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Fast repairing of oxidized OH radical adducts of dAMP and dGMP by phenylpropanoid glycosides from *Scrophularia ningpoensis* Hemsl[1]

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KEY WORDS *Scrophularia ningpoensis* Hemsl; glycosides; antioxidants; deoxyadenine nucleotides; deoxyguanine nucleotides

ABSTRACT

AIM: To investigate the antioxidative activity of the constituents of the roots of *Scrophularia ningpoensis* (Chinese name: Xuanshen). **MEHTODS**: The main compounds from the roots of *Scrophularia ningpoensis* were isolated and identified by chromatography and FABMS, NMR etc. Using the techniques of pulse radiolysis, the electron transfers from iridoid glycosides (IG) or phenylpropanoid glycosides (PG) to oxidized OH radical adducts of 2′-deoxyadenosine-5′-monophosphate acid (dAMP) or 2′-deoxyguanosine-5′-monophosphate acid (dGMP) were observed. **RESULTS**: Two IG: harpagoside and harpagide, two PG: angoroside C and acteoside were obtained as the main hydrophilic constituents of the plant. At 0.1 mmol/L concentration, angoroside C and acteoside were able to repair the oxidized OH adducts dAMP and dGMP significantly. However, harpagoside and harpagide had no such effect. The electron transfer rate constants of angoroside C with dAMP and dGMP were 4.2 $\times 10^8$ and 10.3 $\times 10^8$ L·mol^{-1}·s^{-1}; the electron transfer rate constants of acteoside with dAMP and dGMP were 5.3 $\times 10^8$ and 20.2 $\times 10^8$ L·mol^{-1}·s^{-1}. **CONCLUSION**: PG from *Scrophularia ningpoensis* have a potent antioxidative activity for reducting of the oxidized OH adducts of dAMP and dGMP.

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INTRODUCTION

Hydroxyl radical (OH·) is generated either from radiolysis of water present in the biological tissues or from redox cycling implicated in the toxicity of a wide range of chemicals. It has been recognized as an extremely reactive oxidative species which can attack DNA bases and sugar moieties of nucleotides and cause chemical injury of DNA and other biological targets, leading to strand breaks, cross-links, and modifications in sugars and bases of DNA[1,2]. Pulse radiolysis is a very useful method to observe drugs undergoing electron transfer to OH radicals. The antioxidative activity of many compounds, including phenylpropanoid glycosides (PG)[3], flavones[4] and derivatives of hydroxycinnamic acid[5], have been tested by this method. The roots of *Scrophularia ningpoensis* Hemsl have been used as a famous Chinese medicine named "Xuanshen" for treatment of various inflammatory diseases[6]. In previous studies, many iridoid glycosides (IG) have been reported to be isolated from the above mentioned plant[7]. The IG are regarded as the bioactive source of the plant[8]. However, in our recent works, many PG obtained from the plant[9] have also shown many pharmacological activities[10]. These were ignored in earlier studies because of their difficult purification procedure[8]. In this study, IG: harpagoside and harpagide, PG: angoroside C and acteoside were obtained as the main hydrophilic constituents from the roots of *Scrophularia ningpoensis* (Fig 1). While the antioxidative effect of acteoside (also called verbascoside) was tested by pulse radiolysis[3], angoroside C which exists in large amounts in the plant was not tested by this method. In order to investigate the antioxidative effects of angoroside C and to observe which among the PG and IG is the bioactive antioxidizing agent from *Scrophularia ningpoensis*, the interaction of IG and PG with oxidized

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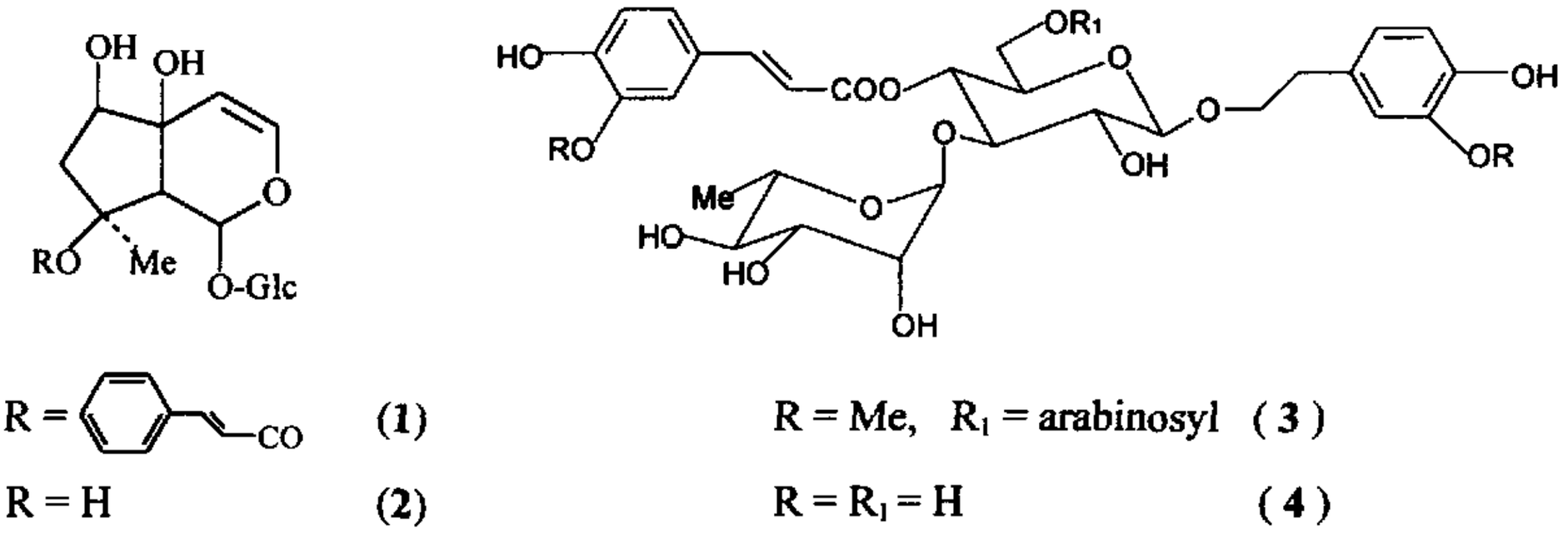

Fig 1. Chemical structures of harpagoside (1), harpagide (2), angoroside C (3), and acteoside (4).

OH radical adducts of dAMP and dGMP was studied by pulse radiolysis.

MATERIALS AND METHODS

Plant materials The roots of *Scrophularia ningpoensis* were collected from Zhejiang province of China. The plant was identified by Dr MA Xiao-Qiang from Shanghai Institute of Materia Medica, Chinese Academy of Sciences, where the voucher specimens (№ 95 – 10) have been deposited.

Extraction and isolation Eight kilogram of powdered roots of the plant were extracted with hot 95 % EtOH (twice), 60 % EtOH (twice) for 1 h each under refluxing. The combined EtOH extracts were concentrated *in vacuo*. The residue was suspended in H_2O and the suspension was shaken successively with ether and *n*-BuOH. A portion of the *n*-BuOH layer residue (140 g) was subjected to column chromatography on macroporous resin DA-201, silica gel and sephadex LH20 with various eluents to obtain harpagoside (0.95 g), harpagide (54 mg), angoroside C (15.4 g) and acteoside (97 mg). The structures of the 4 compounds were assigned by comparing the chemical and spectral data (IR, EIMS, ^{1}HNMR, ^{13}CNMR) with those reported in the literature[9].

Pulse radiolysis experiments The dAMP and dGMP were obtained from Sigma Chemical Co (St Louis, MO, USA). All solutions were prepared using triple distilled water, were buffered with phosphate (2 mmol·L^{-1}), and saturated with high purity nitrous oxide. All experiments were carried out at room temperature.

Pulse radiolysis experiments were conducted by using a linear accelerator providing 10 MeV electron pulse with a duration of 8 ns. The dosimetery of electron pulse was determined by thiocyanate dosimeter containing 0.01 mol·L^{-1} potassium thiocyanate (KSCN) solution saturated with nitrous oxide by taking $\epsilon_{(SCN)2^-} = 7600$ L·mol^{-1}·cm^{-1} at 480 nm. The detailed descriptions of pulse radiolysis equipment and experimental set-up have been described elsewhere[11]. In this work, the dose per electron pulse was 100 Gy.

RESULTS

Reduction of oxidized OH radical adducts of dGMP and dAMP through electron transfer The fast repairing of radical adducts of dGMP and dAMP by IG and PG were investigated by a pulse radiolysis method. The reaction rate constants of OH radical with dGMP and dAMP were determined as 8.2×10^9, 4.1×10^9, 8.0×10^9, 7.2×10^9, and 9.3×10^9 mol·L^{-1}·s^{-1} at pH 7, respectively. In this experiment, the solutions contained 2 mmol·L^{-1} deoxyribonucleotide and 0.1 mmol·L^{-1} the tested IG and PG. The results showed that angoroside C and acteoside were able to repair the oxidized OH adducts of dGMP and dAMP significantly, but harpagoside and harpagide had no such effect.

The transient absorption spectra recorded at 1 μs and 50 μs after pulse radiolysis of 2 mmol·L^{-1} dGMP aqueous solution containing 0.1 mmol·L^{-1} angoroside C and saturated with N_2O at pH 7 are shown in Fig 2a. The transient absorption spectra at 1 μs after electron pulse was predominantly due to OH radical adducts of dGMP. At 50 μs after the pulse, accompanying the decay of the absorption band in wavelength region 390 – 540 nm, a new absorption peak appeared at 360 nm, which was as-

signed as the absorption peak of phenoxyl radical of angoroside C arising from electron transfer reaction of anion radical of angoroside C at 360 nm. The transient absorption spectra recorded at 1 μs and 50 μs after pulse radiolysis of 2 mmol·L^{-1} dGMP aqueous solution containing 0.1 mmol·L^{-1} acteoside and saturated with N_2O at pH 7 are shown in Fig 2b. It indicates that the absorption peak of phenoxyl radical of acteoside was also at 360 nm. In addition, the transient absorption spectra obtained from 2 mmol·L^{-1} dAMP aqueous solution containing 0.1 mmol·L^{-1} angoroside C or acteoside and saturated with N_2O at pH 7 are shown in Fig 2c and 2d.

Reaction rate constants for reactions of angoroside C and acteoside with OH radical adducts of dGMP and dAMP A series of pseudo-first-order rate constants (k_{app}) of formation of angoroside C phenoxyl radical were determined with a fixed concentration (2 mmol·L^{-1}) of deoxyribonucleotide, and altering the concentrations of angoroside C from 0.02 mmol·L^{-1} to 0.1 mmol·L^{-1}. The k_{app} was treated as a function of the concentration of angoroside C, so that a line could be obtained, whose slope was the rate constant (k) of electron transfer reaction of deoxyribonucleotide with angoroside C. The electron transfer rate constants of angoroside C with dAMP and dGMP were 4.2×10^8 and 10.3×10^8 L·mol^{-1}·s^{-1}, respectively. The electron transfer reaction rate constants of deoxyribonucleotide and acteoside could be determined in the same way. The electron transfer rate constants of acteoside with dAMP and dGMP were 5.3×10^8 and 20.2×10^8 L·mol^{-1}·s^{-1}, respectively.

DISCUSSION

The reactions between OH radical, deoxyribonucleotides and PG are shown as follows taking dGMP as an example:

$$OH\cdot + dGMP \longrightarrow [dGMP\text{-}OH]^{\cdot}_{OX} + [dGMP\text{-}OH]^{\cdot}_{red} \quad (1)$$

$$[dGMP\text{-}OH]^{\cdot}_{OX} + PG \longrightarrow [dGMP\text{-}OH]^{-}_{OX} + PG^{\cdot} \quad (2)$$

$$[dGMP\text{-}OH]^{-}_{OX} \xrightarrow{-OH} dGMP \quad (3)$$

In these three steps, step 2 is more important as it determines the electron transfered from PG to oxidized OH radical adduct of deoxyribonucleotide. In this study, the reaction rate of dGMP was faster than that of dAMP, as guanosine is more easily oxidizable than adenosine.

The above-mentioned results indicated that the phenylpropanoid glycosides, which were extracted from

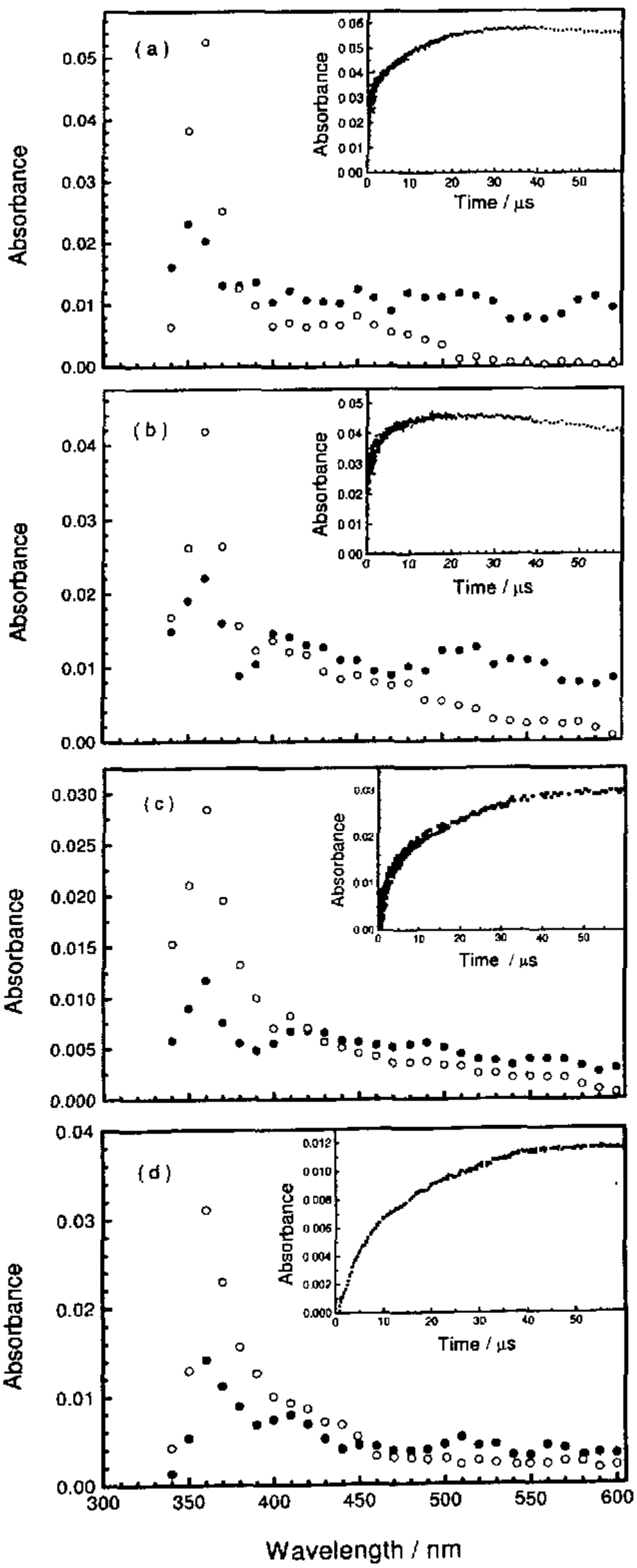

Fig 2. Transient absorption spectra from pulse radiolysis of dAMP or dGMP 2 mmol·L^{-1} and angoroside C or acteoside aqueous solution 0.1 mmol·L^{-1}, saturated with N_2O at pH 7 at 1 μs (●) and 50 μs (○). Insert: Traces of anion radicals of phenylpropanoid glycosides at 360 nm. (a) dGMP and angoroside C; (b) dGMP and acteoside; (c) dAMP and angoroside C; (d) dAMP and acteoside.

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Scrophularia ningpoensis, were able to react with the OH radical adducts of deoxyribonucleotide *via* electron transfer process. Thus reparing the damaged deoxyribonucleotides could be reported. According to Rice-Evans, *et al*[12], the phenolic compounds possess an antioxidative activity. Hence it can be suggested that the repairing activity of phenylpropanoid glycoside compounds seems to be related to the phenolic hydroxy in the structure.

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玄参中苯丙素苷对脱氧核苷酸羟基加成自由基的快速修复作用[1]

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关键词 玄参；糖苷类；抗氧化剂；脱氧腺嘌呤核苷酸类；脱氧鸟嘌呤核苷酸类

目的：探讨玄参化学成分的抗氧化作用. **方法**：应用多种层析方法和化学和光谱方法，对玄参的水溶性主要成分进行分离和结构鉴定. 应用脉冲辐解方法，观察分离得到的主要单体成分对脱氧核苷酸 dAMP 和 dGMP 羟基加成自由基的快速修复过程，测定苯丙素苷与羟基加成自由基的反应速率常数. **结果**：从玄参水溶性部位分得四种主要成分，发现它们属于环烯醚萜苷：哈帕酯苷与哈帕苷，和苯丙素苷：安格洛苷 C 与 acteoside. 脉冲辐解实验中观察到在 0.1 mmol·L^{-1}时，苯丙素苷安格洛苷 C 与 acteoside 对脱氧核苷酸羟基加成自由基产生显著的修复作用，而环烯醚萜苷：哈帕酯苷与哈帕苷在相同条件下作用不明显. 安格洛苷 C 与 dAMP 及 dGMP 间的电子转移速率常数为 4.2×10^8 及 10.3×10^8 L·mol^{-1}·s^{-1}；acteoside 与 dAMP 及 dGMP 间的电子转移速率常数为 5.3×10^8 及 20.2×10^8 L·mol^{-1}·s^{-1}. **结论**：玄参中的苯丙素苷在还原脱氧核苷酸的氧化性羟基加成自由基方面有很好的抗氧化作用.

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