In Vitro Antioxidant Activity and Antigenotoxic Effects of Avenanthramides and Related Compounds

Alison M. Lee-Manion,[†] Ruth K. Price,[†] J. J. Strain,[†] Lena H. Dimberg,[‡]
Kerstin Sunnerheim,[§,#] and Robert W. Welch*,[†]

[†]Northern Ireland Centre for Food and Health, School of Biomedical Sciences, University of Ulster, Coleraine BT52 1SA, United Kingdom, [‡]Department of Food Science, Swedish University of Agricultural Sciences, S-75007 Uppsala, Sweden, and [§]Institute of Chemistry, Department of Organic Chemistry, Uppsala University, S-75121 Uppsala, Sweden. [#]Present address: Department of Natural Sciences, Mid Sweden University, 70 Sundsvall, SE-85170 Sundsvall, Sweden.

Avenanthramides are substituted *N*-cinnamoylanthranilic acids, with hydroxycinnamic acid and anthranilic acid moieties. These alkaloid phenols, which are unique to oats, may confer health benefits via antioxidant or other mechanisms. Synthetic avenanthramides, hydroxycinnamic acids, Tranilast, and ascorbic acid were evaluated for antioxidant activity using two assays, DPPH (2,2-diphenyl-1-picrylhydrazyl) and FRAP (ferric reducing antioxidant potential), and for antigenotoxicity using the Comet assay with stressed human adenocarcinoma colon cells. Of all the compounds tested, *N*-(3′,4′-dihydroxy-(*E*)-cinnamoyl)-5-hydroxyanthranilic acid (**2c**), an abundant oat avenanthramide, generally had the highest activity in all three assays. The drug Tranilast showed antigenotoxic effects, but not antioxidant activity, suggesting that antigenotoxicity is not dependent on antioxidant effects. Overall, results show that avenanthramides exert antioxidant and antigenotoxic activities that are comparable to those of ascorbic acid and which have the potential to exert beneficial physiological effects.

KEYWORDS: Oats; avenanthramides; Tranilast; antioxidants; antigenotoxicity; Comet assay

INTRODUCTION

Although there is substantial evidence that whole-grain cereals are protective against heart disease, certain cancers, and diabetes, the mechanisms underlying these protective effects are unclear (*1*). Oats, which are usually consumed as a whole-grain cereal, are a good source of mixed-linkage soluble fiber $(1{\rightarrow}3)(1{\rightarrow}4)$-$\beta$-D-glucan ($\beta$-glucan or oat gum), and this grain component is associated with the cholesterol-lowering effects of dietary oats and with the ability of oats to modulate postprandial glucose and insulin responses (*2*). However, whole-grain cereals, such as oats, are also relatively high in a wide range of phenolic phytochemicals and other compounds, which also have number of potentially beneficial physiological effects, including antioxidant and anti-inflammatory activities (*3*).

Oats have substantial antioxidant activity, and oat flour was used as a food antioxidant to prevent rancidity before the advent of synthetic antioxidants (*4*, *5*). Oat antioxidants include tocols (tocopherols; tocotrienols), phytic acid, and a wide range of phenolic compounds (*5*, *6*). These phenolic compounds include the relatively simple hydroxycinnamic acids, *p*-coumaric, caffeic, ferulic, and sinapic acids (Figure 1), and the avenanthramides, which are alkaloid phenols, found uniquely in oats (*6*–*9*). Avenanthramides are substituted *N*-cinnamoylanthranilic acids, which comprise hydroxycinnamic acid and anthranilic acid moieties (Figure **2**). In the abbreviations used for the avenanthramides in this paper, the number (1 or 2) denotes anthranilic or 5-hydroxyanthranilic acid and the letter denotes the hydroxycinnamic acid moiety (p, *p*-coumaric; c, caffeic; f, ferulic; s, sinapic) (*10*). The major avenanthramides found in oats are 2p, 2c, and 2f, and 1p, 1c, and 1f have also been identified (*7*, *10*–*12*). However, there are no reports of the sinapic acid derivatives, 1s and 2s, in oats. Avenanthramides are present in oats at substantially higher concentrations than the free hydroxycinnamic acids and are major contributors to the antioxidant activity that has been demonstrated in vitro in oats (*8*, *9*, *13*).
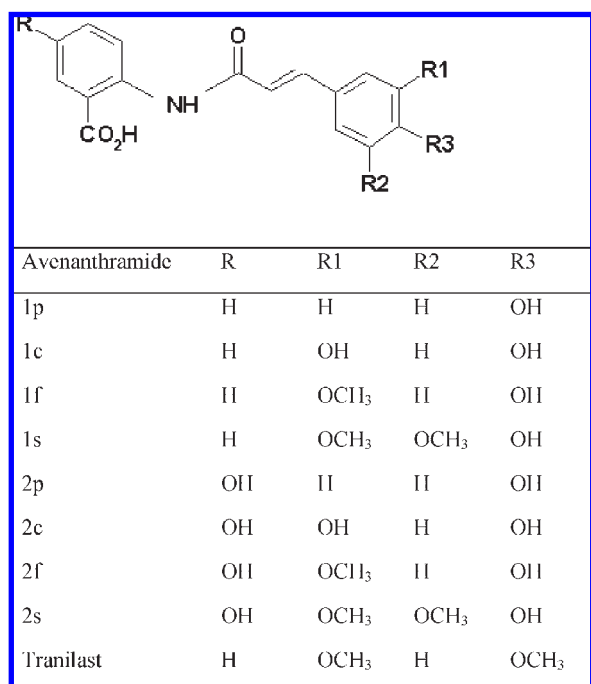
Phenolic antioxidants, including those from whole-grain cereals, have the potential to protect against oxidant damage to biomolecules such as LDL-cholesterol and DNA, which may be involved in the onset and progression of heart disease and cancer (*14*–*16*). Avenanthramides may also have antiatherogenic effects mediated by their anti-inflammatory and antiproliferative properties (*17*–*19*). Furthermore, avenanthramides are structurally very similar to the oral drug Tranilast (**Figure 2**), which has a number of potential therapeutic benefits including anti-inflammatory, antiatherogenic, and anticancer effects (*20*–*22*).

The aims of this study were to evaluate synthetic avenanthramides and related compounds for antigenotoxic effects by assessing protection against DNA damage in a human colon adenocarcinoma cell line, using the Comet assay, and to relate the antigenotoxic activity to antioxidant activity.

*Corresponding author [telephone + 44 (0)28 70324205; fax +44 (0)28 70324965; e-mail rw.welch@ulster.ac.uk].
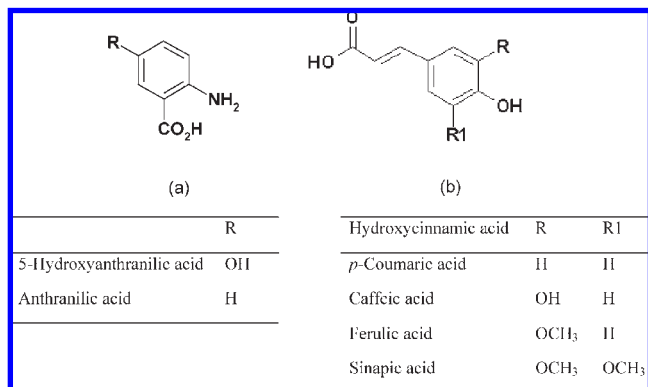
Figure 1. Structures of the (**a**) anthranilic acid and (**b**) hydroxycinnamic acid moieties.

	R
5-Hydroxyanthranilic acid	OH
Anthranilic acid	H

Hydroxycinnamic acid	R	R1
p-Coumaric acid	H	H
Caffeic acid	OH	H
Ferulic acid	OCH$_3$	H
Sinapic acid	OCH$_3$	OCH$_3$

Figure 2. Structures of the avenanthramides and Tranilast.

Avenanthramide	R	R1	R2	R3
1p	H	H	H	OH
1c	H	OH	H	OH
1f	H	OCH$_3$	H	OH
1s	H	OCH$_3$	OCH$_3$	OH
2p	OH	H	H	OH
2c	OH	OH	H	OH
2f	OH	OCH$_3$	H	OH
2s	OH	OCH$_3$	OCH$_3$	OH
Tranilast	H	OCH$_3$	H	OCH$_3$

MATERIALS AND METHODS

Chemicals and Reagents. Caffeic acid, *p*-coumaric acid, ferulic acid, sinapic acid, ascorbic acid, 5-hydroxyanthranilic acid, methanol (HPLC grade), 2,4,6-tripyridyl-*s*-triazine (TPTZ), ferrous sulfate, 2,2-diphenyl-1-picrylhydrazyl (DPPH), low melting point agarose, standard agarose, phosphate-buffered saline tablets (PBS), trypan blue dye, sodium chloride, disodium ethylenediaminetetraacetic acid dihydrate (EDTA), tris[hydroxymethyl]aminomethane (Tris), hydrogen peroxide solution, Triton X-100, sodium hydroxide, and ethidium bromide were all purchased from Sigma Aldrich (Poole, U.K.). Tranilast was purchased from Tocris Cookson Ltd. (Bristol, U.K.). Eight avenanthramides (1p, 1c, 1f, 1s, 2p, 2c, 2f, 2s) were synthesized using a modified version of the method of Mayama et al. (*23*) as outlined in Bratt et al. (*10*). Stock solutions of hydroxycinnamic acids, 5-hydroxyanthranilic acid, Tranilast, and the avenanthramides were prepared in methanol and stored at −70 °C until required. Ascorbic acid solutions were prepared freshly as required in deionized water. Lysis buffer (pH 10) for the Comet assay was made up using 2.5 M NaCl, 0.1 M EDTA, and 10 mM Tris, with 1% Triton X added to the solution immediately before use. HT-29 cells were obtained from the European Collection of Animal Cell Cultures (Salisbury, U.K.), and Dulbecco's Modified Eagle Medium (DMEM), trypsin−EDTA, and penicillin−streptomycin were from Gibco BRL (Eggenstein, Germany).

DPPH Assay. The free radical scavenging capacity of the compounds was evaluated using the DPPH method adapted from Miller et al. (*24*). Dilutions of 0, 75, 100, and 125 μmol/L of the test compounds were made with methanol on the day of analysis. Aliquots (5 mL) of these dilutions were added to 5 mL of DPPH solution (101 μM in methanol), methanol (15 mL), and water (25 mL), giving final concentrations of 0−12.5 μmol/L. After incubation (40 °C; 2 h) in a rotating incubator (SI 50 Stuart Scientific, Surrey, U.K.), the change in absorbance at 515 nm was used to calculate the amount of DPPH reduced by the test compounds as previously described (*24*). Initial absorbance readings for DPPH were around 0.9, and a 10% decrease was typical for the 0 μmol/L concentration. Duplicate analyses at each concentration were performed and results expressed as micromoles of DPPH reduced per micromole of antioxidant, calculated from the mean of the three concentrations tested. DPPH reduced was linearly proportional to the concentration of antioxidants over the ranges tested (data not shown). The coefficient of variation for the DPPH method was 10% ($n = 7$) from the 101 μM DPPH blank solution at time zero.

Ferric Reducing Antioxidant Potential (FRAP) Assay. The FRAP assay measures the combined reducing power of electron-donating antioxidants, using a timed redox-linked ferric−ferrous tripyridyltriazine reaction (*25, 26*). Analysis was carried out on an automatic analyzer (Hitachi 912, Lewes, U.K.), and FRAP values were calculated from a ferrous sulfate (FeSO$_4$·7H$_2$O) standard curve run in parallel. Dilutions (0, 250, 500, 750, and 1000 μmol/L) of the test compounds were prepared in water on the day of analysis. Duplicate analyses at each concentration were performed, and in all cases a linear dose−response relationship was observed over the range of concentrations tested (data not shown). Results were expressed as the parameter "equivalent concentration" 1 (EC$_1$), which is the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 mmol/L FeSO$_4$·7H$_2$O and was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 mmol/L concentration of Fe(II) solution determined using the corresponding equation of the line (*27*). Therefore, the lower the EC$_1$ value, the higher the antioxidant activity. An in-house pooled plasma standard was run with each batch analysis, and the coefficient of variation for the FRAP assay was 2% ($n = 7$).

Comet Assay for Genotoxicity. The Comet assay (single-cell gel electrophoresis) was used to assess potential protective effects of the test compounds against DNA damage in cells stressed with hydrogen peroxide.

Preparation of Test Compounds. Dilutions of the test compounds were made with water to concentrations of 5 and 25 μmol/L antioxidant in 1.56% aqueous methanol. Compounds that were difficult to dissolve were gently heated or sonicated. Aliquots (1 mL) of these dilutions were added to 9 mL of Dulbecco's Modified Eagle Medium (DMEM). The final concentrations of compounds analyzed were therefore 0.5 and 2.5 μmol/L, and the final concentration of methanol in the medium was 0.156%. Duplicate solvent controls (0.156% aqueous methanol) were included in each run.

Adenocarcinoma Cell Line. HT-29 cells (human colon adenocarcinoma cells) were grown in tissue culture flasks with DMEM supplemented with 10% fetal bovine serum and 1% penicillin−streptomycin at 37 °C in a (95%) humidified incubator (5% CO$_2$). On days 3 and 5 cells were trypsinized (1% trypsin, PBS) at 37 °C for a maximum of 10 min and subcultured at a dilution of 1:3. On day 5 cells were trypsinized again and subcultured at a dilution of 2:8. These cells were left to culture for 24 h to form a monolayer, and the medium was decanted in preparation for incubation with the test compounds.

Pretreatment and Stressing of Cells. HT-29 cells were incubated with the medium containing the test compounds (10 mL) for 24 h. Cells were then trypsinized, and cell viability was assessed using trypan blue exclusion. Cell suspensions were prepared in serum-free DMEM at a concentration of 1.5×10^5 cells per milliliter, and the suspensions (450 μL) were stressed by incubation with hydrogen peroxide (50 μL, 750 μM H$_2$O$_2$) for 5 min on ice. Duplicate suspensions from each treatment were included. Unstressed cells that had been treated with the different levels of antioxidant were included to assess if the test solutions caused any damage to the cells.

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Table 1. Antioxidant Activities (DPPH and FRAP Assays) and Antigenotoxic Effects (Comet Assay) of Hydroxycinnamic Acids, 1- and 2-Analogue Avenanthramides, 5-Hydroxycinnamic Acid, Tranilast, and Ascorbic Acid (Mean $\pm$ SD)

| compound	DPPH assay[a] (μmol)	FRAP assay[b] (EC$_1$, μmol/L)	Comet assay[c] (EC$_{50}$, μmol/L)
p-coumaric acid	1.8 $\pm$ 0.2 e	2419 $\pm$ 12.4 j	2.2 $\pm$ 0.6
caffeic acid	5.6 $\pm$ 0.1 b	362 $\pm$ 0.4 d	2.5 $\pm$ 0.1
ferulic acid	3.2 $\pm$ 0.2d	423 $\pm$ 1.2 f	1.9 $\pm$ 0.4
sinapic acid	2.7 $\pm$ 0.0f	334 $\pm$ 3.4 c	3.7 $\pm$ 3.1
avenanthramide 1p	0.0 $\pm$ 0.0 g	2901 $\pm$ 9.3 k	2.4 $\pm$ 0.1
avenanthramide 1c	3.2 $\pm$ 0.2 d	393 $\pm$ 1.7 e	1.5 $\pm$ 0.3
avenanthramide 1f	1.8 $\pm$ 0.0 e	697 $\pm$ 0.9 i	1.1 $\pm$ 0.2
avenanthramide 1s	NT[d]	550 $\pm$ 2.0 g	NT
avenanthramide 2p	5.7 $\pm$ 0.2 ab	343 $\pm$ 12.5 cd	2.6 $\pm$ 0.7
avenanthramide 2c	6.1 $\pm$ 0.3 a	275 $\pm$ 0.1 b	0.9 $\pm$ 0.4
avenanthramide 2f	3.3 $\pm$ 0.1 d	422 $\pm$ 2.2 f	1.6 $\pm$ 0.3
avenanthramide 2s	3.3 $\pm$ 0.2 d	604 $\pm$ 0.9 h	1.3 $\pm$ 0.1
5-hydroxyanthranilic acid	4.9 $\pm$ 0.2 c	234 $\pm$ 0.5 a	1.9 $\pm$ 0.0
Tranilast	0.0 $\pm$ 0.0 g	NR[e]	1.7 $\pm$ 0.1
ascorbic acid	1.7 $\pm$ 0.1 e	412 $\pm$ 0.4 ef	1.6 $\pm$ 0.0

[a] Micromoles of DPPH reduced per micromole of compound $\pm$ SD; higher values denote higher activity. Means followed by the same letter are not significantly different ($P < 0.05$). [b] EC$_1$, concentration of compound having ferric-TPTZ reducing ability equivalent to 1 mmol/L FeSO$_4 \cdot$7H$_2$O; lower values denote higher activity. Means followed by the same letter are not significantly different ($P < 0.05$). [c] EC$_{50}$, concentration of compound decreasing DNA damage by 50% of the H$_2$O$_2$ control; lower values denote higher activity. Means followed by the same letter are not significantly different ($P < 0.05$). [d] NT, not tested. [e] NR, no reaction.

Assessing Cell Damage. After incubation, cells were centrifuged (1000*g*, 3 min) in a microcentrifuge at room temperature. The supernatant was discarded, and the pellet was resuspended in 85 μL of 0.85% low melting point agarose and layered, with coverslips, onto frosted slides precoated with 100 μL of 1% normal melting point agarose. The gel layer was allowed to solidify, coverslips were removed, and slides were immersed in lysis buffer in Coplin jars. Slides that had been treated with H$_2$O$_2$ were kept in separate jars during lysis. Cells were lysed in lysis buffer for 1 h at 4 °C in the dark. After lysis, slides were transferred to an electrophoresis tank and placed in chilled electrophoresis buffer, and the DNA was allowed to unwind for 20 min before a current (25 V; 300 mA) was applied for 20 min. After electrophoresis, gels were washed (3 × 5 min) in neutralizing buffer (0.4 M Tris, pH 7.5) at 4 °C, stained with ethidium bromide (20 μg/mL), and covered with a coverslip. Slides were placed in a humidified airtight box and stored at 4 °C. Analysis was performed within 3 days by measuring the intensity of light in the comet tail, which represents the percentage of tail DNA damage. Gels were analyzed at 400× magnification using a fluorescence microscope, and 50 cells per slide were scored. Tail DNA was recorded using a computerized image analysis system (Komet 3.0, Kinetic Imaging, Liverpool, U.K.). All test and control samples were coded, and the operator was not aware of their identity while conducting the assays. The results are expressed as percent DNA in the comet tail (mean $\pm$ SD), which indicates DNA damage, and as the equivalent concentration 50 (EC$_{50}$), which is the concentration of antioxidant required to reduce the DNA damage, caused by the positive control, by 50%. The coefficient of variation for the Comet assay, which was estimated from the negative control run with each experiment, was 8.4% ($n = 6$).

Statistical Analysis. Results were analyzed by one-way analysis of variance followed by a post hoc test (Tukey) and by using Pearson's correlations (SPSS, version 11.0). Differences were considered to be significant at $P < 0.05$.

RESULTS

DPPH Assay. Table 1 shows the micromoles of DPPH reduced per micromole of the compounds tested, where the greater the
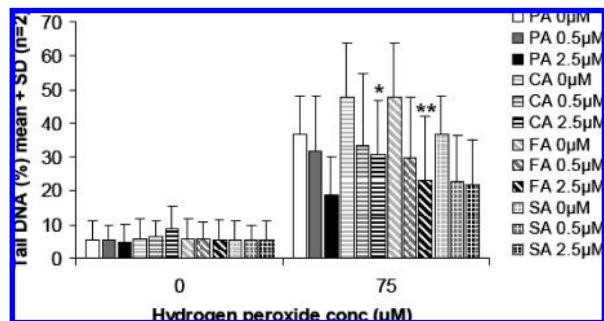
Figure 3. Effects of *p*-coumaric acid (PA), caffeic acid (CA), ferulic acid (FA), and sinapic acid (SA) pretreatment on hydrogen peroxide mediated DNA damage in the colon adenocarcinoma cell line HT-29, assessed by the Comet assay. Bars are means of duplicate analyses $\pm$ SEM. ∗ and ∗∗: significantly different from 0 μM at $P < 0.05$ and $P < 0.01$, respectively.
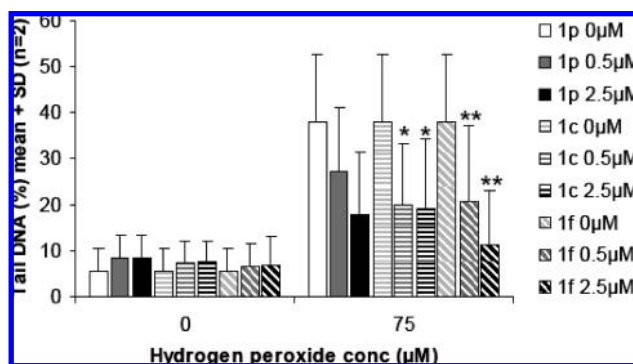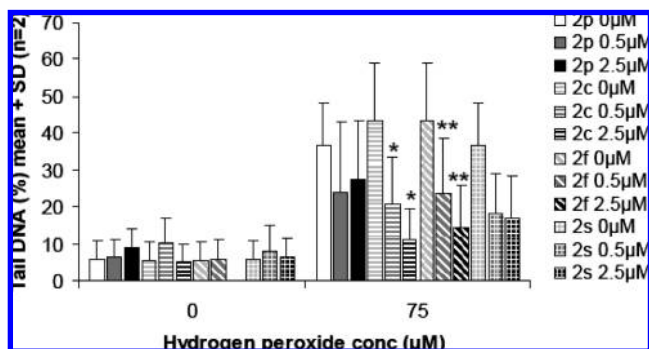
Figure 4. Effects of avenanthramide 1p, 1c, and 1f pretreatment on hydrogen peroxide mediated DNA damage in the colon adenocarcinoma cell line HT-29, assessed by the Comet assay. Bars are means of duplicate analyses $\pm$ SEM. ∗ and ∗∗: significantly different from 0 μM at $P < 0.05$ and $P < 0.01$, respectively.

amount of DPPH reduced, the higher the antioxidant activity. All compounds, except 1p and Tranilast, showed activity in this assay. The most reactive avenanthramides were 2c and 2p followed by 2f, 2s, and 1c, which were similar in activity. Caffeic acid had the highest activity of the hydroxycinnamic acids, followed by ferulic acid > sinapic acid > *p*-coumaric acid. The three 1-analogue avenanthramides (1p, 1c, 1f) each had significantly lower activities than their 2-analogue counterparts, and the activities of all 2-analogue avenanthramides were higher than, or equal to, those of their corresponding hydroxycinnamic acids. All avenanthramides, except 1p, and all of the hydroxycinnamic acids, except *p*-coumaric acid, had significantly higher activities than ascorbic acid.

FRAP Assay. Table 1 shows the EC$_1$ values for the compounds tested. The lower the EC$_1$ value, the higher the antioxidant activity of the compound. All compounds tested, except Tranilast, demonstrated antioxidant activity in the FRAP assay. 5-Hydroxyanthranilic acid and avenanthramide 2c had the highest antioxidant activities within this system, whereas *p*-coumaric acid and avenanthramide 1p had by far the lowest activities. All of the 1-analogue avenanthramides had lower activities than their corresponding 2-analogue avenanthramides and hydroxycinnamic acids, with the exception of 1s, which had a higher activity than 2s. In this system the only avenanthramides that had significantly higher activities than ascorbic acid were 2p and 2c.

Comet Assay. Results of the Comet assays are shown in Figures 3−6. Cells that were not stressed with hydrogen peroxide showed about 5% damage, and this was not influenced by the addition of

Figure 5. Effects of avenanthramide 2p, 2c, 2f, and 2s pretreatment on hydrogen peroxide mediated DNA damage in the colon adenocarcinoma cell line HT-29, assessed by the Comet assay. Bars are means of duplicate analyses ± SEM. ∗ and ∗∗: significantly different from 0 μM at $P < 0.05$ and $P < 0.01$, respectively.
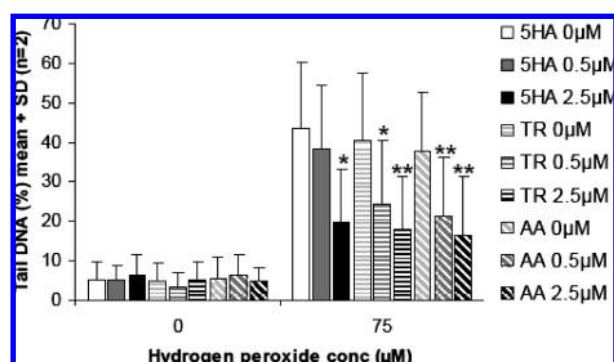
Figure 6. Effects of 5-hydroxyanthranilic acid (5HA), Tranilast (TR), and ascorbic acid (AA) pretreatment on hydrogen peroxide mediated DNA damage in the colon adenocarcinoma cell line HT-29, assessed by the Comet assay. Bars are means of duplicate analyses ± SEM. ∗ and ∗∗: significantly different from 0 μM at $P < 0.05$ and $P < 0.01$, respectively.

any test compounds. In H_2O_2-stressed cells DNA damage was significantly ($P < 0.05$) lower than in the control for 2c, 2f, 1c 1f, Tranilast, and ascorbic acid at both concentrations tested, and for caffeic, ferulic, and 5-hydroxyanthranilic acid at the higher concentration tested. The EC_{50} values for each of the compounds tested are presented in **Table 1**. The two compounds that proved the most protective against damage to the HT-29 cells were avenanthramides 2c and 1f. With the exception of 1p and 2p, the avenanthramides had a more protective effect than their corresponding hydroxycinnamic acids and showed a protection higher than or equal to that of ascorbic acid.

DISCUSSION

A number of methods are available for assessing antioxidant activity, and these methods vary substantially in terms of complexity, chemistry, and other factors (*28−30*). Although the DPPH and FRAP methods used in the present study employ different chemistries and conditions, both methods measure the ability of compounds to reduce the oxidant present through their electron-donating ability (*28−30*). Thus, it is not surprising that these two methods gave similar rankings of antioxidant activity for the compounds tested and that there was a significant overall correlation between the results obtained by the two methods for the avenanthramides and hydroxycinnamic acids ($r = -0.74$; $P < 0.01$, 10 df).

With the exception of 2s, the 2-analogue avenanthramides showed greater antioxidant activity than the 1-analogues in both assays. Previous results showed that the 2-analogues had greater antioxidant activity in the linoleic acid assay, but in contrast found that the 1- and 2-analogues had similar activities in the DPPH assay (*10, 31*). In the present study, the antioxidant activity of the three major avenanthramides in oats decreased in the order 2c > 2p > 2f in both the DPPH and FRAP assays. This confirms the role of 2c as the most potent antioxidant of the major oat avenanthramides, but is in contrast to previous studies in which the activity was found to decrease in the order 2c > 2f > 2p in the DPPH assay (*10,31,32*) and the linoleic acid assay (*10*). The lack of consistency between studies for the DPPH assay may be due to variations in reaction conditions, such as the timings or concentrations used (*10, 31, 32*). A study that assessed a range of compounds using both the FRAP and DPPH assays found that activity increased in the order ascorbic acid < ferulic acid < caffeic acid, which is similar to the present results (*33*).

The Comet assay assesses the effectiveness of compounds to protect cells from genotoxic damage. Thus, the use of HT-29 human colon adenocarcinoma cells may provide a more physiologically relevant assay than the DPPH and FRAP assays. Correlations between the results from the DPPH and FRAP assays and the results from the Comet assay for the avenanthramides and hydroxycinnamic acids were nonsignificant (DPPH, $r = -0.12$, ns, 10 df; FRAP, $r = 0.15$, ns, 10 df). This suggests that the antigenotoxicity observed is not directly related to antioxidant activity. The lack of a relationship is exemplified by avenanthramides 2c and 1f, which showed similar large protective effects in the Comet assay but which gave divergent responses in the DPPH and FRAP assays. Nevertheless, the antigenotoxic effects of the three major oat avenanthramides decreased in the order 2c > 2f > 2p, which is the same as that observed in previous in vitro antioxidant assays (*10, 31, 32*). Thus, despite the overall lack of significant correlations between the Comet assay and the DPPH and FRAP assays, antioxidant activity may play a role in the antigenotoxic effects of the avenanthramides. However, Tranilast, which has no hydroxyl groups and was unresponsive in the DPPH and FRAP assays, showed a significant dose response in the Comet assay, indicating that protective effects can be found in the absence of antioxidant activity.

The antioxidant activity of avenanthramides and related compounds has been related to a range of structural factors including the number and position of hydroxyl groups and the presence of amide bonds (*10, 31*). The present results showed that 5-hydroxyanthranilic acid, which is the anthranilic acid moiety of the 2-series avenanthramides, had high activity in all three assays. This adds support to the suggestion that the anthranilic acid moiety might be as important as the cinnamic acid moiety for the biological effects exerted by avenanthramides (*31*).

Other studies with in vitro cell systems have shown that synthetic avenanthramide 2c can exert potentially beneficial effects on the development of atherosclerosis by inhibiting the proliferation of rat and human aortic smooth muscle cells and human aortic endothelial cells at concentrations ranging from 20 to 220 μmol/L (*18, 34*). However, in the present study, 2c at a concentration of only 0.9 μmol/L decreased DNA damage by 50% in stressed HT-29 adenocarcinoma cells.

Data on the absorption, distribution, metabolism, and excretion of avenanthramides are scant. However, a study in which hamsters were given single doses of a phenol-rich extract of oat bran showed that avenanthramides 2p and 2f were detectable in the plasma at a total concentration of 0.7 μmol/L, but apparent

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bioavailability was very low (1.3%) in comparison with simpler phenolics (*35*). Although it is not clear if physiologically significant plasma concentrations can be obtained from the consumption of oat products, in vivo studies with rats have shown that incorporating 2c in the diet at 0.1 g/kg favorably affected some markers of physiological oxidant stress (*36*).

Furthermore, a bioavailability study in humans with avenanthramide-rich mixtures prepared from oats, which included 2p, 2f, and 2c, showed that postprandial plasma levels peaked at about 2 h, when the total plasma avenanthramide concentration after the higher dose was ∼0.37 μmol/L (*37*). This plasma concentration, obtained postprandially with a relatively high dose of avenanthramides, was somewhat lower than the lowest concentration of 2c (0.5 μmol /L) that was effective at decreasing DNA damage in stressed HT-29 cells in vitro in the present study. However, it is notable that the concentration that was effective here in vitro was of the same order of magnitude as that found in plasma in the previous human study (*37*). Furthermore, prolonged consumption of oat avenanthramides may lead to higher concentrations in other body tissues. For example, concentrations of isoflavonoids in prostatic fluid have been found to be up to 10 times higher than plasma concentrations (*38*).

A range of antioxidants including tocopherols, ascorbic acid, and phenolics such as ferulic acid have been shown to act synergistically to decrease oxidative damage in biological systems (see, e.g., refs *39* and *40*). Thus, when oats are consumed, there may be synergistic interactions in vivo between avenanthramides and other oat antioxidants, such as tocopherols and tocotrienols, or with other antioxidants, such as ascorbic acid. This possibility is supported by data from ex vivo and in vitro studies which showed that ascorbic acid potentiated the antioxidant effects of avenanthramides, leading to decreased oxidation of LDL-cholesterol (*35*).

Of the avenanthramides, 2c is usually the most abundant in oats (*9*, *41*) and 2c also showed the highest antioxidant activities and demonstrated the strongest protective effects in stressed adenocarcinoma cells in the present study. Interestingly, in comparisons with ascorbic acid, avenanthramide 2c showed a greater antigenotoxic effect in stressed cells, 2c and 2p gave significantly greater activities in the FRAP assay, and 2c, 2p, and 2f had antioxidant activities that were substantially greater in the DPPH assay. Further studies are needed to assess the metabolism and distribution of avenanthramides in animal models and to evaluate the effects of longer term intake of oat-rich diets by humans on biomarkers such as the oxidizability of LDL-cholesterol and lymphocyte DNA damage. However, overall, these results suggest that avenanthramides from oats may play a role in the protective effects of whole-grain foods against chronic diseases.

ABBREVIATIONS USED

FA, ferulic acid; CA, caffeic acid; PA, *p*-coumaric acid; SA, sinapic acid; TR, Tranilast; 5HA; 5-hydroxyanthranilic acid; AA, ascorbic acid; 1c, *N*-(3,4-dihydroxy-(*E*)-cinnamoyl)anthranilic acid; 1f, *N*-(4-hydroxy-3-methoxy-(*E*)-cinnamoyl)anthranilic acid; 1p, *N*-(4-hydroxy-(*E*)-cinnamoyl)anthranilic acid; 1s, *N*-(4-hydroxy-3, 5-dimethoxy-(*E*)-cinnamoyl)anthranilic acid; 2c, *N*-(3′,4′-dihydroxy-(*E*)-cinnamoyl)-5-hydroxyanthranilic acid; 2f, *N*-(4′-hydroxy-3′-methoxy-(*E*)-cinnamoyl)-5-hydroxyanthranilic acid; 2p, *N*-(4′-hydroxy-(*E*)-cinnamoyl)-5-hydroxyanthranilic acid; 2s, *N*-(4′-hydroxy-3′,5′-dimethoxy-(E)-cinnamoyl)-5-hydroxyanthranilic acid; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant potential; TPTZ, tripyridyltriazine.

LITERATURE CITED

(1) Slavin, J. L.; Jacobs, D.; Marquart, L.; Wiemer, K. The role of whole grains in disease prevention. *J. Am. Diet. Assoc.* **2001**, *101*, 780–785.

(2) Wood, P. J. Cereal β-glucans in diet and health. *J. Cereal Sci.* **2007**, *46*, 230–238.

(3) Issa, A. Y.; Volate, S. R.; Wargovich, M. J. The role of phytochemicals in inhibition of cancer and inflammation: new directions and perspectives. *J. Food Compos. Anal.* **2006**, *19*, 405–419.

(4) Welch, R. W. The chemical composition of oats. In *The Oat Crop: Production and Utilisation*; Welch, R. W., Ed.; Chapman and Hall: London, U.K., 1995; pp 279−320.

(5) Peterson, D. M. Oat antioxidants. *J. Cereal Sci.* **2001**, *33*, 115–129.

(6) Collins, F. W. Oat phenolics: structure, occurrence and function. In *Oats: Chemistry and Technology*; Webster, F. H., Ed.; American Association of Cereal Chemists: St. Paul, MN, 1986; pp 227−295.

(7) Collins, F. W. Oat phenolics − avenanthramides, novel substituted *N*-cinnamoylanthranilate alkaloids from oat groats and hulls. *J. Agric. Food Chem.* **1989**, *37*, 60–66.

(8) Dimberg, L. H.; Theander, O.; Lingnert, H. Avenanthramides − a group of phenolic antioxidants in oats. *Cereal Chem.* **1993**, *70*, 637–641.

(9) Dimberg, L. H.; Molteberg, E. L.; Solheim, R.; Frolich, W. Variation in oat groats due to variety, storage and heat treatment 1. Phenolic compounds. *J. Cereal Sci.* **1996**, *24*, 263–272.

(10) Bratt, K.; Sunnerheim, K.; Bryngelsson, S.; Fagerlund, A.; Engman, L.; Andersson, R. E.; Dimberg, L. H. Avenanthramides in oats (*Avena sativa* L.) and structure−antioxidant activity relationships. *J. Agric. Food Chem.* **2003**, *51*, 594–600.

(11) Collins, F. W.; Mullin, W. J. High-performance liquid-chromatographic determination of avenanthramides, *N*-aroylanthranilic acid alkaloids from oats. *J. Chromatogr.* **1988**, *445*, 363–370.

(12) Peterson, D. M.; Emmons, C. L.; Hibbs, A. H. Phenolic antioxidants and antioxidant activity in pearling fractions of oat groats. *J. Cereal Sci.* **2001**, *33*, 97–103.

(13) Emmons, C. L.; Peterson, D. M.; Paul, G. L. Antioxidant capacity of oat (*Avena sativa* L.) extracts. 2. In vitro antioxidant activity and contents of phenolic and tocol antioxidants. *J. Agric. Food Chem.* **1999**, *47*, 4894–4898.

(14) Szeto, Y. T.; Benzie, I. F. F. Effects of dietary antioxidants on human DNA ex vivo. *Free Radical Res.* **2002**, *36*, 113–118.

(15) Chen, C. Y.; Milbury, P. E.; Kwak, H. K.; Collins, F. W.; Samuel, P.; Blumberg, J. B. Avenanthramides and phenolic acids from oats are bioavailable and act synergistically with vitamin C to enhance hamster and human LDL resistance to oxidation. *J. Nutr.* **2004**, *134*, 1459–1466.

(16) Parikka, K.; Rowland, I. R.; Welch, R. W.; Wähälä, K. In vitro antioxidant activity and antigenotoxicity of 5-*n*-alkylresorcinols. *J. Agric. Food Chem.* **2006**, *54*, 1646–1650.

(17) Liu, L.; Zubik, L; Collins, F. W.; Marko, M.; Meydani, M. The antiatherogenic potential of oat phenolic compounds. *Atherosclerosis* **2004**, *175*, 39–49.

(18) Nie, L.; Wise, M. L.; Peterson, D. M.; Meydani, M. Avenanthramide, a polyphenol from oats, inhibits vascular smooth muscle cell proliferation and enhances nitric oxide production. *Atherosclerosis* **2006**, *186*, 260–266.

(19) Guo, W.; Wise, M. L.; Collins, F. W.; Meydani, M. Avenanthramides, polyphenols from oats, inhibit IL-1β-induced NF-κB activation in endothelial cells. *Free Radical Biol. Med.* **2008**, *44*, 415–429.

(20) Kagitani, S.; Ueno, H.; Hirade, S.; Takahashi, T.; Takata, M.; Inoue, H. Tranilast attenuates myocardial fibrosis in association with suppression of monocyte/macrophage infiltration in DOCA/salt hypertensive rats. *J. Hypertens.* **2004**, *22*, 1007–1015.

(21) Ward, M. R.; Agrotis, A.; Kanellakis, P.; Hall, J.; Jennings, G.; Bobik, A. Tranilast prevents activation of transforming growth factor-β system, leukocyte accumulation, and neointimal growth in porcine coronary arteries after stenting. *Arterioscler., Thromb. Vasc. Biol.* **2002**, *22*, 940–948.

(22) Yashiro, M.; Murahashi, K.; Matsuoka, T.; Nakazawa, K.; Tanaka, H.; Osaka, H.; Koyama, T.; Ohira, M.; Chung, K. H. Y. S. Tranilast (*N*-3,4-dimethoxycinamoyl anthranilic acid): a novel inhibitor of

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invasion-stimulating interaction between gastric cancer cells and orthotopic fibroblasts. *Anticancer Res.* **2003**, *23*, 3899–3904.

(23) Mayama, S.; Tani, T.; Ueno, T.; Hirabayashi, K.; Nakashima, T.; Fukami, H.; Mizuno, Y.; Irie, H. Isolation and structure elucidation of genuine oat phytoalexin, avenalumin-I. *Tetrahedron Lett.* **1981**, *22*, 2103–2106.

(24) Miller, H. E.; Rigelhof, F.; Marquart, L.; Prakash, A.; Kanter, M. Whole-grain products and antioxidants. *Cereal Foods World* **2000**, *45*, 59–63.

(25) Benzie, I. F. F.; Strain, J. J. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Anal. Biochem.* **1996**, *239*, 70–76.

(26) Benzie, I. F. F.; Szeto, Y. T. Total antioxidant capacity of teas by the ferric reducing/antioxidant power assay. *J. Agric. Food Chem.* **1999**, *47*, 633–636.

(27) Pulido, R.; Bravo, L.; Saura-Calixto, F. Antioxidant activity of dietary polyphenols as determined by a modified ferric reducing/ antioxidant power assay. *J. Agric. Food Chem.* **2000**, *48*, 3396–3402.

(28) Frankel, E. N.; Meyer, A. S. Review: The problems of using one dimensional methods to evaluate multifunctional food and biological antioxidants. *J. Sci. Food Agric.* **2000**, *80*, 1925–1941.

(29) Huang, D.; Ou, B.; Prior, R. L. Review: The chemistry behind antioxidant capacity assays. *J. Agric. Food Chem.* **2005**, *53*, 1841–1856.

(30) Prior, R. L.; Wu, X.; Schaich, K. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J. Agric. Food Chem.* **2005**, *53*, 4290–4302.

(31) Fagerlund, A.; Sunnerheim, K.; Dimberg, L. H. Radical scavenging and antioxidant activity of avenanthramides. *Food Chem.* **2009**, *113*, 550–556.

(32) Peterson, D. M.; Hahn, M. J.; Emmons, C. L. Oat avenanthramides exhibit antioxidant activities in vitro. *Food Chem.* **2002**, *79*, 473–478.

(33) Stratil, P.; Klejdus, B.; Kubáň, V. Determination of total content of phenolic compounds and their antioxidant activity in vegetables − evaluation of spectrophotometric methods. *J. Agric. Food Chem.* **2006**, *54*, 607–616.

(34) Nie, L.; Wise, M.; Peterson, D.; Meydani, M. Mechanism by which avenanthramide-c, a polyphenol of oats, blocks cell wall cycle progression in vascular smooth muscle cells. *Free Radical Biol. Med.* **2006**, *41*, 702–708.

(35) Chen, C. Y.; Milbury, P. E.; Kwak, H. K.; Collins, F. W.; Samuel, P.; Blumberg, J. B. Avenanthramides and phenolic acids from oats are bioavailable and act synergistically with vitamin C to enhance hamster and human LDL resistance to oxidation. *J. Nutr.* **2004**, *134*, 1459–1466.

(36) Ji, L. L.; Lay, D.; Chung, E. H.; Fu, Y.; Peterson, D. Effects of avenanthramides on oxidant generation and antioxidant enzyme activity in exercised rats. *Nutr. Res. (N.Y.)* **2003**, *23*, 1579–1590.

(37) Chen, C. Y.; Milbury, P. E.; Collins, F. W.; Blumberg, J. B. Avenanthramides are bioavailable and have antioxidant activity in humans after acute consumption of an enriched mixture from oats. *J. Nutr.* **2007**, *137*, 1375–1382.

(38) Morton, M. S.; Chan, P. S.; Cheng, C.; Blacklock, N.; Matos-Ferreira, A.; Abranches-Monteiro, L.; Correia, R.; Lloyd, S.; Griffiths, K. Lignans and isoflavonoids in plasma and prostatic fluid in men: samples from Portugal, Hong Kong, and the United Kingdom. *Prostate* **1997**, *32*, 122–128.

(39) Kagan, V. E.; Serbinova, E. A.; Forte, T.; Scita, G.; Packer, L. Recycling of vitamin-E in human low-density lipoproteins. *J. Lipid Res.* **1992**, *33*, 385–397.

(40) Trombino, S.; Serini, S.; Di Nicuolo, F.; Celleno, L.; Ando, S.; Picci, N.; Calviello, G.; Palozza, P. Antioxidant effect of ferulic acid in isolated membranes and intact cells: synergistic interactions with α-tocopherol, β-carotene, and ascorbic acid. *J. Agric. Food Chem.* **2004**, *52*, 2411–2420.

(41) Bryngelsson, S.; Dimberg, L. H.; Kamal-Eldin, A. Effects of commercial processing on levels of antioxidants in oats (*Avena sativa* L). *J. Agric. Food Chem.* **2002**, *50*, 1890–1896.