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Photoreactivity of Anthraquinones for the Analysis of Ginsenosides Using Photoreduction Fluorescence Detection-HPLC

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The photoreactivity of twelve anthraquinone derivatives was examined to evaluate its usefulness as a photo-reagent for the analysis of ginsenosides using photoreduction fluorescence (PRF) detection method. Among the tested compounds, 2-*tert*-butylanthraquinone (TBAQ), 2-chloroanthraquinone (CAQ) and anthraquinone (AQ) showed good characteristics as photoreagents. The detection limits of ginsenoside Rg_1 by PRF-HPLC method using TBAQ, CAQ or AQ as a photo-reagent were found to be ca. 35 ng, 50 ng and 50 ng, respectively.

Key words : Anthraquinone derivatives, Photoreduction fluorescence detection, Analysis of ginsenoside, HPLC chemical Ionization

INTRODUCTION

Photoreduction fluorescence detection HPLC (PRF-HPLC) was first introduced by Birks and Gandelman for the analysis of alcohols, carbohydrates and cardiac glycosides (Gandelman *et al.*, 1982; 1983a; 1983b). Recently we applied this method to the analysis of ginsenosides and saikosaponins (Kim *et al.*, 1992; Park *et al.*, 1995; Shin *et al.*, 1996).

In PRF-HPLC method, anthraquinone derivatives react with proton donor compounds such as alcohol, amine and ether to form highly fluorescent 9,10-dihydroxyanthracene derivatives (AQH_2) in anaerobic condition (Carson *et al.*, 1973; Loelt *et al.*, 1983). Since the amount of AQH_2 produced is proportional to the amount of analyte, one can quantitize the analyte by measuring the fluorescence intensity of AQH_2.

In this study, we examined photoreactivity of twelve anthraquinone derivatives to evaluate its applicability to the analysis of ginsenosides using HPLC-PRF detection.

MATERIALS AND METHODS

Materials

Anthraquinone (AQ), 2,3-dimethylanthraquinone (DMAQ), 2-methylanthraquinone (MAQ), anthraquinone-1,5-disulfonate (AQ15DS), anthraquinone-2,6-disulfonate (AQ26DS), 2-*tert*-butylanthraquinone (TBAQ), 2-(hydroxymethyl)-anthraquinone (HMAQ), 2-chloroanthraquinone (CAQ), 2-aminoanthraquinone (AAQ), 2-ethylanthraquinone (EAQ) and anthrone were purchased from Aldrich (U.S.A.) and were used after recrystallization in acetonitrile. Aloe-emodin (AE) was isolated from *Aloe vera* in our laboratory (Fig. 1). Acetonitrile (Merck, Germany) was of HPLC grade, and HPLC grade water was prepared using Millipore Super Q RO-60 (U.S.A.). Ginsenoside Rg_1 was isolated from ginseng in our laboratory.

Instruments

A Hitachi L-6000 pump (Hitachi, Japan) equipped with a 20 µl loop injector (model 7125, Rheodyne, USA), Hitachi F-1050 fluorescence detector (excitation : 400 nm, emission : 500 nm, Hitachi, Japan) was used. FC 4870A flow conditioner (Pickering laboratories, USA) was used as a pulse damper for flow injection analysis. Lichrosorb NH_2 column (250 mm×4 mm, 10 µm, Merck, Germany) was used for the analysis of ginsenosides, and acetonitrile/water (80/20) mixture with a photoreagent was used as mobile phase.

Photochemical reactor

The 40~70 cm long PTFE capillary tube (0.3 mm i.d.×1.5 mm o.d., Alltech associate, USA) was coiled around a 10W-UV lamp (2.5 cm×32 cm, cylinder type, Sam-gong co., Korea) and was wrapped with aluminium foil to increase the photon flux to the tube by reflection. The photoreactor was installed between

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column and fluorescence detector and purged with nitrogen to remove oxygen. The irradiation time was checked by varying the flow rate of the eluent. Details of the reactor system were reported in our previous paper (Park *et al.*, 1995).

Photoreactivity of anthraquinone derivatives to 2-propanol

Test solutions were prepared by adding 1 ml of 2-propanol to the 24 ml of each anthraquinone solution which contains 1.0×10^{-3}M of anthraquinone in 80% acetonitrile aqueous solution. Twenty microliter of test solution was injected to the HPLC system which consists of injector, damper, photoreactor with 70 cm PTFE reaction coil and fluorescence detector. Acetonitrile/water (80/20) solution was used as an eluent. The fluorescence intensities of test solutions were measured to evaluate the photoreactivity of anthraquinone derivatives.

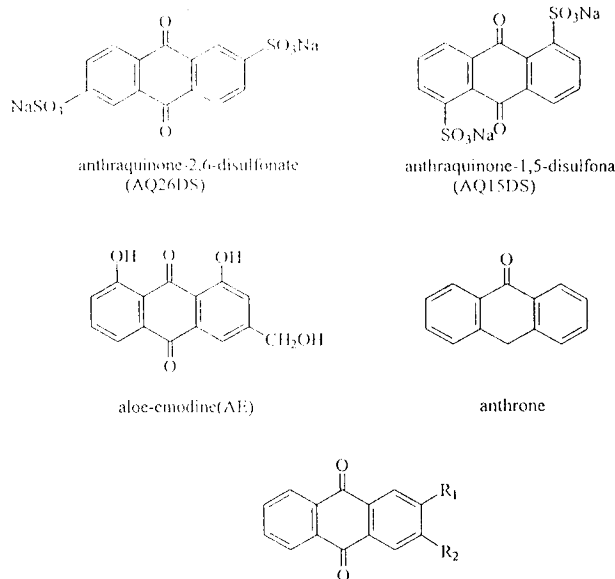
Photoreactivity of anthraquinone derivatives to ginsenoside Rg_1

Anthraquinone derivative was dissolved in the HPLC eluent (80% acetonitrile) with the concentration from 2×10^{-4}M to 2×10^{-5}M. The response of injected ginsenoside Rg_1 (5 μg) was examined to find out the optimal chromatographic parameters. The length of photoreaction coil was 40 cm and the reaction time was about 2.2 sec. In case of DMAQ, the photoreaction time was about 5.6 sec using 70 cm reaction coil.

RESULTS

Photoreactivity of anthraquinone derivatives to 2-propanol

For the preliminary evaluation of photoreactivity of anthraquinones, 2-propanol was used as an analyte. Since photoreaction time is one of the key factors which affect the signal intensity, its effect to the signal intensity was tested. As shown in Fig. 2, the highest signal intensity was observed at ca. 2.3 sec for AQ, MAQ, EAQ, CAQ, TBAQ and AQ26DS, and at ca. 5.6 sec for HMAQ and DMAQ. AAQ, AQ 15DS, AE and anthrone showed little activity.



anthraquinone derivatives	R_1	R_2
anthraquinone (AQ)	H	H
2,3-dimethylanthraquinone (DMAQ)	CH_3	CH_3
2-chloroanthraquinone (CAQ)	Cl	H
2-methylanthraquinone (MAQ)	CH_3	H
2-ethylanthraquinone (EAQ)	CH_2CH_3	H
2-tert-butylanthraquinone (TBAQ)	$C(CH_3)_3$	H
2-(hydroxymethyl)anthraquinone (HMAQ)	CH^2OH	H
2-aminoanthraquinone (AAQ)	NH_2	H

Fig. 1. The structure of anthraquinone derivatives

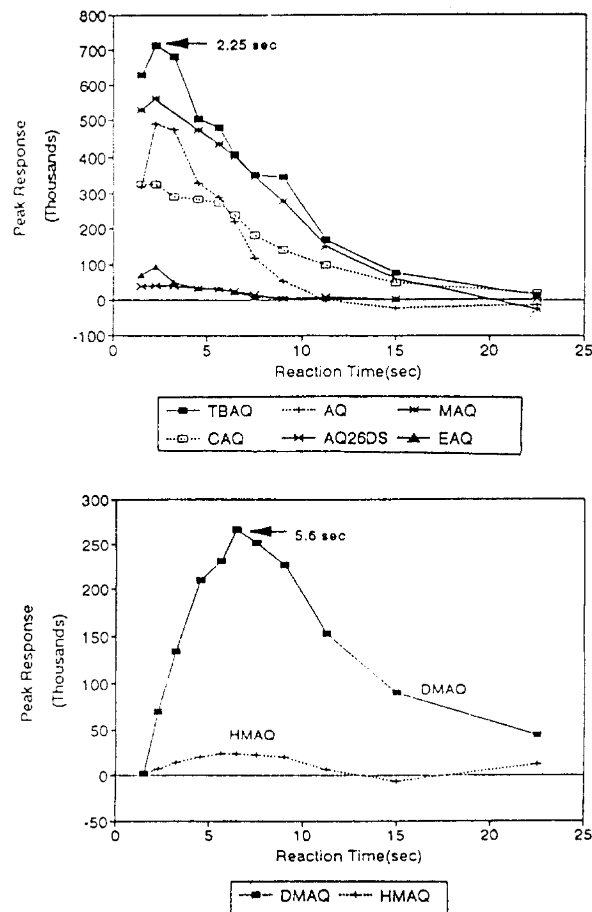


Fig. 2. Photoreduction reactivity of anthraquinone derivatives to propanol by flow injection analysis. 10W UV lamp with 70 cm PTFE tube, eluent : acetonitrile/water=80/20, 20 l of 1×10^{-3}M AQ derivative with 0.05 M 2-propanol in acetonitrile/water (80/20) was injected into HPLC.

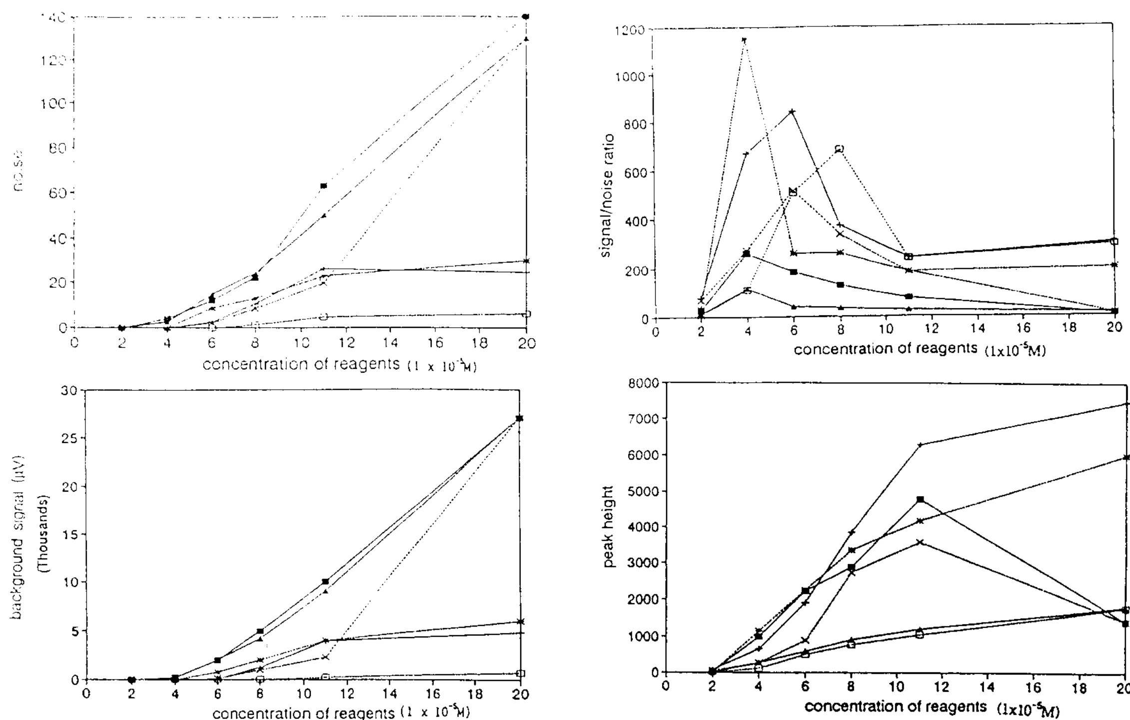


Fig. 3. The effect of concentration of photo-reagent to the peak response of ginsenoside Rg_1. 10W UV lamp with 40 cm PTFE tube, reaction time : 2.2 sec, eluent : CH_3CN/H_2O=80/20 with AQ derivatives (2×10^{-5}M-2×10^{-4}M), ginsenoside Rg_1 (5 μg) was injected into HPLC (■—■, MAQ; +—+, AQ; *—*, TBAQ; □—□, CAQ; ×—×, EAQ; ▲—▲, DMAQ)

Long reaction time resulted in reduced peak response due to the degradation of the dihydroxyanthracene compound produced (Gandelman *et al.*, 1983a; 1983b). Among twelve reagents, AQ, MAQ, CAQ and TBAQ showed superior photoreactivity to HMAQ, AAQ, AQ15DS, AE and anthrone.

Photoreactivity of anthraquinone derivatives to ginsenoside Rg_1

Fig. 3 shows the effect of concentration of photoreagents on the peak intensity of ginsenoside Rg_1, on the background signal level, on the S/N ratio and on the noise level. In general, high concentration of reagent resulted in the increase of peak height, as well as background and noise level.

MAQ, DMAQ and EAQ showed strong peak intensity, but S/N ratios were low due to the high background signal and noise. AQ, CAQ and TBAQ showed low noise and background signal, consequently high S/N ratio. CAQ showed the lowest background signal and noise, but signal intensity was relatively small. AQ and TBAQ showed best S/N ratio.

Among twelve anthraquinone derivatives, AQ, CAQ and TBAQ were found to be good photoreactive reagents for ginsenosides in PRF-HPLC. Table I shows the detection limit of ginsenoside Rg_1 (S/N=3) analyzed in optimal concentration of these reagents.

Table I. Comparison of detection limits of ginseonisde Rg_1 (S/N=3)

detection method	detection limit
PRF(AQ)	50 ng
PRF(TBAQ)	35 ng
PRF(CAQ)	50 ng
UV	100 ng
RI	2000 ng

DISSCUSSION

AQ15DS which has no C2-substituent did not show photoactivity to ginsenoside Rg_1, while other C2-substituted anthraquinones showed photoreactivity, which was consistent with Moore's report (Moore *et al.*, 1987). Among C2-sustituted anthraquinones, alkyl- or chlorine-substituted compounds showed high reactivity, but amino- or hydroxy-substituted compound showed little reactivity. This phenomena may be arised from the intra-molecular hydrogen abstraction reaction by amino or hydroxy group (Inoue *et al.*, 1982; Flom *et al.*, 1985). Among C2-alkylated anthraquinones, higher alkyl homologue showed better reactivity, namely TBAQ showed higher S/N ratio than MAQ, EAQ or DMAQ.

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