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Original article

Thiaflavan scavenges radicals and inhibits DNA oxidation: A story from the ferrocene modification

Hai-Wang Lai, Zai-Qun Liu*

Department of Organic Chemistry, College of Chemistry, Jilin University, Changchun 130021, China

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ABSTRACT

4-Thiaflavan is a sulfur-substituted flavonoid with a benzoxathiin scaffold. The aim of this work is to compare abilities of sulfur and oxygen atom, hydroxyl groups, and ferrocene moiety at different positions of 4-thiaflavan to trap radicals and to inhibit DNA oxidation. It is found that abilities of thiaflavans to trap radicals and to inhibit DNA oxidation are increased in the presence of ferrocene moiety and are further improved by the electron-donating group attaching to thiaflavan skeleton. It can be concluded that the ferrocene moiety plays the major role for thiaflavans to be antioxidants even in the absence of phenolic hydroxyl groups. On the other hand, the antioxidant effectiveness of phenolic hydroxyl groups in thiaflavans can be improved by the electron-donating group. The influences of sulfur and oxygen atoms in thiaflavans on the antioxidant property of *para*-hydroxyl group exhibit different manners when the thiaflavans are used to trap radicals and to inhibit DNA oxidation.

1. Introduction

Flavonoid (structure shown in Scheme 1) is an important compound for natural polyphenols and is widely found in plants [1]. The biological and pharmacological properties of flavonoids are attributed to phenolic hydroxyl groups at different positions in flavonoid [2]. It has been proved that the bond dissociation energy (BDE) of O–H in hydroxyl group at B ring is about 10 kcal mol^{-1} lower than that at A ring. This result indicates that hydroxyl groups at B ring play the key role in scavenging radicals and exhibiting antioxidant effectiveness [3]. Some flavonoid derivatives are also shown in Scheme 1 [4]. For example, when the double bond between C-2 and C-3 in flavonoid is converted into single bond, the generated flavan is also natural polyphenol [5]. The antioxidant properties of flavans have been estimated in various experimental systems [6]. In addition, ferrocene-appended naphthoquinones exhibit high antiplasmodial activities [7], and copper (II) complexes of curcumin and *N*-ferrocenylmethyl-*L*-amino acids possess high photocytotoxicities [8]. The aforementioned results reveal that ferrocenyl group is able to improve the biological activities of natural compounds, and this encourages us to explore whether ferrocenyl group can also varies the antioxidant effects of thiaflavans and to investigate positions of ferrocenyl group and sulfur atom for being a mutual antioxidant.

2. Chemistry

Scheme 1 outlines that sulfur atom can be applied to replace CH_2 at 4-position in flavan, producing sulfur-contained benzoxathiin. This sulfur-involved flavan is also called as 4-thiaflavan, which behaves as a heterocyclic nucleus for designing many drugs [9].

The sulfur atom in thiaflavan can decrease the BDE of O–H attaching to its *para*-position. As shown as the structure **I** in Scheme 1, the single electron at the oxygen atom in –OH may be transferred to sulfur atom in order to use the sulfur atom for accommodating the single electron and to stabilize the peroxyl radical [10]. Similarly, the structure **II** in Scheme 1 outlines that the oxygen atom at 1-position can also stabilize the phenoxyl radical at 6-position. Therefore, it is worthy to compare the ability of hydroxyl group at 6- and 7-position to scavenge radicals and to inhibit the oxidation of biological species. Therefore, thiaflavan is an appropriate model compound for the investigation on the effect of sulfur atom on phenoxyl radical at ring A [11]. Although abilities of hydroxyl group at 5-position to quench 2,2′-diphenyl-1-picrylhydrazyl radical (DPPH) [12] and to inhibit glutathione/ferric ion-induced oxidation of DNA have been reported [13], the study on antioxidant abilities of hydroxyl groups at 6- and 7-position are not usually found. A straight carbon chain is used to attach to ring A of thiaflavan,

* Corresponding author.
E-mail address: zaiqun-liu@jlu.edu.cn (Z.-Q. Liu).

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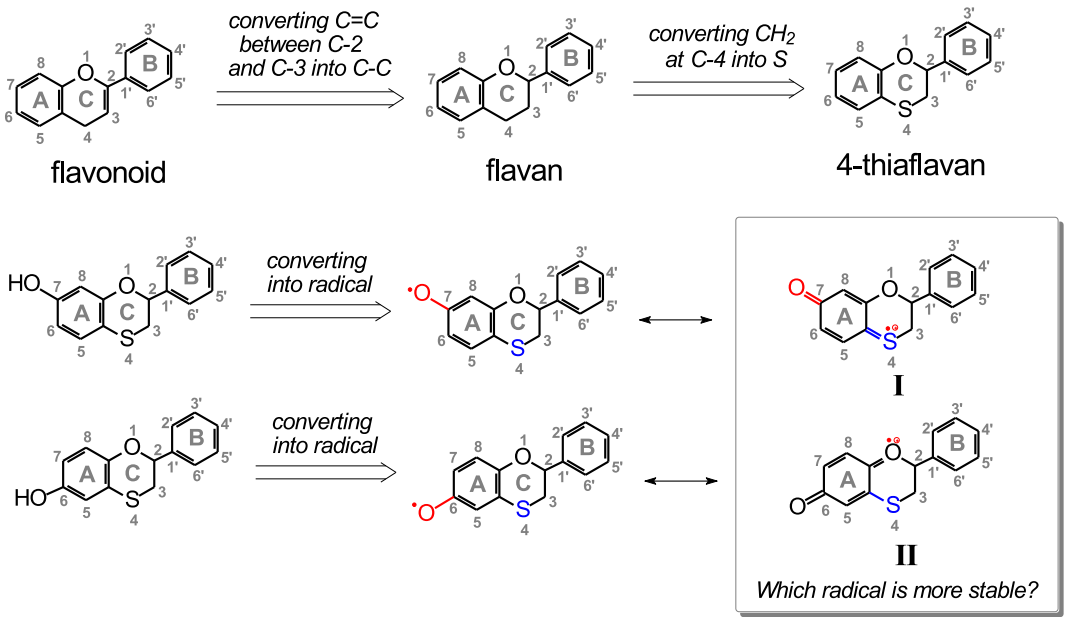

Scheme 1. Structures of flavonoid-related compounds and radicals deriving from 4-thiaflavan.

forming an amphiphilic antioxidant [14], and the carbon chain is employed to attach to ring C, producing a tocopherol-like antioxidant [15]. These results encourage us to apply an organometallic group to attach ring B and to estimate the antioxidant property of thiaflavan.

3. Pharmacology

The inhibition effect on DNA oxidation and the ability to scavenge radicals are characteristic properties of an antioxidant. So, the aim of this work is to clarify effects of hydroxyl groups at ring A and B and ferrocene moiety at ring B on the antioxidant effectiveness of thiaflavans. Therefore, as shown in Scheme 2, thiaflavans **1** to **11** are synthesized following a description in a literature (protocol A) [16], and thiaflavans **12** and **13** are prepared following the synthetic protocol B [17]. Then, the radical-scavenging properties are compared by quenching 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) cationic radical ($ABTS^{+\bullet}$), DPPH, and galvinoxyl radical, respectively [18]. The antioxidant effectiveness of these thiaflavans are also estimated inhibiting 2,2′-azobis(2-amidinopropane hydrochloride) (AAPH)-induced oxidation of DNA [19].

4. Results and discussion

4.1. Scavenging radicals

$ABTS^{+\bullet}$, DPPH, and galvinoxyl radical are radical resources, which are usually employed to evaluate the property of an antioxidant in scavenging radicals. The reaction of an antioxidant with $ABTS^{+\bullet}$, DPPH, and galvinoxyl radical reveals the ability of the antioxidant to reduce radical and to donate its hydrogen to N- or O-centered radicals, respectively. Some methods are designed to express the ability of the antioxidant to quench these radicals [20]. But kinetic methods are not usually found because the operation to measure the rate constant ($\boldsymbol{k}$) of the antioxidant to quench these radicals are so complicated [21]. But Figs. 1S, 2S and 3S show that some thiaflavans are able to decrease the concentrations of these radicals rapidly, while other thiaflavans cannot react with these radicals. Hence, it is necessary to find a convenient way to express the results from the aforementioned figures.

We have provided a method for expressing the relationship between the concentration of a radical and the reaction period [22]. Briefly, when $ABTS^{+\bullet}$ is taken as the example, the rate constant ($\boldsymbol{k}$) of the reaction between an antioxidant and $ABTS^{+\bullet}$ can be calculated by equation (1) when [$ABTS^{+\bullet}$], [antioxidant], and the corresponding reaction rate ($\boldsymbol{r}$) are known.

$$-\frac{\mathrm{d}[\mathrm{ABTS}^{+\bullet}]}{\mathrm{d}t} = \boldsymbol{r} = \boldsymbol{k}[\mathrm{ABTS}^{+\bullet}][\mathrm{antioxidant}] \quad (1)$$

The chemical kinetic equation (1) is also available at $t = 0$ and can be expressed as equation (2), in which $[\mathrm{ABTS}^{+\bullet}]_{t=0}$ and $[\mathrm{antioxidant}]_{t=0}$ refer to concentrations of the antioxidant and $ABTS^{+\bullet}$ at the beginning of the reaction.

$$\boldsymbol{r}_0 = \boldsymbol{k}[\mathrm{ABTS}^{+\bullet}]_{t=0}[\mathrm{antioxidant}]_{t=0} \quad (2)$$

In view of the treatment of the experimental data in Fig. 1S, the concentration of $ABTS^{+\bullet}$ and the corresponding time-point (t) are input into a statistical software in order to find quantitative relationship of [$ABTS^{+\bullet}$] $\sim t$. As a result, the double exponential function is the most suitable function for expressing the relationship of [$ABTS^{+\bullet}$] $\sim t$ and listed in Table 1S.

$$[\mathrm{ABTS}^{+\bullet}] = A\boldsymbol{e}^{-\frac{t}{a}} + B\boldsymbol{e}^{-\frac{t}{b}} + C \quad (3)$$

Moreover, the differential operation is performed to equation (3) for expressing the relationship between the reaction rate ($\boldsymbol{r}$) and the time (t), $-\mathrm{d}[\mathrm{ABTS}^{+\bullet}]/\mathrm{d}t \sim t$ (equation (4)).

$$-\frac{\mathrm{d}[\mathrm{ABTS}^{+\bullet}]}{\mathrm{d}t} = \boldsymbol{r} = \frac{A}{a}\boldsymbol{e}^{-\frac{t}{a}} + \frac{B}{b}\boldsymbol{e}^{-\frac{t}{b}} \quad (4)$$

The reaction rate at $t = 0$ ($\boldsymbol{r}_0$) can be calculated following equation (4) when the reaction time (t) is assigned to 0. The rate constant ($\boldsymbol{k}$) is thereby calculated by using equation (2) and collected in Table 1. This method is also used to treat the