ELSEVIER

Food Chemistry 70 (2000) 437–441

Food Chemistry

www.elsevier.com/locate/foodchem

Antioxidant activity of anthraquinones and anthrone

Gow-Chin Yen [a,*], Pin-Der Duh [b], Da-Yon Chuang [a]

[a] *Department of Food Science, National Chung Hsing University, 250, Kuokuang Road, Taichung 402, Taiwan*
[b] *Department of Food Health, Chia Nan College of Pharmacy and Science, Tainan 717, Taiwan*

Received 23 October 1999; received in revised form 14 February 2000; accepted 14 February 2000

Abstract

The antioxidant properties of anthraquinones (AQs) and anthrone were evaluated using different model systems. The antioxidant activity of these compounds (200 ppm) on the inhibition of peroxidation of linoleic acid was found to be in the order of BHA (96%), anthrone (95%), alizarin (93%) > aloe-emodin (78%) > rhein (71%) > emodin (36%) > anthraquinone (8%). Chrysophanol accelerated the peroxidation of linoleic acid. Anthrone and alizarin exhibited a reducing power, although the other AQs did not show any reducing power. AQs and anthrone exhibited a weak chelating ability on iron (II). At a concentration of 0.25 mg/ml, the scavenging effects of anthrone, aloe-emodin and emodin, on hydroxyl radicals produced by the Fenton reaction were 26.2, 16.6 and 41.8%, respectively. However, at the same concentration, anthraquinone, alizarin, chrysophanol and rhein accelerated the production of hydroxyl radicals. These results suggest that the antioxidant mechanism, for both emodin and aloe-emodin, possibly depends on scavenging hydroxyl radicals. The strong activity shown by anthrone could be due to its reducing power and scavenging effects on hydroxyl radicals. The pro-oxidant activity exhibited by chrysophanol might be due to the enhanced production of free radicals.

Keywords: Antioxidant activity; Anthraquinones; Anthrone; Free radical

1. Introduction

Antioxidants are used to preserve foods by retarding discoloration, rancidity, or deterioration due to autoxidation. However, synthetic antioxidants have been reported to be carcinogenic (Imaida, Fukushima, Shivai, Ohtani, Nakanishi & Ito, 1983). Hence, several attempts to replace synthetic antioxidants with natural antioxidants have been developed. Antioxidative substances obtained from natural sources, such as oilseed, grains, beans, vegetables, fruits, leaf waxes, bark, roots, spices, hulls and seaweeds, have been investigated (Duh, Yen & Yen, 1992; Milovanoric, Jovanovic, Radovic & Vrbaski, 1996; Namiki, 1990; Oomah & Mazza, 1996; Yen, Chen & Duh, 1998).

The seeds of *Cassia tora* L., called jue ming zi in Chinese, have been conventionally used in traditional Chinese medicine for several centuries. Traditionally, jue ming zi has been used to improve visual acuity and to remove "heat" from the liver. This herb has been reported to contain many active components, including chrysophanol, emodin, aloe-emodin, rhein, physcion, obtusin, aurantio-obtusin, rubrofusarin, torachryson, and toralactone (Duke, 1992). The basic chemical structures of anthraquinones and anthrones are shown in Fig. 1. The anthraquinones, with two ketone groups added at position C9 and position C10, occur naturally in plants. Anthraquinones and anthrones, of which the latter is reduced from the former, are referred to as anthraquinoids. The anthraquinones, accompanied by an additional functional group on the phenyl group, are effective against certain diseases (Brown, 1980; Huang, Chang, Tung, Wu & Foegh, 1992; Malterud, Farbrot, Huse & Sund, 1993; Mitsuda, Yasumoto & Iwami, 1966). In our previous study, the extracts of jue ming zi and emodin were determined to exhibit antioxidant activity (Yen et al., 1998). However, it remains unclear whether anthraquinoids possess antioxidant activity.

The present paper describes the inhibitory effects of anthraquinones and related compounds on the peroxidation of linoleic acid emulsion. The correlation between the structure of anthraquinoids and their inhibitory effect, as well as their effects on chelating metal

* Corresponding author. Fax: 886-4-285-4378.
E-mail address: gcyen@mail.nchu.edu.tw (G.-C. Yen).

0308-8146/00/$ - see front matter
PII: S0308-8146(00)00108-4

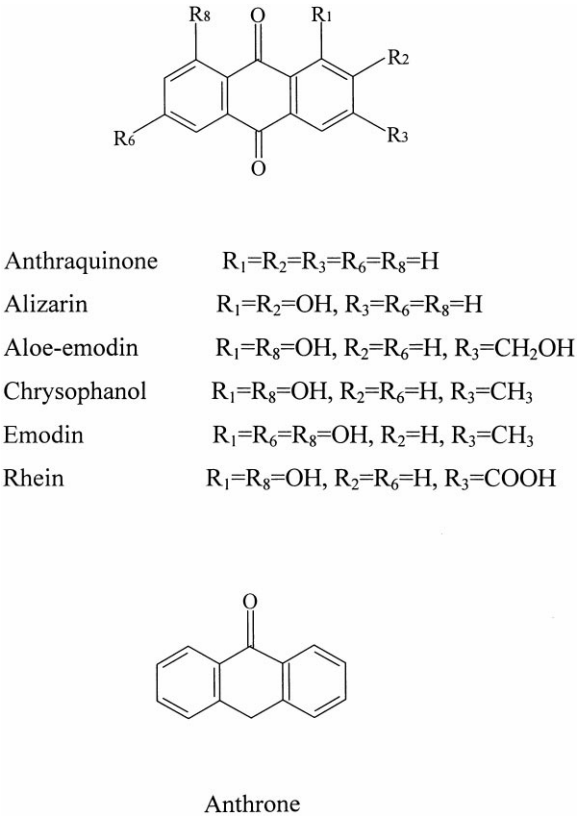

Fig. 1. Basic chemical structures of anthraquinones and anthrone.

ions, scavenging hydroxyl radicals and their reducing capacity, are also discussed.

2. Materials and methods

2.1. Materials

Anthraquinone was purchased from Fluka (Buchs, Switzerland). Anthrone, alizarin, aloe-emodin, rhein, emodin, chrysophanol, butylated hydroxyanisole, α-tocopherol, and 5,5-dimethyl pyrrolidine-*N*-oxide were obtained from Sigma Chemical Co. (St. Louis, MO). Selected compounds tested were dissolved in dimethyl sulfoxide (E. Merck Co., Darmstadt, Germany).

2.2. Antioxidant activity in a linoleic acid system

Antioxidant activity assay was carried out by using the linoleic acid system. A sample solution containing each sample (0.5 ml, 2.0 mg/ml), a solution of linoleic acid emulsion (2.5 ml, 0.02 M), and 0.2 ml phosphate buffer (pH 7.0, 0.2 M) was mixed thoroughly. The reaction mixture was incubated at 37°C in the dark, and the degree of oxidation was measured according to the thiocyanate method (Mitsuda et al., 1966), by sequentially adding ethanol (4.7 ml, 75%), ammonium thiocyanate (0.1 ml, 30%), sample solution (0.1 ml) and ferrous chloride (20 mM in 3.5% HCl) solution (0.1 ml). After the mixture was stirred for 3 min, the peroxide value was determined by reading the absorbance at 500 nm, and the percent inhibition of linoleic acid peroxidation was calculated as (%) = [1-(absorbance of the sample at 500 nm)/(absorbance of control at 500 nm)] × 100. All tests were run in duplicate, and analyses of all samples were run in triplicate and averaged.

2.3. Reducing power

The reducing power of extract was determined according to the method of Oyaizu (1986). Samples (2.5 ml, 0–10 mg/ml) in phosphate buffer (2.5 ml, 0.2 M, pH 6.6) were added to potassium ferricyanide (2.5 ml, 1.0%) and the mixture was incubated at 50°C for 20 min. Trichloroacetic acid (2.5 ml, 10%) was added, and the mixture was centrifuged at $650 \times g$ for 10 min. The supernatant (5.0 ml) was mixed with distilled water (5.0 ml) and ferric chloride (1.0 ml, 0.1%) , and then the absorbance was read spectrophotometrically at 700 nm. Higher absorbance of the reaction mixture indicated greater reducing power.

2.4. Chelating of metal ions

The chelating of ferrous ions by the sample was estimated by the method of Dinis, Madeira and Almeida (1994). Briefly, samples (0.2 mg) were added to a solution of 2.0 mM ferrous chloride (0.1 ml) and 3.7 ml methanol. The reaction was initiated by the addition of 5 mM ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the resulting solution was then measured spectrophometrically at 562 nm. All tests and analyses were run in replicate and averaged.

2.5. Hydroxyl radical scavenging activity

The hydroxyl radical reacts rapidly with the nitrone spin trap DMPO; the resultant DMPO-OH adduct can be detected by use of electron paramagnetic resonance (EPR) spectroscopy. The spectrum was recorded 2.5 min after samples (0.2 ml) were mixed with H_2O_2 (10.0 mM, 0.2 ml), Fe^{2+} (10.0 mM, 0.2 ml) and DMPO (0.3 M, 0.2 ml) in phosphate buffer (pH 7.2). Parameters of EPR spectrometer (Bruker ER 200D 10/12) were set at the following conditions: receiver gain, 8×10^5; modulation amplitude, 1.0 G; scan time, 200 s; field, 3478.9±50 G; time constant, 0.5 s (Shi, Dalal & Jain, 1991). The data are the average of two determinations.

2.6. Statistical analysis

Statistical analysis was carried out by the Statistical Analysis System (Statistical Analysis System Institute

[SAS], 1985) software package. Analysis of variance was performed by ANOVA procedures. Significant differences between means were determined by Duncan's multiple range tests.

3. Results and discussion

The data of percentage inhibition of linoleic acid peroxidation, determined by the thiocyanate method, at 37°C after the addition of different authraquinones, anthrone and other antioxidants, are plotted in Fig. 2. The order of potency of these compounds was found to be: BHA (96%), anthrone (95%), alizarin (93%) > α-tocopherol (79%), aloe-emodin (78%) > rhein (71%) > emodin (36%) > anthraquinone (8%) > chrysophanol (−23%). BHA, anthrone and alizarin exhibited the strongest activity, with no significant differences ($P > 0.05$) in antioxidant activity to be found among them. Of these compounds, anthrone, which lacks a ketone group at the position C-10 , showed the strongest antioxidant activity when compared with the other of anthraquinoids. No significant difference ($P > 0.05$) was found between alizarin and anthrone, indicating that hydroxylation at positions C-1 and C-2 in the case of alizarin contributes to the inhibition, even though alizarin contained a ketone group at position C-10. The antioxidant activity of aloe-emodin was stronger than that of rhein indicating that the carboxyl group in rhein, resulting from the oxidation of aloe-emodin, lowered its antioxidant effects. Chrysophanol acts as a prooxidant in the model system. This observation is in agreement with the study by Hartman and Goldstein (1989). However, the mechanism of pro-oxidation of chrysophanol remains unclear.

Some literature (Yen & Duh, 1993) has reported that reducing power was associated with antioxidant activity. Pitotti, Elizalde and Anese (1995) noted that the antioxidative effect of Maillard reaction products (MRP) were shown to be concomitant with the development of reducing power. Thus, it is necessary to determine the reducing power of anthraquinones and anthrone in order to elucidate the relationship between their antioxidant effect and their reducing power. The reducing power of different anthraquinones and anthrone are shown in Fig. 3. The reducing power of anthrone and alizarin increased with an increase in concentration; however, the remaining anthraquinones showed no reducing capacity. According to Figs. 2 and 3, the greater reducing power of anthrone and alizarin correlates well with their marked antioxidative action, indicating that their reducing power contributes to their antioxidant activity. However, the other anthraquinones, which also possessed antioxidant activity, showed almost no reducing power, suggesting that reducing power of other anthraquinoids, except for anthrone and alizarin, can not be considered as contributing to their antioxidative effect.

Fig. 4 shows the chelating effect of different anthraquinones and anthrone on ferrous ions. Selected anthraquinones at 0.5 mg/ml show a 0–15% chelating effect on Fe^{2+}. Of these compounds, anthraquinone did not show any chelating effect, and anthrone showed nearly no effect. Apparently, these compounds exhibited weak chelating effects on metal ions. This observation corresponds well with the report of Weng and Gordon

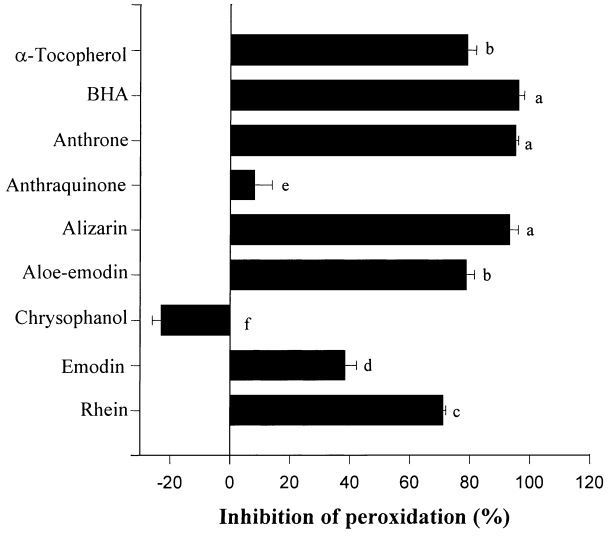

Fig. 2. Antioxidant activity of anthraquinones and anthrone. *Inhibition of peroxidation (%) = [1–(absorbance of sample at 500 nm)/(absorbance of control at 500 nm)]×100. $^{a-f}$. Each value is the mean± standard derivation of three replicate analyses. Values in each column with different letters are significantly different ($P < 0.05$). **The concentrations of various anthraquinones and anthrone were 0.2 mg/ml.

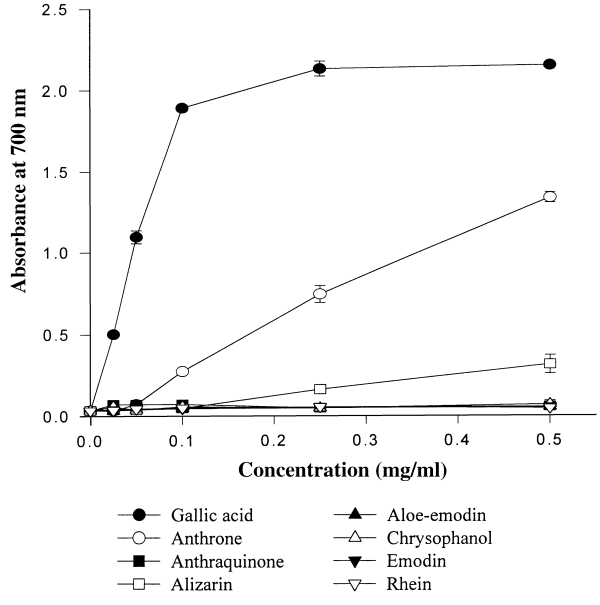

Fig. 3. Reducing power of anthraquinones and anthrone.

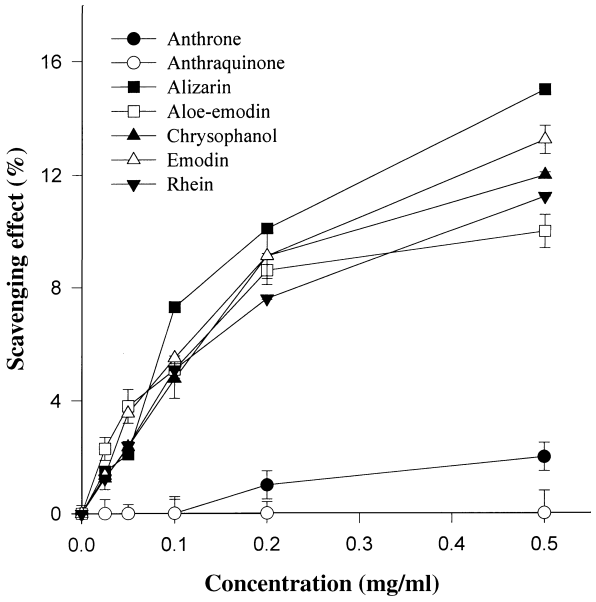

Fig. 4. Chelating effects of anthraquinones and anthrone on Fe^{2+}.

(1992), who noted that certain quinone-derivatives, isolated from Tanshen (*Salvia miltiorrhiza* Bunge), showed weak chelating effects on Fe^{2+}. Apparently, the chelation efficiency of a compound on Fe^{2+} is dependent on the number of hydroxyl groups. Moreover, the orthodihydroxyl, at positions C-1 and C-2 in alizarin, results in an increasing chelating effect. This suggests that hydroxyl substitution in the *ortho* position is desirable.

The scavenging activity of different anthraquinones and anthrone on the hydroxyl radical was evaluated by the EPR spectra of DMPO-OH spin adducts (data not shown). At a concentration of 0.25 mg/ml, anthrone, aloe-emodin and emodin showed 26.2, 16.6 and 41.8% scavenging effects, respectively, on hydroxyl radicals produced by the Fenton reaction (Table 1). However, anthraquinone, alizarin, chrysophanol and rhein, at 0.25 mg/ml, accelerated the formation of hydroxyl radicals. Among these compounds, emodin exhibited the strongest scavenging effect on hydroxyl radicals. Emodin has been known to be pharmacologically potent and this characteristic was reported to be associated with its scavenging of hydroxyl radicals (Huang, Lee, Lee, Chao, Chen & Chu, 1991). On the other hand, the prooxidant ability of chrysophanol may derive from its greater formation of hydroxyl radicals.

In conclusion, the basic chemical structure of anthrone exhibited the role of electron acceptor, and the *ortho*-dihydroxy substituent in alizarin, polyhydroxyl group at position C1, C6 and C8 with methylation at position C3 (emodin), and polyhydroxyl group at position C1 and C8 with hydroxylmethylation at position C3 (aloe-emodin) are multifunctional antioxidants, combining both chain-breaking and metal-chelating properties. In general, the greater reducing power of anthrone and greater reducing power as well as metal chelating activity of alizarin, may relate to their marked antioxidant activity. On the other hand, the significant scavenging effect of emodin and aloe-emodin on hydroxyl radicals may contribute to their antioxidant activity. However, it is not easy to relate the data presented in the four model tests because of differences in test methods, substances and antioxidant concentrations. In the present work the antioxidant properties of anthraquinones depend on both hydroxyl substitution in the structure and on the methods of assessment.

Table 1
Effects of anthraquinones and anthrone on EPR spectrum signal intensity of DMPO-OH spin adducts

Sample[a]	Relative EPR signal intensity[b]	Scavenging effect (%)[c]
Control	100	0.00
Anthrone	73.8	26.2
Anthraquinone	153	−53.1
Alizarin	123	−23.3
Aloe-emodin	83.4	16.6
Chrysophanol	218	−117.3
Emodin	58.2	41.8
Rhein	130	−30.1

[a] The concentration of anthraquinones and anthrone was 0.25 mg/ml.
[b] Relative EPR signal intensity (%) = $\{[h\Delta H^2(\text{sample})/h\Delta H^2(\text{dpph})]/[h\Delta H^2(\text{control})/h\Delta H^2(\text{dpph})]\} \times 100$. h = The height of the peak. ΔH = The width of the peak.
[c] Scavenging effects (%) = 100-relative EPR signal intensity (%). Data are the average of two determinations.

Acknowledgements

This research work was supported in part by the National Science Council, Republic of China under grant NSC 88-2313-B005-005.

References

Brown, J. P. (1980). A review of the genetic effects of naturally occurring flavonoids, anthraquinones and related compounds. *Mutation Research, 75*, 243–277.

Dinis, T. C. P., Madeira, V. M. C., & Almeida, L. M. (1994). Action of phenolic derivatives (acetaminophen, salicylate, and 5-aminosalicylate) as inhibitors of membrane lipid peroxidation and as peroxyl radical scavengers. *Archives of Biochemistry and Biophysics, 315*, 161–169.

Duh, P. D., Yen, D. B., & Yen, G. C. (1992). Extraction and identification of an antioxidative component from peanut hulls. *Journal of the American Oil Chemists' Society, 69*, 814–818.

Duke, J.A. (1992). *Handbook of phytochemical constituents of GRAS herbs and other economic plants*. Boca Raton, FL: CRC Press (pp. 143–144).

Hartman, P. E., & Goldstein, M. A. (1989). Superoxide generation by photomediated redox cycling of anthraquinones. *Environ. Mol. Mutat., 14*, 42–47.

Huang, H. C., Chang, J. H., Tung, S. F., Wu, R. T., & Foegh, M. L. (1992). Immunosuppressive effect of emodin, a free radical generator. *European Journal of Pharmacology, 211*, 359–364.

Huang, H. C., Lee, C. R., Lee, Chao, P. D., Chen, C. C., & Chu, S. H. (1991). Vasorelaxant effect of emodin, an anthraquinone from a Chinese herb. *Journal of Pharmacology, 205*, 289–294.

Imaida, K., Fukushima, T., Shivai, T., Ohtani, M., Nakanishi, K., & Ito, N. (1983). Promoting activities of butylated hydroxyanisole and bultylated hydroxytoluene on 2-stage urinary bladder carcinogenesis and inhibition of γ-glutamyl transpeptidase-positive foci development in the liver of rats. *Carcinogenesis, 4*, 895–899.

Malterud, K. E., Farbrot, T. L., Huse, A. E., & Sund, R. B. (1993). Antioxidant and radical scavenging effects of anthraquinone and anthrones. *Pharmacology, 47*, 77–85.

Milovanoric, M., Jovanovic, P. J., Radovic, B. V., & Vrbaski, Z. (1996). Antioxidant effects of flavonoids of *Anthriscus sylvestris* in lard. *Journal of the American Oil Chemists' Society, 73*, 773–776.

Mitsuda, H., Yasumoto, K., & Iwami, K. (1966). Antioxidative action of indole compounds during the autoxidation of linoleic acid. *Eiyo to Shokuryo, 19*, 210–214.

Namiki, M. (1990). Antioxidants/antimutagens in foods. *Critical Reviews in Food Science and Nutrition, 29*, 925–932.

Oyaizu, M. (1986). Antioxidative activity of browning products of glucosamine fractionated by organic solvent and thin-layer chromatography. *Nippon Shokuhin Kogyo Gakkaishi, 35*, 771–775.

Oomah, B. D., & Mazza, G. (1996). Flavonoids and antioxidative activities in buchwheat. *Journal of Agriculture and Food Chemistry, 44*, 1746–1750.

Pitotti, A., Elizalde, B. E., & Anese, M. (1995). Effect of caramelization and Maillard reaction products on peroxidase activity. *Journal of Food Biochemistry, 18*, 445–457.

Statistical Analysis System Institute (1985). *SAS user's guide: statistics*. Cary, NC: author.

Shi, X., Dalal, N. S., & Jain, A. C. (1991). Antioxidant behaviour of caffeine efficient scavenging of hydroxyl radicals. *Food and Chemical Toxicology, 29*, 1–6.

Weng, X. C., & Gordon, M. H. (1992). Antioxidant activity of quinones extracted from Tanshen (*Salvia miltriorrhiza* Bunge). *Journal of Agricultural and Food Chemistry, 40*, 1331–1336.

Yen, C. C., Chen, H. H., & Duh, P. D. (1998). Extraction and identification of an antioxidative component from Jue Ming Zi (*Cassia tora* L.). *Journal of Agricultural and Food Chemistry, 46*, 820–824.

Yen, C. C., & Duh, P. D. (1993). Antioxidative properties of methanolic extracts from peanut hulls. *Journal of the American Oil Chemists' Society, 70*, 383–386.