms intramolecular hydrogen bond, which is beneficial to stabilizing benzene-oxygen free radical. Ortho-phenolic dihydroxyl free radical is stabilized through resonance by forming ortho-bezoquinone structure. That is the reason ring A and/or B with dihydroxyl group can remarkably increase the ·OH-scavenging activity.

The structural analysis shows that the unique difference among quercetin, heliosin, and hyperoside lies in 3 position in ring C, on which there exists hydroxyl group, digalactose, and galactose respectively. The observation on ·OH-scavenging effects shows that hydroxyl group is better than glucoside, and disaccharide is slightly stronger than monosacchride, suggesting that hydroxyl group or glucoside on 3 position in ring C is related to ·OH-scavenging ability. Corylifolin and corylifolinin belong to flavanones and chalcones respectively. The main structural difference between corylifolin and corylifolinin lies in that ring C of the latter is an open circular. But the scavenging activity of the former is much higher than that of the latter. It is suggested that scavenging effect of flavanones is more prominent than that of chalcones, if the number and position of the hydroxyl groups are similar. Similarly, the comparison of the structure and effect between kaempferol and genistein, belonging to flavonols and isoflavones respectively, shows that the scavenging activity of flavanols is much higher than that of isoflavones, if the number and position of the phenolic hydroxyl groups are similar. The above results reveal

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Tab 1. Effects of twelve natural flavonoids on scavenging ·OH. $n = 3$. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* control.

Flavonoids	Concentration (g/L)	Chemiluminescence intensity (CP10s)	Chemiluminescence inhibition rate (%)	IC_{50}/g·L^{-1} (95 % CL)[1]
Control	0	32568 ± 729	0	–
Quercetin	5	27715 ± 1034^c	14.9	12.1
	10	17912 ± 581^c	45.0	9.9 – 14.5
	15	14279 ± 290^c	56.1	
	20	12995 ± 673^c	60.1	
	25	12278 ± 438^c	62.3	
Heliosin	0.75	29930 ± 1204^a	8.1	15.8
	5	23775 ± 812^c	27.7	14.0 – 19.2
	12.5	17391 ± 498^c	46.6	
	25	14884 ± 205^c	54.3	
	100	3517 ± 187^c	89.2	
Hyperoside	2.5	26217 ± 731^c	19.5	19.5
	6.3	22765 ± 1120^c	30.1	16.8 – 27.4
	12.5	18596 ± 370^c	42.9	
	25	14362 ± 348^c	55.9	
	75	10617 ± 351^c	67.4	
Kaempferol	5	28269 ± 1102^c	13.2	20.1
	20	16382 ± 295^c	49.7	13.6 – 29.0
	30	14395 ± 373^c	55.8	
	40	13320 ± 361^c	59.1	
	80	12083 ± 297^c	62.8	
Baicalin	10	22504 ± 738^c	30.9	34.6
	25	18433 ± 349^c	43.4	28.4 – 43.4
	50	14265 ± 267^c	56.2	
	100	8109 ± 253^c	75.1	
	200	6481 ± 275^c	80.1	
Corylifolin	12.5	28985 ± 768^b	11.0	66.8
	25	24784 ± 1289^c	23.9	63.2 – 74.4
	50	19215 ± 927^c	41.0	
	75	15340 ± 431^c	52.9	
Lysionotin	38	30125 ± 1162^a	7.5	187
	50	28530 ± 830^c	12.4	147 – 235
	100	24165 ± 1017^c	25.8	
	200	15535 ± 416^c	52.3	
Matteucinol	13	27096 ± 513^c	16.8	211
	38	24361 ± 661^c	25.2	165 – 284
	150	18270 ± 782^c	43.9	
	250	15079 ± 658^c	53.7	
Corylifolinin	50	27618 ± 1129^c	15.2	262
	120	22175 ± 1362^c	31.9	190 – 346
	200	18726 ± 1071^c	42.5	
	300	14981 ± 679^c	54.0	
Genistein	50	26738 ± 1150^c	17.9	708
	150	20681 ± 997^c	36.5	498 – 994
	300	18766 ± 1219^c	42.5	
	900	15665 ± 783^c	51.9	
Nobilelin[2]		without inhibitory effect		
Corylifolin-Ac[2]		without inhibitory effect		

[1] 95 % CL: 95 % confidence limits. [2] The data are negligible and not given.
IC_{50}(95 % CL) were determined by log-probit analysis.

that the structural types of flavonoids themselves can influence their ˙OH-scavenging activity.

Bors *et al*[9] proposed that three structural groups were important determinants for free radical scavenging. The *o*-dihydroxyl structure in ring B is the radical target site for all flavonoids (a). The 2,3-double bond in conjunction with 4-oxo function is responsible for electron delocalization (b). And the additional presence of both 3- and 5-hydroxyl groups is for maximal radical scavenging potentials and strongest radical absorption (c). Our present ˙OH-scavenging experiments, at least partly, support this idea. Our results reveal that quercetin is the strongest scavenger among the investigated flavonoids, for its structure satisfies all the determinants proposed by Bors *et al*. Heliosin and hyperoside also show a high scavenging activity, for their structures satisfy determinants (a) and (b). Kaempferol is also a strong scavenger, for its structure satisfies determinants (b) and (c). Therefore, these four flavonoids are more efficient in ˙OH-scavenging effects than the rest ones, which satisfy most determinant (b).

Compared with ESR, the chemiluminescence method needs less quantity of test compound and is of high sensitivity, in addition to simpler and less expensive, though it can not detect free radicals directly. Authors have compared the ˙OH-scavenging effects of quercetin and tea polyphenols by using chemiluminescence method, ESR, and pulse radiolysis[10]. The results from these three methods are proved to be identical[10], and the chemiluminescence method developed by our laboratory is feasible and reliable.

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天然黄酮类化合物清除羟自由基的构效关系

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关键词 黄酮类化合物；羟自由基；构效关系；化学发光

目的：研究 12 种天然黄酮类化合物清除羟自由基(˙OH)的构效关系. **方法**：使用产生并能灵敏检测˙OH的抗坏血酸-$CuSO_4$-酵母-H_2O_2 的化学发光体系. **结果**：槲皮素、泽漆新苷、金丝桃苷、山奈素、黄芩苷、补骨脂甲素、石吊兰素、映山红素、补骨脂乙素和染料木素能有效地清除˙OH，抑制体系化学发光，它们的半数抑制浓度(IC_{50})值(95 %可信限)分别是：12.1 (9.9-14.5) g/L, 15.8 (14.0-19.2) g/L, 19.5 (16.8-27.4) g/L, 20.1 (13.6-29.0) g/L, 34.6 (28.4-43.4) g/L, 66.8 (63.2-74.4) g/L, 187 (147-235) g/L, 211 (165-284) g/L, 262 (190-346) g/L 和 708 (498-994) g/L，而川陈和补甲-Ac 则无效. **结论**：(1) 黄酮类酚羟基是清除˙OH的主要活性基团；(2) B 环上羟基和 A 环上羟基都是清除˙OH的重要活性基团；(3) 位于 A 环和/或 B 环上的邻二羟基能大大提高相应环的清除˙OH活性；(4) 对槲皮素、泽漆新苷、金丝桃苷、黄芩苷、石

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吊兰素和映山红素的 IC_{50} 值进行比较，发现它们的 B 环 3′,4′位上的羟基具有高清除 ·OH 活性；它们的 B 环上羟基清除活性高于 A 环上羟基；它们的 C 环 3 位上的羟基或糖苷与清除 ·OH 的能力也有相关性；(5)黄酮类化合物的种类也影响其清除 ·OH 活性.

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