Relationship Between Structure and Antioxidative Properties of Some 3-Formylchromone Derivatives

F. Šeršeň[1,*], D. Loos [2], L. Mezešová[3] and M. Lácová[2]

[1]*Institute of Chemistry, Faculty of Natural Sciences, Comenius University in Bratislava, 842 15 Bratislava, Slovakia;* [2]*Department of Organic Chemistry, Faculty of Natural Sciences, Comenius University in Bratislava, 842 15 Bratislava, Slovakia;* [3]*Institute for Heart Research, Slovak Academy of Science, 814 38 Bratislava, Slovakia*

Abstract: Flavonoids, which generally exhibit very good antioxidant properties, contain the chromone unity. The work elucidates the relation between chemical structure of chromones and their ability to scavenge DPPH radicals. The work deals with antioxidative properties of some hydroxy derivatives of 3-formylchromones (without substituent, 6-hydroxy-, 7-hydroxy-, 7,8-dihydroxy-). It was found that the last two derivatives scavenge DPPH radicals, whereas the first two ones do not. It was demonstrated that the presence and location of hydroxyl groups play a crucial role for antioxidative activity of 3-formylchromones. The scavenging of DPPH radicals runs through H^+ abstraction from hydroxyl groups of formylchromones. The DPPH scavenging by 3-formylchromones with hydroxyl group in the 7th position is connected with the formation of more stable form of anion than in the case of 6-hydroxy-3-formylchromone. Calculation heats of formations of studied formylchromone anions confirmed this fact. All studied 3-formylchromones did not scavenge HO$^\bullet$ radicals, what supports H^+ abstraction mechanism of DPPH scavenging.

Key Words: Antioxidant, chromone, DPPH, flavonoid, hydroxyl radical.

1. INTRODUCTION

Flavonoids are reach group of natural polyphenolic compounds from plant sources. More than 5000 individual flavonoids are known [1,2]. The chemical structure of flavonoids consists of the unity benzo-γ-pyrone (synonymous with chromone) where aromatic substituent most often attached at C2 position. Flavonoids exhibit various biological activities as are: cardiotonic, hepatoprotective, antineoplastic, antiulceral, anti-inflamatory, antimicrobial, antifungal, and antiviral [1-3]. The radical scavenging effect by flavonoids is notoriously known [1-6]. The presence of C2-C3 double bond or presences of hydroxyl groups (in positions C3, C7, C4`) are usually listed as condition for good antioxidant and antiradical activity [5,6]. Also, antiphotosynthetic properties of some 3-formylchromones were reported in our previous work [7].

The aim of this work is to find a relationship between chemical structure and antioxidative properties of some 4-formylchromones. Chemical structure of studied chromones is presented in Fig. (**1**).

2. MATERIAL AND METHODS

2.1. Reagents and Equipments

Methanol, chloroform, 2,2-diphenyl-1-picrylhydrazyl (DPPH), 5,5-dimethyl-1-pyrroline-*N*-oxid (DMPO), and 30% H_2O_2 were purchased from Sigma-Aldrich Ltd. The EPR spectra were registered by a X-band (~9.3 GHz) equipment ERS 230 (ZWG Akad. Wiss. Berlin, Germany). The absorption spectra were recorded by Specord UV-VIS (C. Zeiss Jena, Germany). Derivatives of 3- formylchromones were prepared according to the work by Lácová *et al*. 1995 [8].

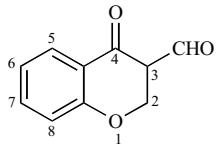

Fig. (1). Structural formulae of 3-formylchromones.

2.2. DPPH Assay

The scavenging of DPPH radicals by studied chromones was carried out according to our previous work [9] Into the methanolic or chloroformic DPPH solutions, different amounts of the tested substances were added, maintaining the final DPPH concentration always of 0.9 10^{-4} mol.dm^{-3}. After 20 min. absorbance at 517 nm was measured. These measurements were carried out at a laboratory temperature of 25°C.

2.3. Hydroxyl Radical Assay

The scavenging of hydroxyl radicals by studied samples was determined by EPR spectroscopy according to our previous work [9]. Briefly, the spontaneous degraded hydrogen peroxide at 25°C was used as a source of HO$^\bullet$ radicals. As HO$^\bullet$ radicals possess very short lifetime, they cannot be detected readily by the continuous wave EPR spectrometer. Therefore, a DMPO spin trap captured them and the EPR spectra of the resulting spin adduct were subsequently recorded. The aqueous reaction solution contained 0.05 mol.dm^{-3} of DMPO, 0.05 mol.dm^{-3} of H_2O_2 and different amounts of the studied chromone. The EPR measurements were carried out at a modulation amplitude 0.1 mT, and microwave power 5 mW. The EPR spectra were recorded 20 min. after H_2O_2 addition.

*Address correspondence to this author at the Comenius University in Bratislava, Faculty of Natural Sciences, Institute of Chemistry, Mlynská dolina CH2, 842 15 Bratislava, Slovakia; Tel: +421-7-60296412;
E-mail: sersen@fns.uniba.sk

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3. RESULTS AND DISCUSSION

The effect of studied chromones on the methanolic DPPH solution was accompanied by a bleaching reaction. The free radical scavenging activity was calculated using the following equation:

scavenging activity (%) = $100(A_a - A_b)/A_a$

where A_a is the absorbance of the DPPH solution without the addition of the tested samples, A_b is the absorbance of the mixture containing both the tested sample and DPPH. SC_{50} values calculated from a regression curve denoted the concentration of sample required to scavenge 50% of DPPH radical. The values of the average square deviation (r^2) were also calculated.

It was found that some of the studied chromones were quite effective scavengers of DPPH radicals. Their SC_{50} values are presented in Table **1**. From this table it is evident that the crucial factor for DPPH scavenging by studying 3-formylchromones is the presence of hydroxyl groups in 7 and 8 positions, where the most effective derivative was 7,8-dihydroxy-3-formylchromone. On the other hand, it is very interested that 6-hydroxy-3-formylchromone exhibited the zero affectivity of DPPH scavenging, whereas chromone with OH group in position 7 exhibited relatively high efficiency (0.667 10^{-3} mol dm^{-3}).

Table 1. SC_{50} Values of DPPH Scavenging by Studied 3-Formylchromones

3-formylchromne	SC_{50} [mmol dm^{-3}]	SC_{50} [μg/ml]	r^2
no substituent	inactive	inactive	
6-hydroxy-	inactive	inactive	
7-hydroxy-	0.667	126.75	0.941
7,8-dihydroxy-	0.104	21.47	0.954

There exist essentially two theories for the mechanism of DPPH scavenging by any of the antioxidants. The first theory is based on hydrogen atom transfer from antioxidant (ArOH) to DPPH (Fig. **2**) [10].

The second theory is based on the H^+ abstraction by solvent effect from antioxidant molecules and the following trapping by DPPH molecules [11] according to Fig. (**3**). Total enthalpies of individual reactions for 7-hydroxy-3-formyl-chromone were calculated by quantum-chemical method AM1 [12, 13] and they are presented in Fig. (**3**). As can be seen from these values, the determining step of this process is reaction (1).

The third possibility of reaction DPPH with antioxidant molecule is a combination of both the above-mentioned processes [14]. The properties of antioxidant and solvent determine, which mechanism takes place in the reaction of DPPH with an antioxidant.

An experiment with DPPH scavenging in chloroform was carried out in order to find if DPPH scavenging takes place according to Fig. (**2**) or to Fig. (**3**). It was found that any studied chromone did not scavenge DPPH radicals in chloroform. Based on the above-mentioned results, we suggest that the DPPH scavenging in methanol runs according to Fig. (**4**) i.e. through H^+ abstraction. The H^+ abstraction is followed by the creation of two mesomeric structures of chromone anions (Fig. **4**).

As is shown in Fig. (**4**), chromone derivatives, which have hydroxyl in the 7th position, are able to create more stable form of anion (Fig. **4A**) as 6-hydroxy-3-formyl-chromone (Fig. **4B**). The calculation heats of formations by quantum-chemical method AM1 [13, 14] supported this fact. In Table **2** are presented the formation heats (E) for suggested mesomeric structures. From this table is evident that anion of 7-hydroxy-3-formylchromone is by 35 kJ/mol more stable as anion of 6-hydroxy-3-formylchromone. Table **2** also shows that hydroxy groups decrease energy of formation for relevant anions, whereby both anion mesomeric structures presented in Fig. (**4A**) are equally stable.

We have made an attempt with the scavenging of $HO^\bullet$ radicals by studied formylchromones. It was found that all studied 3-formylchromones did not scavenge $HO^\bullet$ radicals. This fact also supports the second theory, i.e. H^+ abstraction from formylchromone molecule. The energy of formation for radicals is much larger than for anions as was proved *via* calculation by quantum-chemical method AM1.

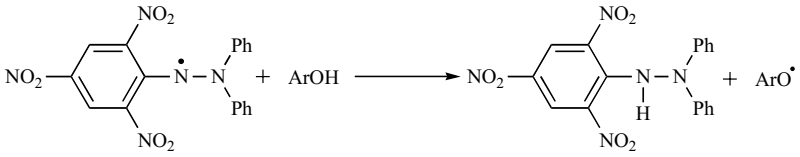

Fig. (2). The scheme of reaction of DPPH with an antioxidant *via* $H^\bullet$ transfer.

$ArOH \rightarrow ArO^- + H^+$	$\Delta H = 669$ kJ/mol	(1)
$ArO^- + DPPH^\bullet \rightarrow ArO^\bullet + DPPH^-$	$\Delta H = -49$ kJ/mol	(2)
$DPPH^- + H^+ \rightarrow H\text{-}DPPH$	$\Delta H = -560$ kJ/mol	(3)
$DPPH^\bullet + ArO^\bullet \rightarrow$ product	$\Delta H = 25$kJ/mol	(4)

Fig. (3). Schemes of reaction of DPPH with an antioxidant *via* H^+ abstraction.

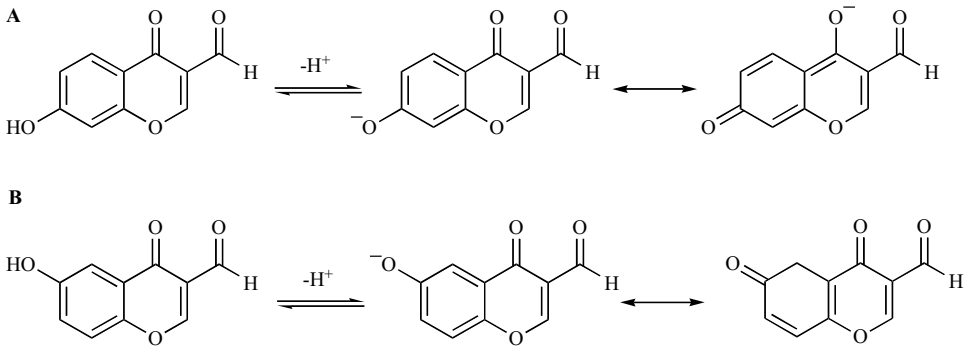

Fig. (4). Mesomeric structures of 7-hydroxy-3-formylchromone (A) and 6-hydroxy-3-formylchromone (B) after H^+ abstraction.

SUMMARY

Recently, numerous authors investigated the antioxidant activity of flavonoids [4-6,15-17]. In general, the radical scavenging activity of flavonoids depends on the molecular structure, the number and location of hydroxyl groups. It is known that the presence of 3-OH, 5-OH and 7-OH groups increases the radical scavenging activity of flavonoids. Flavonoids with more of OH groups in its chromone part exhibit greater antioxidative effect. Cao *et al*. [17] found that 6-hydroxyflavone exhibited lower values of ORAC (for $ROO^{\bullet}$ radicals) than flavonoids with OH groups in position 5 and 7. Their finding is in accordance with our antioxidative test. In this article, we are explaining this fact by the scavenging mechanism of DPPH radicals. The antiradical inactivity of 6-hydroxy-3-formylchromone is caused by the impossibility to form stable forms of anion after H^+ abstraction from formylchromone.

Table 2. Formation Heats (E) for Suggested Mesomeric Structures of 3-Formylchromones

3-formylchromone	E [kJ/mol]	ΔE [kJ/mol]
6-hydroxy-	-405.091	
C5 anion	-557.142	-152.051
O6 anion	-557.139	-152.048
7,8-dihydroxy-	-590.792	
C5 anion	-735.084	-144.291
O7 anion	-778.082	-187.29
O8 anion	-735.069	-144.277
O4 anion	-778.082	-187.29
7-hydroxy-	-412.713	
O4 anion	-592.364	-179.651
O7 anion	-592.364	-179.651

Oi – denotes an oxygen atom on the i^{th} carbon atom.

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