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5-Alk(en)ylresorcinols as the major active components in wheat bran inhibit human colon cancer cell growth

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ABSTRACT

We and others have found that wheat bran oil is the active constituent in wheat bran for colon cancer prevention. However, the active components in wheat bran oil are still unknown. Using human colon cancer cells (HCT-116 and HT-29) as the guiding assays, we further purified the active components from wheat bran using column chromatography. In this study, we identified that a fraction containing 5-*n*-alk(en)ylresorcinols had the strongest inhibitory effect on the proliferation of human HCT-116 and HT-29 colon cancer cells. Further purification led to the identification of 14 5-alk(en)ylresorcinols. Among them, **7**, (10′*Z*,13′*Z*,16′*Z*)-5-(nonadeca-10′,13′,16′-trienyl)resorcinol, is a novel compound and **5**, **6**, **9**, **10**, and **13** were purified as individual compounds for the first time. The identification and structural elucidation of these compounds were based on 1D and 2D NMR and tandem mass spectral analyses. All these compounds (**1**–**14**) except **10** were evaluated for growth inhibition of human colon cancer cell lines (HCT-116 and HT-29). Our results indicate that increasing the length of the side chain will diminish the inhibitory activity, and the existence of a double bond and a carbonyl group will strengthen such an activity.

1. Introduction

Colorectal cancer (CRC) is the third most common form of cancer in men and women in the United States and the second leading cause of cancer-death.[1] Epidemiological studies have suggested that dietary cereal fibers may reduce the risk of colon cancer.[2–6] Human and animal studies have found that wheat bran (WB) is the only cereal bran that shows consistent protection against colon cancer in laboratory animal models, and oat and corn bran may enhance carcinogenesis.[7–9]

Although most of the human and animal studies show a correlation between WB consumption and the reduction of the risk of colon cancer, the mechanism by which WB protects against colon tumor development has not been fully explored. Most of the mechanisms are proposed based on the physiological function of fiber, such as its fecal dilution effect and its ability to accelerate the transit of fecal material through the colon.[10] It has also been suggested that the fermentation products of WB fiber in the colon, such as butyric acid and orthophenolic acids, are protective agents.[10,11] These proposed mechanisms are a subject of debate.[10] For instance, oat bran produced higher levels of luminal butyric acid than did WB, even though WB showed a protective effect on colon cancer and oat bran enhanced colon carcinogenesis.[12]

We and others have found that WB oil is the active constituent for colon cancer prevention. Dr. Reddy's group has previously compared the effects of specific WB fractions, including WB, dephytinized WB (WB-P), defatted WB (WB-F), dephytinized and defatted WB (WB-PF), and WB-PF fortified with 2% WB oil and/or with 0.4% phytate, on colon carcinogenesis in an azoxymethane (AOM)-induced rat tumor model.[13] The results demonstrated for the first time that removal of lipid and lipid-soluble components from WB increased colon tumorigenesis compared to the WB control group (10% WB), whereas fortification of WB-PF diet with WB oil significantly increased its inhibitory activity. It is also noteworthy that the degree of inhibition of colon carcinogenesis in the rats fed a WB oil-fortified diet exceeded that seen with WB diet, because WB oil intake in animals fed WB-PF fortified with bran oil was about 4 times higher than in those fed the WB control diet. The inhibitory effect of WB oil on tumorigenesis was also observed in our studies in the $Apc^{min/+}$ mouse model.[14] Our results showed that the mice in the 2% WB oil treated group had significantly fewer total tumors in the small intestine than those in the control group ($p < 0.0001$) with 35.6% inhibition. Two percent WB oil also significantly inhibited ($p < 0.0001$) the formation of tumors when analyzed by size, with the greatest inhibition on large tumors (>2 mm, 46.7%).

To identify the major active components in WB oil, the present study investigated the purification and structure elucidation of the major active components in WB oil using HCT-116 and HT-29 human colon cancer cells as the guiding assays.

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2. Results and discussion

2.1. Cell growth inhibition by different fractions of WB oil

In order to purify and identify the active components from WB oil, we fractionated WB oil through column chromatography into eight fractions. Based on the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT) assay, fraction 7 (F7) had the highest growth inhibitory activity on HCT-116 human colon cancer cells after treatment for 48 h (Fig. 1). Similar results were observed on HT-29 human colon cancer cells (Fig. 1). Further liquid chromatography/mass spectrometry/ultraviolet (LC/MS/UV) analysis indicated that the major components in F7 are 5-alk(en)ylresorcinol (AR) analogues with compounds **3** and **8** as the most abundant components (Fig. 2).

2.2. Purification and structure elucidation of compounds 1–14

F7 was applied to a series of chromatographic steps to yield 12 AR-related analogues, **1**–**10**, **12** and **14** (Fig. 3). The amount of compounds **11** and **13** in F7 are so tiny, we purified them from fraction 8 (F8). The purified compounds include one new, (10′*Z*,13′*Z*,16′*Z*)-5-(nonadeca-10′,13′,16′-trienyl)resorcinol (**7**), and 13 known alk(en)ylresorcinols (ARs) (**1–6**, and **8**–**14**). Among the known compounds, (12′*Z*)-5-(nonadeca-12′-enyl)resorcinol (**5**), (10′*Z*,13′*Z*)-5-(nonadeca-10′,13′-dienyl)resorcinol (**6**), (16Z′)-5-(heneicos-16′-enyl)resorcinol (**9**), (12′*Z*)-5-(Heneicos-12′-enyl)resorcinol (**10**), and 5-(2′-oxotricosyl)resorcinol (**13**) were purified for the first time as individual compounds. Their structures were previously predicted from the mixture using mass spectrometry. This is the first report on the purification and structure elucidation using 1 dimensional (1D) and 2 dimensional (2D) nuclear magnetic resonance (NMR) of these compounds.

The structures of compounds **1**–**4**, **8**, **11**–**12**, and **14** were identified by comparison of their NMR and MS data with those reported in the literature.[15–17] They are 5-*n*-heptadecylresorcinol (**1**), (12′Z)-5-(heptadec-12′-enyl)resorcinol (**2**), 5-*n*-nonadecylresorcinol (**3**), (14′*Z*)-5-(nonadeca-14′-enyl)resorcinol (**4**), 5-*n*-heneicosylresorcinol (**8**), 5-(2′-oxoheneicosyl)resorcinol (**11**), 5-*n*-tricosylresorcinol (**12**), and 5-*n*-pentadecylresorcinol (**14**). The positions of the double bond in **2** and **4** and the carbonyl group in **11** were established based on the key cross peaks observed in their heteronuclear multiple bond correlation (HMBC) spectra as shown in Fig. 4.

Compound **5** was shown to have the molecular formula $C_{25}H_{42}O_2$ based on positive atmospheric-pressure chemical ionization (APCI)-MS (*m*/*z* 375 $[M+H]^+$), ^1H and ^{13}C NMR data, which is the same as that of (14′*Z*)-5-(nonadeca-14′-enyl)resorcinol (**4**). Similar to **4**, the ^1H and ^{13}C NMR data of **5** had the signals of one double bond (δ_H 5.51, m, 2H and δ_C 130.7, d, 2C). The only difference between **4** and **5** is the position of double bond. The HMBC spectrum of **4** showed correlations between H-15′ (δ_H 5.48) with C-16′ (δ_C 27.6) and C-17′ (δ_C 32.6); H-16′ (δ_H 2.10) with C-14′/15′ (δ_C 130.7), C-17′ (δ_C 32.6), and C-18′ (δ_C 23.0); and H-19′ (δ_H 0.88) with C-17′ (δ_C 32.6) and C-18′ (δ_C 23.0) (Fig. 4). This indicated that the double bond in **4** is at $\Delta^{14'}$. The MS/MS spectrum of **4** had fragment ions at *m*/*z* 277 $[M-C_7H_{14}+H]^+$ and 333 $[M-C_3H_6+H]^+$ arising from the cleavage at C'_{12}–C'_{13} and C'_{16}–C'_{17} by classical McLafferty type rearrangement (Fig. 5), respectively, which was in good agreement with the result from its NMR spectrum. However, the HMBC spectrum of **5** could not determine the position of double bond indicating that this double bond is located in the center of the side chain. In a similar manner with that of **4**, the MS/MS spectrum of **5** showed fragment ions at m/z 249 $[M-C_9H_{16}+H]^+$ and 305 $[M-C_5H_{12}+H]^+$ attributing to the cleavage at C'_{10}–C'_{11} and C'_{14}-C'_{15} (Fig. 5), respectively, suggesting the position of the double bond in **5** is at $\Delta^{12'}$. Therefore, compound **5** was identified as (12′*Z*)-5-(nonadeca-12′-enyl)resorcinol, which was previously reported from wheat and rye whole flour based on mass spectrometry.[18]

Compound **6** had a molecular formula of $C_{25}H_{40}O_2$ based on positive APCI-MS (*m*/*z* 373 $[M+H]^+$), ^1H and ^{13}C NMR data, which is two mass units less than that of (14′*Z*)-5-(nonadeca-14′-enyl)res-

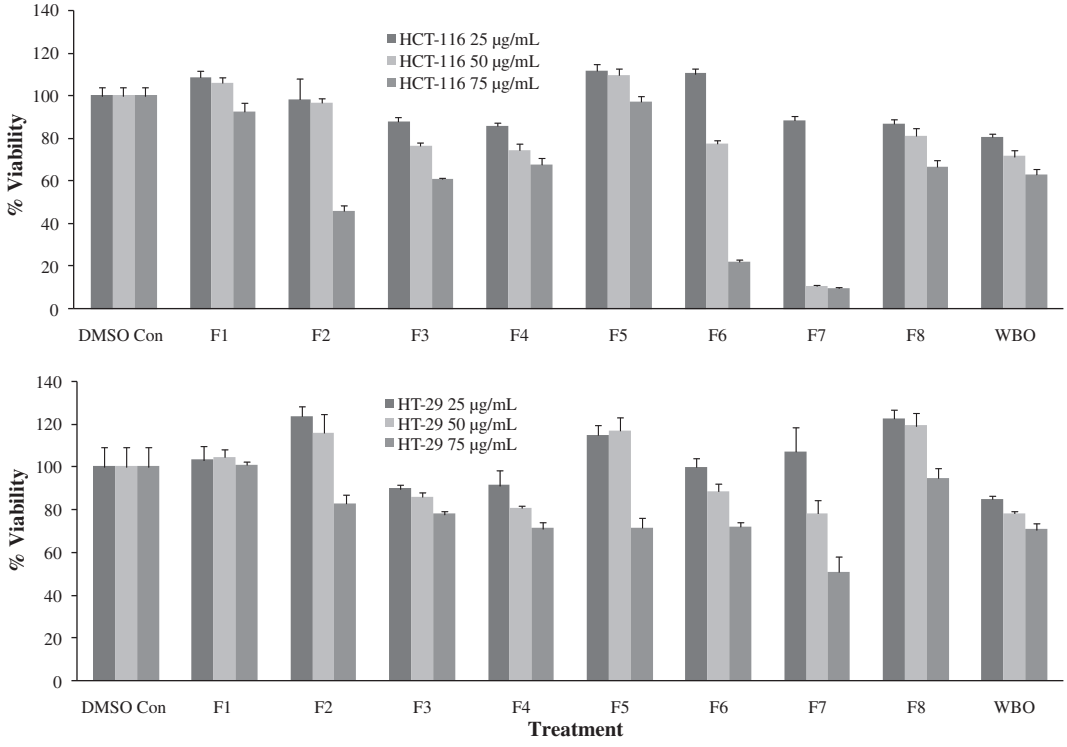
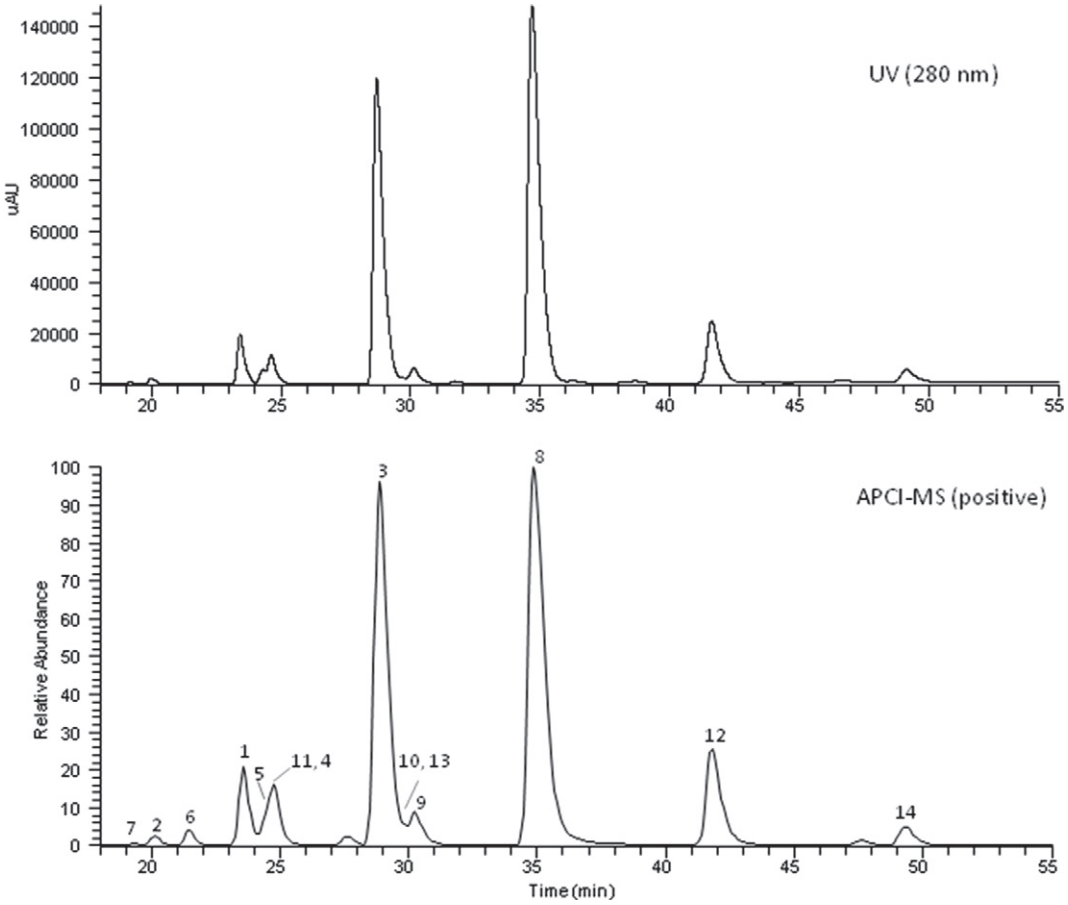


Figure 1. Effects of eight subfractions and wheat bran oil (WBO) on the growth of human colon cancer cells (HCT-116 and HT-29). Cells were treated with three different concentrations (25, 50, and 75 μg/mL) of test agents for 48 h in the presence of 10% FBS at 37 °C. Cell growth inhibition was determined by MTT assay ($n = 8 \pm SD$).



peaks	APCI $[M+H]^+$	MS/MS
5-n-heptadecylresorcinol (**1**)	349	111 (100), 123
(12′Z)-5-(heptadec-12′-enyl)resorcinol (**2**)	347	123 (100), 165, 249, 305
5-n-nonadecylresorcinol (**3**)	377	111 (100), 123
(14′Z)-5-(nonadeca-14′-enyl)resorcinol (**4**)	375	123 (100), 277, 333
(12′Z)-5-(nonadeca-12′-enyl)resorcinol (**5**)	375	123 (100), 249, 305
5-(nonadeca-10′,13′-dienyl)resorcinol (**6**)	373	123 (100), 221, 260, 275, 317
5-(nonadeca-10′,13′,16′-trienyl)resorcinol (**7**)	371	123 (100), 261, 275, 301, 315, 355
5-n-heneicosylresorcinol (**8**)	405	123 (100)
(16Z′)-5-(heneicos-16′-enyl)resorcinol (**9**)	403	123 (100), 305, 361
(12′Z)-5-(heneicos-12′-enyl)resorcinol (**10**)	403	123 (100), 249, 305
5-(2′-oxoheneicosyl)resorcinol (**11**)	419	123, 165 (100)
5-n-tricosylresorcinol (**12**)	433	123 (100)
5-(2′-oxotricosyl)resorcinol (**13**)	447	123, 151, 165 (100)
5-n-pentadecylresorcinol (**14**)	461	123, 137 (100)

Figure 2. Chemical profile of fraction 7 generated from UV at 280 nm and positive LC/APCI-MS, and structure information of compounds **1**–**14** generated from LC/MS/MS in positive APCI source.

orcinol (**4**). This indicates that compound **6** has two double bonds. The ^{1}H NMR spectra of **6** (Table 1) featured broad olefinic proton multiplets centered at δ 5.51 (4H, m) and one bisallylic methylene at δ 2.95 (2H, t, J = 5.4 Hz). The positions of the two double bonds were established at $\Delta^{10'}$ and $\Delta^{13'}$ by key HMBC correlations (Fig. 4) detected as H-16′/ C-14′, C-17′, C-18′, and H-12′/ C-10′, C-11′, C-13′, C-14′. Usually, the chemical shifts of carbons next to a *trans* (*E*) double bond appear at δ 32–33, whereas those of a *cis* (*Z*) double bond appear at δ 27–28.[19] The two allylic methylenes at δ 27.9 (C-9′/ C-15′, overlapped) and the resonance at δ 26.5 (C-12′) for the bisallylic methylene were in agreement with the *cis* (*Z*) geometry. Thus, **6** was identified as (10′*Z*,13′*Z*)-5-(nonadeca-10′,13′-dienyl)resorcinol (Fig. 3), which was previously reported from rye whole flour based on mass spectrometry.[18,20]

Compound **7** had a molecular formula of $C_{25}H_{38}O_2$ determined by positive APCI-MS (m/z 371 $[M+H]^+$), ^{1}H and ^{13}C NMR data, which is two mass units less than that of (10′*Z*,13′*Z*)-5-(nonadeca-10′,13′-dienyl)resorcinol (**6**), indicating that this compound

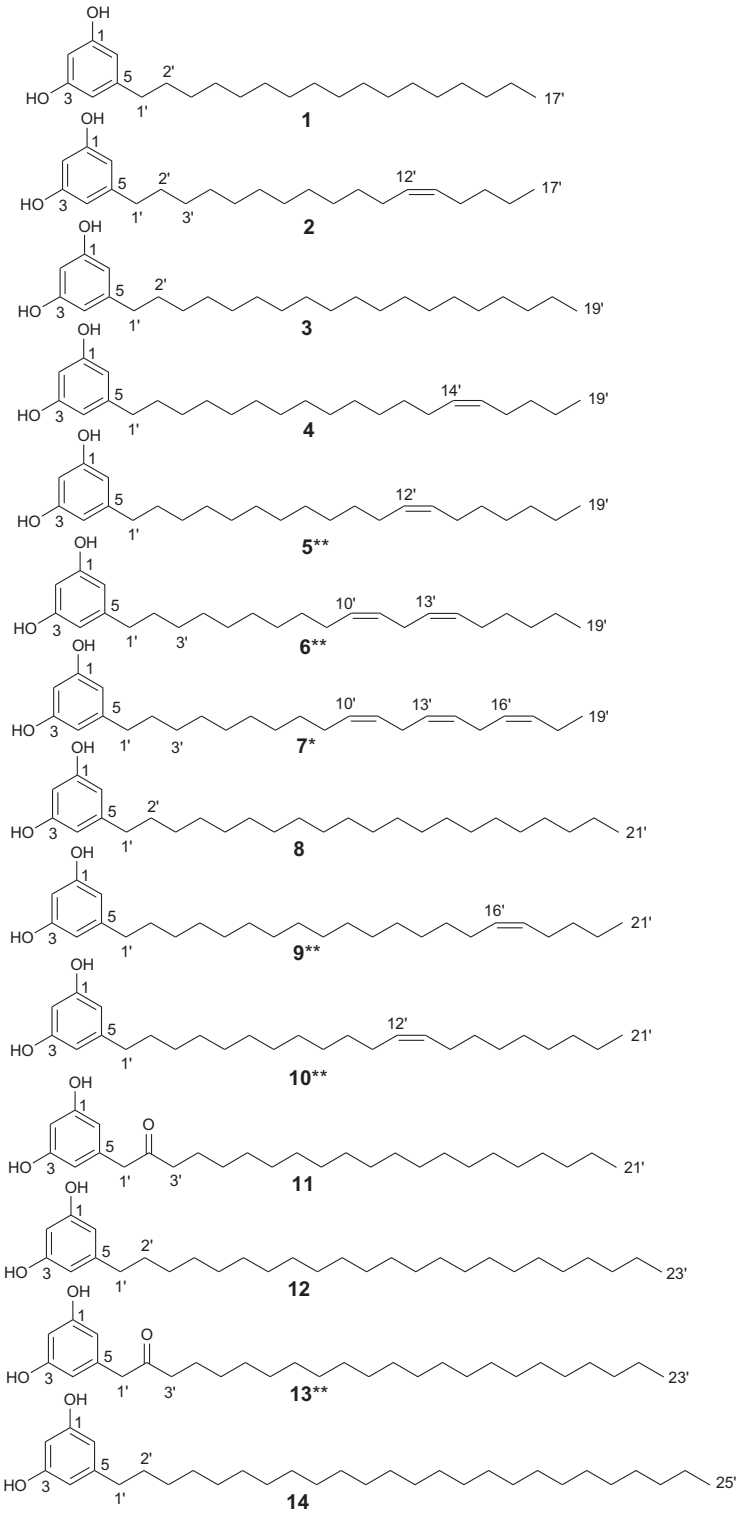


Figure 3. Chemical structures of the major components (**1**–**14**) purified from fraction 7. *new compound, and ** compound purified from nature for the first time.

has three double bonds. Its ^{1}H NMR had two sets of broad olefinic proton multiplets centered at δ 5.51 (4H, m) and 5.46 (2H, m), and two typical bisallylic methylene groups centered at δ 2.96 (2H, m) and 2.93 (2H, m) (Table 1). The HMBC spectrum showed the cross peaks between C-17′/H-19′, H-18′, H-15′, C-13′(14′)/H-15′, H-12′, H-11′, H-16′, and C-10′/H-12′, H-9′ (Fig. 4), suggesting the positions of the three double bonds are at $\Delta^{10\prime}$, $\Delta^{13\prime}$ and $\Delta^{16\prime}$ with two bisallylic methylene groups (δ_H 2.96/δ_C 26.5; and δ_H 2.93/δ_C 26.3) located at positions 12′ and 15′, respectively. The chemical shifts of C-9′ (δ 28.0), C-12′ (δ 26.5), C-15′ (δ 26.3), and C-18′ (δ 21.3) were in agreement with *Z* geometry.[19] Hence the structure of **7** was elucidated as (10′*Z*,13′*Z*,16′*Z*)-5-(nonadeca-10′,13′,16′-trienyl)resorcinol (Fig. 3), which, to our knowledge, is a novel compound.

Compounds **9** and **10** had the same molecular formula $C_{27}H_{46}O_2$, based on the analysis of their positive APCI-MS (*m*/*z* 403 $[M+H]^+$), ^{1}H and ^{13}C NMR data, which is two mass units less

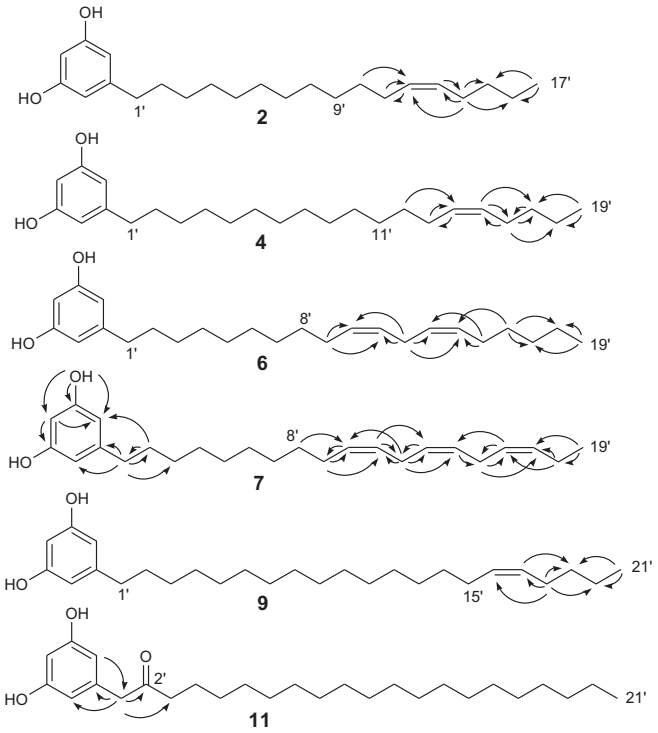


Figure 4. Significant ^{1}H–^{13}C long-range correlations ($^{1}H \curvearrowright {}^{13}C$) in the HMBC spectra of **2**, **4**, **6**, **7**, **9**, and **11**.

than that of 5-*n*-heneicosylresorcinol (**8**), indicating that they had one double bond in their structures when compared with **8**. The double bond position of analogue **9** was determined at $\Delta^{16'}$ according to the observation of the key HMBC cross peaks between H-18′/C-16′(17′), C-19′, C-20′ and H-21′/C-20′, C-19′. The *Z* geometry of **9** was assigned based on the observation that the chemical shifts of two allylic methylene groups were at δ 27.6 (C-15′/18′, overlapped).[19] All of these features suggested that compound **9** was (16Z′)-5-(heneicos-16′-enyl)resorcinol, which was prevously reported from wheat and rye whole flour based on the analysis of its tandem mass.[18] The position of the double bond in **10** could not be determined by its key HMBC correlations indicating this double bond is located in the center of the side chain. The analysis of MS/MS spectrum of **10** provided fragment ions at *m*/*z* 249 $[M-C_{11}H_{22}+H]^+$ and 305 $[M-C_7H_{14}+H]^+$ due to the cleavage at C'_{10}–C'_{11} and C'_{14}–C'_{15} by classical McLafferty type rearrangement (Fig. 5), respectively, suggesting the position of the double bond in **10** is at $\Delta^{12'}$. Therefore, the structure of **10** was proposed to be (12′*Z*)-5-(heneicos-12′-enyl)resorcinol (Fig. 3), which was first reported from wheat and rye whole flour based on tandem mass analysis.[18]

Compound **11** was identified to be 5-(2′-oxoheneicosyl)resorcinol[17,20] based on the analysis of its positive APCI-MS (*m*/*z* 419 $[M+H]^+$), ^{1}H and ^{13}C NMR data. The position of carbonyl group at C-2′ was established by key HMBC correlations (Fig. 4) detected between H-1′ with C-2′, C-5 and C-4(6). The ^{1}H NMR and ^{13}C NMR data of compound **13** were very similar to those of **11**. The only difference is the length of the side chain. The molecular weight of 13 (APCI-MS, *m*/*z* 447 $[M+H]^+$) was 28 mass units (two CH_2 units) higher than that of **11**. Its ^{13}C NMR also showed 29 carbons instead of 27 in **11**. All of these features suggested that **13** is 5-(2′-oxotricosyl)resorcinol, which was previously reported from wheat and rye grains on the basis of mass analysis.[20,21]

2.3. Cell growth inhibition by purified compounds

All 14 analogues (**1**–**14**) except **10** were evaluated for growth inhibitory activities against human colon cancer cells (HCT-116 and HT-29) using MTT assays (Fig. 6). Among the five dominant

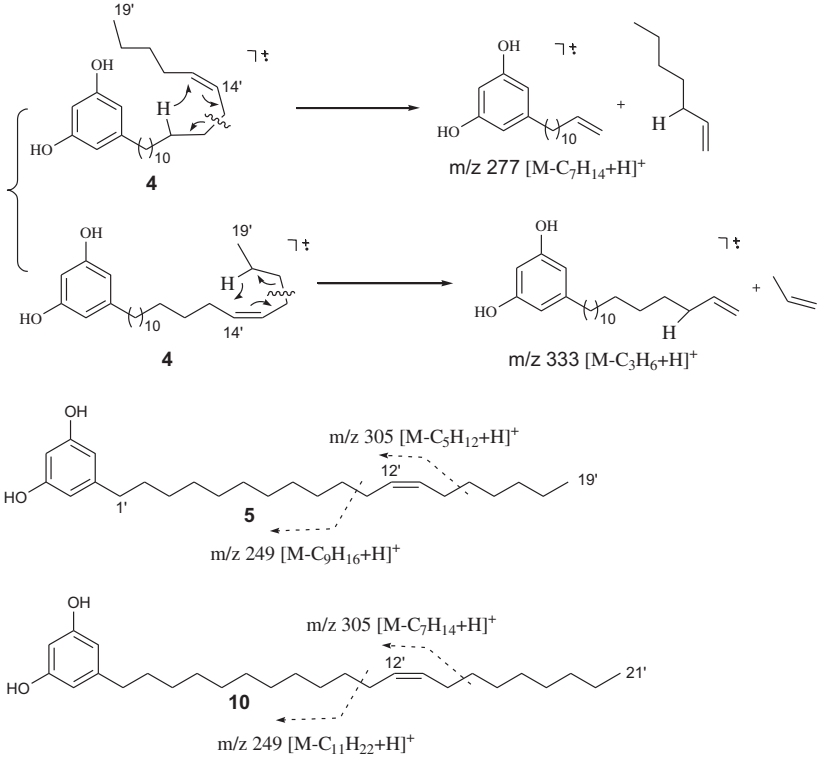


Figure 5. Formation mechanism of the key fragment ions of **4**, **5,** and **10**.

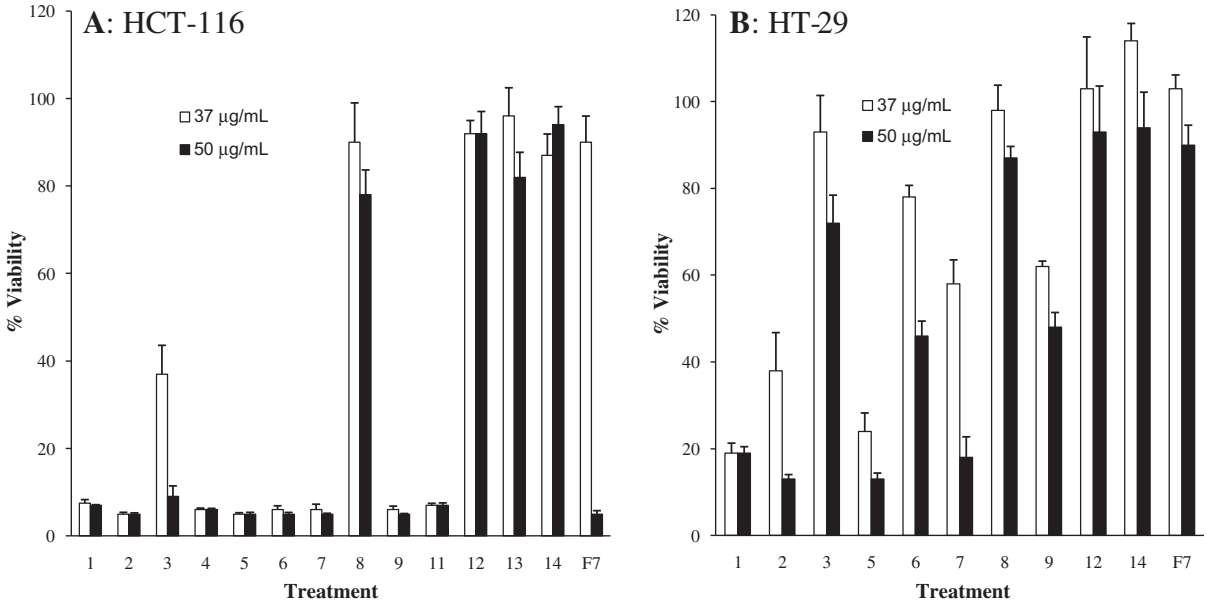
Table 1
^{1}H NMR (600 MHz) and ^{13}C NMR (150 MHz) Spectroscopic Data of **2**, **4–7**, and **9–10** (in Py-d5)

Position	2		4		5		6		7		9		10	
	δ_H, *J*	δ_C	δ_H, *J*	δ_C	δ_H, *J*	δ_C	δ_H, *J*	δ_C	δ_H, *J*	δ_C	δ_H, *J*	δ_C	δ_H, *J*	δ_C
1,3		160.8 C		160.7 C		160.7 C		160.7 C		160.8 C		160.7 C		160.7 C
2	6.93, t, 2.0	102.1 CH	6.94, t, 1.9	102.1 CH	6.93, t, 2.0	102.1 CH	6.93, t, 2.0	102.1 CH	6.94, t, 2.0	102.1 CH	6.93, t, 1.9	102.1 CH	6.93, t, 1.9	102.1 CH
4,6	6.80, d, 2.0	108.3 CH	6.80, d, 1.9	108.3 CH	6.80, d, 2.0	108.3 CH	6.80, d, 2.0	108.3 CH	6.80, d, 2.0	108.3 CH	6.80, d, 1.9	108.3 CH	6.80, d, 1.9	108.3 CH
5		146.3 C		146.2 C		146.2 C		146.2 C		146.3 C		146.2 C		146.2 C
1′	2.65, t, 7.5	36.9 CH_2	2.65, t, 7.6	36.9 CH_2	2.65, t, 7.6	36.9 CH_2	2.65, t, 7.5	36.9 CH_2	2.65, t, 7.4	36.9 CH_2	2.65, t, 7.6	36.9 CH_2	2.65, t, 7.6	36.9 CH_2
2′	1.70, m	32.2 CH_2	1.71, m	32.2 CH_2	1.70, m	32.2 CH_2	1.71, m	32.1 CH_2	1.70, m	32.2 CH_2	1.71, m	32.2 CH_2	1.71, m	32.2 CH_2
3′	1.29, m	30.0 CH_2	1.28, m	30.0 CH_2	1.27, m	30.0 CH_2	1.26, m	30.0 CH_2	1.27, m	30.0 CH_2	1.28, m	30.0 CH_2	1.28, m	30.0 CH_2
4′	1.32, m	30.3 CH_2	1.32, m	30.3 CH_2	1.32, m	30.3 CH_2	1.28, m	30.3 CH_2	1.28, m	30.3 CH_2	1.32, m	30.3 CH_2	1.32, m	30.3 CH_2
5′	1.32, m	30.3 CH_2	1.32, m	30.3 CH_2	1.32, m	30.3 CH_2	1.28, m	30.3 CH_2	1.28, m	30.3 CH_2	1.32, m	30.3 CH_2	1.32, m	30.3 CH_2
6′	1.32, m	30.3 CH_2	1.32, m	30.3 CH_2	1.32, m	30.3 CH_2	1.28, m	30.3 CH_2	1.28, m	30.3 CH_2	1.32, m	30.3 CH_2	1.32, m	30.3 CH_2
7′	1.32, m	30.3 CH_2	1.32, m	30.3 CH_2	1.32, m	30.3 CH_2	1.28, m	30.3 CH_2	1.28, m	30.3 CH_2	1.32, m	30.3 CH_2	1.32, m	30.3 CH_2
8′	1.32, m	30.3 CH_2	1.32, m	30.3 CH_2	1.32, m	30.3 CH_2	1.36, m	30.4 CH_2	1.37, m	30.4 CH_2	1.32, m	30.3 CH_2	1.32, m	30.3 CH_2
9′	1.32, m	30.3 CH_2	1.32, m	30.3 CH_2	1.32, m	30.3 CH_2	2.13, m	27.9 CH_2	2.12, m	28.0 CH_2	1.32, m	30.3 CH_2	1.32, m	30.3 CH_2
10′	1.39, m	30.3 CH_2	1.32, m	30.3 CH_2	1.40, m	30.3 CH_2	5.52, m	130.7 CH	5.51, m	131.1 CH	1.32, m	30.3 CH_2	1.40, m	30.3 CH_2
11′	2.11, m	27.6 CH_2	1.32, m	30.3 CH_2	2.12, m	27.9 CH_2	5.52, m	128.8 CH	5.51, m	128.6 CH	1.32, m	30.3 CH_2	2.12, m	27.9 CH_2
12′	5.49, m	130.7 CH	1.40, m	30.3 CH_2	5.51, m	130.7 CH	2.95, t, 5.4	26.5 CH_2	2.96, t, 5.4	26.5 CH_2	1.32, m	30.3 CH_2	5.51, m	130.6 CH
13′	5.49, m	130.7 CH	2.10, m	27.6 CH_2	5.51, m	130.7 CH	5.51, m	130.9 CH	5.52, m	129.1 CH	1.32, m	30.3 CH_2	5.51, m	130.6 CH
14′	2.11, m	27.6 CH_2	5.48, m	130.7 CH	2.12, m	27.9 CH_2	5.51, m	130.9 CH	5.52, m	129.1 CH	1.39, m	30.3 CH_2	2.12, m	27.9 CH_2
15′	1.32, m	32.6 CH_2	5.48, m	130.7 CH	1.40, m	30.3 CH_2	2.13, m	27.9 CH_2	2.93, t, 5.1	26.3 CH_2	2.10, m	27.6 CH_2	1.40, m	30.3 CH_2
16′	1.28, m	23.0 CH_2	2.10, m	27.6 CH_2	1.32, m	30.3 CH_2	1.36, m	30.4 CH_2	5.46, m	128.0 CH	5.48, m	130.6 CH	1.32, m	30.3 CH_2
17′	0.88, t, 7.1	14.6 CH_3	1.32, m	32.6 CH_2	1.25, m	32.5 CH_2	1.28, m	32.2 CH_2	5.46, m	132.6 CH	5.48, m	130.6 CH	1.32, m	30.3 CH_2
18′			1.29, m	23.0 CH_2	1.27, m	23.3 CH_2	1.26, m	23.2 CH_2	2.09, m	21.3 CH_2	2.10, m	27.6 CH_2	1.32, m	30.3 CH_2
19′			0.88, t, 7.1	14.6 CH_3	0.87, t, 7.0	14.7 CH_3	0.87, t, 6.9	14.6 CH_3	0.95, t, 7.6	14.9 CH_3	1.35, m	32.6 CH_2	1.25, m	32.5 CH_2
20′											1.32, m	23.0 CH_2	1.26, m	23.3 CH_2
21′											0.88, t, 7.0	14.6 CH_3	0.87, t, 7.0	14.7 CH_3
-OH	11.27, s		11.28, s		11.28, s		11.28, s		11.28, s		11.28, s		11.28, s	

ARs, 5-*n*-heptadecylresorcinol (**1**, C17:0; IC_{50} 18.94 μg/mL) had the strongest inhibitory effect on the growth of HCT-116 cells followed by 5-*n*-nonadecylresorcinol (**3**, C19:0; IC_{50} 36.19 μg/mL). Whereas 5-*n*-heneicosenylresorcinol (**8**, C21:0), 5-*n*-tricosylresorcinol (**12**, C23:0), and 5-*n*-pentadecylresorcinol (**14**, C25:0) had almost no inhibitory effects (Fig. 6), indicating that increasing the length of the side chain will diminish the observed inhibitory effects (ranged from C_{17} to C_{25}). Similar results were observed on the HT-29 human colon cancer cells (Fig. 6). We also observed that alk(en)ylresorcinols had better inhibitory effects than related alkylresorcinols especially on HCT-116 cells (Fig. 6). Compounds **4–7**, as members of C_{19} family owning unsaturated side chains, showed much stronger inhibitory effects than that of 5-*n*-nonadecylresorcinol (**3**, C19:0). Similarly, compound **9** (C21:1; IC_{50} 22.28 μg/mL) had much better activity than that of heneicosenylresorcinol (**8**, C21:0). The presence of a carbonyl group in the alkyl chain enhanced the growth inhibitory effect of compound **11** (IC_{50} 16.63 μg/mL), which had much better inhibitory effects than **8**.

2.4. Discussion

We have previously found that 2% WB oil could significantly inhibit tumorigenesis in the *Apc*$^{min/+}$ mouse model.[14] The objective of the present study is to purify and identify the active components in WB oil using human colon cancer cells (HCT-116 and HT-29) as the guiding assay. We fractionated WB oil through column chromatography into eight subfractions and identified fraction 7 containing ARs as the most active fraction against the proliferation of human HCT-116 and HT-29 colon cancer cells. Further purification led to the identification of 14 ARs. Among them, **7**, (10′*Z*,13′*Z*,16′*Z*)-5-(nonadeca-10′,13′,16′-trienyl)resorcinol, is a novel compound and **5**, **6**, **9**, **10**, and **13** were purified as individual compounds for the first time. To our knowledge, this is the first report on the purification and structure elucidation of **5–7**, **9**, **10**, and **13** using 1D and 2D NMR. All the purified compounds (**1–14**) except 10 were evaluated for the growth inhibition on human colon cancer cell lines (HCT-116 and HT-29). Our results indicate that increasing the length of the side chain will diminish the inhibitory activity, and the exis-



C: IC_{50} of test agents (μg/mL)

	1	2	3	4	5	6	7	8	9	11	12	13	14	F7
HCT-116	18.94	20.03	36.19	17.65	11.57	27.06	20.03	> 50	22.28	16.63	> 50	> 50	> 50	42.22
HT-29	31.15	35.59	> 50	---	30.42	48.28	39.54	> 50	46.89	---	> 50	---	> 50	> 50

Figure 6. Cell growth inhibition in HCT-116 (**A**) and HT-29 (**B**) human colon cancer cell lines by purified compounds at 37 and 50 μg/mL and their IC_{50} (**C**). Cells were treated with 5, 10, 25, 37, and 50 μg/mL of the test compounds for 48 h in the presence of 10% FBS at 37 °C. Cell growth inhibition was determined by MTT assay ($n = 8 \pm SD$). IC_{50} was calculated using GraphPad Prism.

tence of a double bond and a carbonyl group will strengthen such an activity.

Evidence on the anticancer effect of ARs is very limited and most studies have been carried out using cancer cell lines. It has been reported that AR C15:0 could significantly inhibit the growth of BT-20 (IC_{50}: 6.25 μg/mL), FM3A (IC_{50}: 2.80 μM), and MCF-7 (IC_{50}: 37.0 μM) breast cancer cells, NCI-H460 lung cancer cells (IC_{50}: 34.2 μM), and HeLa epithelioid cervix carcinoma cells (IC_{50}: 4.02 μg/mL).[22–25] Arisawa et al. compared the cytotoxicity of ARs with different lengths of side chain (C7:0–C19:0) on KB cells.[23] They found that C13:0 was the most active AR and that both increasing and decreasing the chain length would decrease the activity, indicating that the chain length played an important role on the cytotoxicity of ARs against KB cells. This is consistent with our observation on the effect of ARs against human colon cancer cells. Gasiorowski et al. attributed the anticancer effect of ARs to their ability to increase apoptosis in genotoxically damaged cell.[26] To our knowledge, this is the first study to identify ARs as the major active components in WB oil to inhibit the growth of human colon cancer cells. Our finding that one of the dominant ARs in WB, 5-*n*-nonadecylresorcinol (**3**, C19:0), can inhibit the growth of human colon cancer cells is of significant importance. This indicates that ARs may play important role to the observed in vivo efficacy of WB. Further studies on the in vivo efficacy of ARs could significantly contribute to our understanding of the colon cancer preventive effect of WB.

Specific and precise information on the relationship between WB and human colon cancer is still lacking in spite of many epidemiological and laboratory studies. In epidemiological studies, many results are inconsistent. In addition to confounding factors that are difficult to correct for, the lack of an accurate assessment tool to measure WB intake hampers the ability to determine the relationship between WB and human colon cancer risk. These problems could be resolved by using a biomarker as an objective measure of whole grain wheat intake. ARs are present in significant amounts only in whole grain wheat and rye products.[25] Many studies have shown that ARs and their metabolites can be used as the biomarkers to reflect whole grain wheat and rye intake.[25,27,28] ARs were found to be absorbed by rats, pigs, and humans.[25] Rats fed a single dose of radiolabeled ARs excreted ~66% in the feces as intact ARs and 34% in the urine as metabolized ARs.[25] ARs concentrations in pig plasma increase rapidly after a meal containing ARs and persist in plasma for at least 5 days.[29] Feeding trials in humans have shown measurable levels of ARs and/or their metabolites in plasma or urine samples after whole-grain wheat and rye consumption.[27,30–33] It has been reported that cereal fiber intake during a habitual diet correlates significantly with all plasma ARs and urinary AR metabolites in women.[28] Landberg et al. found that plasma ARs concentrations were significantly higher after the whole-grain diet period than after the refined-grain period and were well correlated with average daily ARs intake estimated by self-reported weighed food records.[33] Therefore, if ARs are the active components in WB for colon cancer prevention, they would be the ideal exposure biomarkers to correlate the consumption of whole grain wheat and human colon cancer risks.

3. Conclusions

In conclusion, using human colon cancer cells (HCT-116 and HT-29) as the guiding assays, we identified that a fraction containing ARs had the strongest inhibitory effect on the proliferation of human HCT-116 and HT-29 colon cancer cells. Further purification

led to the identification of 14 ARs. Among them, **7**, (10′Z,13′Z,16′Z)-5-(nonadeca-10′,13′,16′-trienyl)resorcinol, is a novel compound and **5**, **6**, **9**, **10**, and **13** were purified as individual compounds for the first time. All these compounds (**1**–**14**) except **10** were evaluated for growth inhibition of human colon cancer cell lines (HCT-116 and HT-29). Our results indicate that increasing the length of the side chain will diminish the inhibitory activity, and the existence of a double bond and a carbonyl group will strengthen such an activity.

4. Experimental

4.1. General

4.1.1. Materials

Analytical (250 μm thickness, 2–25 μm particle size) and preparative TLC plates (2000 μm thickness, 2–25 μm particle size) were purchased from Sigma (St. Louis, MO) and Sorbent Technologies (Atlanta, GA), respectively. LC/MS-grade solvents and other reagents were obtained from Fisher Scientific (Pittsburgh, PA).

4.1.2. HPLC analysis

Waters preparative HPLC system with 2545 binary gradient module, Waters 2767 sample manager, Waters 2487 autopurification flow cell, Waters fraction collector III, dual injector module, and 2489 UV/Visible detector was used to purify compounds **1**–**14**. A Phenomenex Gemini-NX C_{18} column (250 mm × 30.0 mm i.d., 5 μm) was used with a flow rate of 20.0 mL/min. The wavelength of the UV detector was set at 280 nm. Water and methanol were used as mobile phases A and B.

An HPLC–ECD/UV system (ESA, Chelmsford, MA) consisting of an ESA model 584 HPLC pump, an ESA model 542 autosampler, an ESA organizer, and an ESA 526 UV detector was used to monitor the combination of fractions collected from the preparative HPLC. Chromatographic analysis was performed on a Gemini C_{18} column (150 mm × 4.6 mm id, 5 μm, Phenomenex) at a flow rate of 1.0 mL/min. The mobile phase consisted of solvent A (95% H_2O + 5% MeOH + 0.2% AA) and solvent B (100% MeOH + 0.2% AA). The wavelength of the UV detector was set at 280 nm. Isocratic elution was used to analyze compounds **1** (t_R 19.0 min), **5** (t_R 20.0 min), and **4** (t_R 21.0 min) using 88% B in 25 min; compounds **3** (t_R 21.0 min), **10** (t_R 22.5 min), and **9** (t_R 24.0 min) using 90% B in 30 min; and compounds **7** (t_R 22.0 min), **2** (t_R 26.0 min), and **6** (t_R 27.5 min) using 84% B in 30 min. Compounds 11 (t_R 16.30 min) and 13 (t_R 17.30 min) were analyzed using a gradient system (90–92% B from 0 to 5 min; 92% B from 5 to 10 min; 92–100% B from 10 to 18 min; 100% B from 18 to 26 min; and then re-equilibrated to 90% B from 26 to 30 min).

4.1.3. LC/MS analysis

LC/PDA/MS analysis was carried out with a Thermo-Finnigan Spectra System which consisted of an Accela high speed MS pump, an Accela refrigerated autosampler, an Accela photodiode array (PDA) detector, and an LCQ Fleet ion trap mass detector (Thermo Electron, San Jose, CA, USA) incorporated with an atmospheric pressure chemical ionization (APCI) interface. A Gemini-NX C_{18} column (50 mm × 2.0 mm id, 3 μm, Phenomenex) was used for the analysis of fraction 7 and its major components with a flow rate of 0.2 mL/min. The binary mobile phase system consisted of 5% aqueous methanol with 0.2% acetic acid as solvent A and 95% aqueous methanol with 0.2% acetic acid as solvent B. The column was eluted with isocratic phase A for 3 min followed by a gradient progress (100 to 88% A from 3 to 10 min; 88 to 0% A from 10 to 50 min; 0% A from 50 to 65 min, then 100% A from 65 to 70 min). The injection volume was 10 μL for each sample. The LC eluent was introduced into the APCI interface. The positive ion polarity mode was set for the APCI source and nitrogen gas was used as the sheath and auxiliary gases. Optimized source parameters include APCI capillary temperature (280 °C), APCI vaporizer temperature (325 °C), capillary voltage (37 V), sheath gas flow rate (20 units), auxiliary gas flow rate (5 units), and tube lens (100 V). The structural information of compounds **1**–**14** were obtained by tandem mass spectrometry (MS/MS) through collision-induced dissociation (CID) with a relative collision energy setting of 35%. Data acquisition was performed with Xcalibur version 2.0 (Thermo Electron, San Jose, CA, USA).

4.1.4. NMR analysis

^1H (600 MHz), ^{13}C (150 MHz), ^1H-^{13}C HMQC (heteronuclear multiple quantum correlation), and HMBC NMR spectra were acquired on a Bruker 600 MHz instrument. All compounds were analyzed in pyridine-d5.

4.2. Fractionation of WB oil

WB (423 kg, provided by ConAgra Food Ingredients Company, NJ) was extracted twice by 95% ethanol at 40 °C to obtain 42.4 kg ethanol extract. Part of the ethanol extract (1 kg) was then suspended in water and partitioned with ethyl acetate to obtain the WB oil (800 g). The WB oil fraction (250 g) was chromatographed on a normal phase silica gel column eluted with a stepwise gradient of hexane/ethyl acetate [20:1] to give fraction 1 (148.5 g), 10:1 to obtain fractions 2 (10.1 g), 3 (6.7 g), and 4 (6.2 g), 5:1 to give fractions 5 (8.5 g), 6 (3.5 g), and 7 (11.5 g), and then methanol to give fraction 8 (29.7 g).

4.3. Purification of compounds 1–14

Fraction 7 (2.0 g) was subjected to reverse phase C_{18} Silica gel medium pressure column chromatography (30 × 360 mm, 60 Å, Sigma, St. Louis, MO) eluted with a gradient of MeOH–H_2O (80:20, 90:10, 95:5, and 100:0, 1000 mL each) to give ten subfractions ($F7_1$–$F7_{10}$). Repeated purification of $F7_2$ (40.0 mg) by preparative HPLC (pre-HPLC) (88% aqueous methanol in 60 min) followed by preparative TLC (CH_2Cl_2/MeOH: 30:1) afforded **7** (3.0 mg, t_R 35.3 min), **2** (4.0 mg, t_R 39.6 min), and **6** (2.5 mg, t_R 44.3 min). $F7_4$ (150.0 mg) was further purified by pre-HPLC (90% aqueous methanol in 60 min) to give **1** (15.0 mg, t_R 49.4 min), **5** (5.0 mg, t_R 53.1 min), and **4** (10.1 mg, t_R 53.2 min). $F7_6$ (300.0 mg) was separated by pre-HPLC eluted with MeOH/H_2O (92% in 60 min) to yield **3** (100.0 mg, t_R 44.9 min), **10** (1.0 mg, t_R 49.0 min), and **9** (13.1 mg, t_R 49.9 min). Similarly, $F7_8$ (100.0 mg), $F7_9$ (80.0 mg) and $F7_{10}$ (50.0 mg) were further purified by pre-HPLC (95% aqueous methanol in 60 min) to give **8** (30.0 mg), **12** (20.0 mg) and **14** (10.0 mg), respectively. F8 (30.0 g) was applied to silica gel column chromatography and eluted with a gradient of chloroform and methanol (100:1 to 0:1) to obtain 13 subfractions (F8A–F8M). F8C (2.0 g) was further separated into five subfractions ($F8C_1$–$F8C_5$) when loaded into reverse phase C_{18} Silica gel medium pressure column with a gradient of MeOH–H_2O (90:10, 95:5, and 100:0, 1000 mL each). $F8C_3$ (460 mg) was further purified by pre-HPLC (92% aqueous methanol in 60 min) in combination with pre-TLC (chlorform/methanol: 30:1) to produce **11** (5 mg, t_R 45.1 min) and **13** (20 mg, t_R 56.4 min). The spectroscopic data of all compounds (**1**–**14**) are listed below.

4.3.1. 5-*n*-Heptadecylresorcinol (1)

White powder; ^1H NMR (600 MHz, Py-D_5) δ 6.94 (1H, t, J = 1.8 Hz, H-2), 6.80 (2H, d, J = 1.8 Hz, H-4/6), 2.65 (2H, t, J = 7.6 Hz, H-1′), 1.71 (2H, m, H-2′), 1.32–1.28 (24H, m, ranged from H-3′ to H-14′), 1.26 (2H, m, H-15′), 1.27 (2H, m, H-16′), and 0.88 (3H, t, J = 7.0 Hz, H-17′); ^{13}C NMR (125 MHz, Py-D_5) δ 160.8 (s,

C-1/3), 102.1 (d, C-2), 108.3 (d, C-4/6), 146.3 (s, C-5), 37.0 (t, C-1′), 32.2 (t, C-2′), 30.1 (t, C-3′), 30.5–30.0 (from C-4′ to C-14′), 32.6 (t, C-15′), 23.4 (t, C-16′), and 14.7 (q, C-17′); APCI-MS *m/z* 349 $[M+H]^+$.

4.3.2. (12′*Z*)-5-(Heptadec-12′-enyl)resorcinol (2)

White powder; ^1H (600 MHz, Py-D_5) and ^{13}C (150 MHz, Py-D_5) NMR, see **Table 1**; positive APCI-MS, *m/z* 347 $[M+H]^+$.

4.3.3. 5-*n*-Nonadecylresorcinol (3)

White powder; ^1H NMR (600 MHz, Py-D_5) δ 6.98 (1H, t, J = 2.0 Hz, H-2), 6.81 (2H, d, J = 2.0 Hz, H-4/6), 2.64 (2H, t, J = 7.7 Hz, H-1′), 1.70 (2H, m, H-2′), 1.32–1.28 (28H, m, ranged from H-3′ to H-16′), 1.26 (2H, m, H-17′), 1.27 (2H, m, H-18′), and 0.89 (3H, t, J = 6.9 Hz, H-19′); ^{13}C NMR (125 MHz, Py-D_5) δ 160.8 (s, C-1/3), 102.2 (d, C-2), 108.4 (d, C-4/6), 146.3 (s, C-5), 37.0 (t, C-1′), 32.3 (t, C-2′), 30.2 (t, C-3′), 30.5–30.0 (from C-4′ to C-16′), 32.7 (t, C-17′), 23.5 (t, C-18′), and 14.8 (q, C-19′); positive APCI-MS, m/*z* 377 $[M+H]^+$.

4.3.4. (14′*Z*)-5-(Nonadeca-14′-enyl)resorcinol (4)

White powder; ^1H (600 MHz, Py-D_5) and ^{13}C (150 MHz, Py-D_5) NMR, see **Table 1**; positive APCI-MS, *m/z* 375 $[M+H]^+$.

4.3.5. (12′*Z*)-5-(nonadeca-12′-enyl)resorcinol (5)

White powder; ^1H (600 MHz, Py-D_5) and ^{13}C (150 MHz, Py-D_5) NMR, see **Table 1**; positive APCI-MS, *m/z* 375 $[M+H]^+$.

4.3.6. (10′*Z*,13′*Z*)-5-(Nonadeca-10′,13′-dienyl)resorcinol (6)

White powder; ^1H (600 MHz, Py-D_5) and ^{13}C (150 MHz, Py-D_5) NMR, see **Table 1**; positive APCI-MS, *m/z* 373 $[M+H]^+$.

4.3.7. (10′*Z*,13′*Z*,16′*Z*)-5-(Nonadeca-10′,13′,16′-trienyl)resorcinol (7)

White powder; ^1H (600 MHz, Py-D_5) and ^{13}C (150 MHz, Py-D_5) NMR, see **Table 1**; positive APCI-MS, *m/z* 371 $[M+H]^+$.

4.3.8. 5-*n*-Heneicosylresorcinol (8)

White powder; ^1H NMR (600 MHz, Py-D_5) δ 6.96 (1H, t, J = 1.9 Hz, H-2), 6.81 (2H, d, J = 1.9 Hz, H-4/6), 2.65 (2H, t, J = 7.3 Hz, H-1′), 1.70 (2H, m, H-2′), 1.33–1.28 (32H, m, ranged from H-3′ to H-18′), 1.26 (2H, m, H-19′), 1.27 (2H, m, H-20′), and 0.89 (3H, t, J = 6.9 Hz, H-21′); ^{13}C NMR (125 MHz, Py-D_5) δ 160.8 (s, C-1/3), 102.1 (d, C-2), 108.3 (d, C-4/6), 146.3 (s, C-5), 37.0 (t, C-1′), 32.3 (t, C-2′), 30.1 (t, C-3′), 30.5–30.0 (from C-4′ to C-18′), 32.6 (t, C-19′), 23.4 (t, C-20′), and 14.8 (q, C-21′); positive APCI-MS, *m/z* 405 $[M+H]^+$.

4.3.9. (16*Z*′)-5-(Heneicos-16′-enyl)resorcinol (9)

White powder; ^1H (600 MHz, Py-D_5) and ^{13}C (150 MHz, Py-D_5) NMR, see Table 1; positive APCI-MS, 403 $[M+H]^+$.

4.3.10. (12′*Z*)-5-(Heneicos-12′-enyl)resorcinol (10)

White powder; ^1H (600 MHz, Py-D_5) and ^{13}C (150 MHz, Py-D_5) NMR, see **Table 1**; positive APCI-MS, *m/z* 403 $[M+H]^+$.

4.3.11. 5-(2′-Oxoheneicosyl)resorcinol (11)

White powder; ^1H NMR (600 MHz, Py-D_5) δ 6.96 (1H, t, J = 2.2 Hz, H-2), 6.86 (2H, d, J = 2.2 Hz, H-4/6), 3.79 (2H, s, H-1′), 2.56 (2H, t, J = 7.3 Hz, H-3′), 1.61 (2H, m, H-4′), 1.21 (2H, m, H-5′),1.32–1.27 (26H, m, ranged from H-6′ to H-18′), 1.24 (2H, m, H-19′), 1.26 (2H, m, H-20′), and 0.88 (3H, t, J = 7.0 Hz, H-21′); ^{13}C NMR (125 MHz, Py-D_5) δ 161.0 (s, C-1/3), 103.1 (d, C-2), 109.3 (d, C-4/6), 138.2 (s, C-5), 51.0 (t, C-1′), 208.6 (C=O, C-2′), 42.3 (t, C-3′), 24.5 (t, C-4′), 29.8 (t, C-5′), 30.5–30.0 (from C-6′ to C-18′), 32.5 (t, C-19′), 23.4 (t, C-20′), and 14.7 (q, C-21′); positive APCI-MS, m/*z* 419 $[M+H]^+$.

4.3.12. 5-*n*-Tricosylresorcinol (12)

White powder; ^1H NMR (600 MHz, Py-D_5) δ 6.94 (1H, t, J = 1.9 Hz, H-2), 6.80 (2H, d, J = 1.9 Hz, H-4/6), 2.65 (2H, t, J = 7.6 Hz, H-1′), 1.71 (2H, m, H-2′), 1.33–1.28 (36H, m, ranged from H-3′ to H-20′), 1.26 (2H, m, H-21′), 1.27 (2H, m, H-22′), and 0.88 (3H, t, J = 7.0 Hz, H-23′); ^{13}C NMR (125 MHz, Py-D_5) δ 160.7 (s, C-1/3), 102.1 (d, C-2), 108.3 (d, C-4/6), 146.3 (s, C-5), 36.9 (t, C-1′), 32.2 (t, C-2′), 30.0 (t, C-3′), 30.5–30.0 (from C-4′ to C-20′), 32.5 (t, C-21′), 23.4 (t, C-22′), and 14.7 (q, C-23′); positive APCI-MS, m/*z* 433 $[M+H]^+$.

4.3.13. 5-(2′-Oxotricosyl)resorcinol (13)

White powder; ^1H NMR (600 MHz, Py-D_5) δ 6.96 (1H, t, J = 2.1 Hz, H-2), 6.85 (2H, d, J = 2.1 Hz, H-4/6), 3.79 (2H, s, H-1′), 2.56 (2H, t, J = 7.4 Hz, H-3′), 1.61 (2H, m, H-4′), 1.21 (2H, m, H-5′),1.32–1.27 (30H, m, ranged from H-6′ to H-20′), 1.24 (2H, m, H-21′), 1.26 (2H, m, H-22′), and 0.89 (3H, t, J = 7.1 Hz, H-23′); ^{13}C NMR (125 MHz, Py-D_5) δ 161.1 (s, C-1/3), 103.1 (d, C-2), 109.3 (d, C-4/6), 138.3 (s, C-5), 51.0 (t, C-1′), 208.6 (C=O, C-2′), 42.3 (t, C-3′), 24.5 (t, C-4′), 29.8 (t, C-5′), 30.5–30.0 (from C-6′ to C-20′), 32.6 (t, C-21′), 23.4 (t, C-22′), and 14.7 (q, C-23′); positive APCI-MS, 447 $[M+H]^+$.

4.3.14. 5-*n*-Pentadecylresorcinol (14)

White powder; ^1H NMR (600 MHz, Py-D_5) δ 6.94 (1H, t, J = 1.8 Hz, H-2), 6.80 (2H, d, J = 1.8 Hz, H-4/6), 2.65 (2H, t, J = 7.6 Hz, H-1′), 1.71 (2H, m, H-2′), 1.33–1.28 (40H, m, ranged from H-3′ to H-22′), 1.26 (2H, m, H-23′), 1.27 (2H, m, H-24′), and 0.88 (3H, t, J = 6.9 Hz, H-25′); ^{13}C NMR (125 MHz, Py-D_5) δ 160.8 (s, C-1/3), 102.1 (d, C-2), 108.3 (d, C-4/6), 146.3 (s, C-5), 36.9 (t, C-1′), 32.2 (t, C-2′), 30.1 (t, C-3′), 30.5–30.0 (from C-4′ to C-22′), 32.6 (t, C-23′), 23.4 (t, C-24′), and 14.7 (q, C-25′); positive APCI-MS, m/*z* 461 $[M+H]^+$.

4.4. Cell culture studies

Cell growth inhibition was determined by the MTT assay.[34] The cells (5000 cells/well) were plated in 96-well microtitre plates and allowed to attach for 24 h at 37 °C. The test samples (in DMSO) were added to McCoy's 5A medium (containing 10% fetal bovine serum, 1% penicillin/streptomycin, 1% amphotericin B, and 1% glutamine) to desired final concentrations. After culturing for 48 h, the medium was aspirated, and the cells were treated with 100 μL fresh medium containing 2.41 mmol/L MTT. Following incubation for 3 h at 37 °C, the MTT-containing medium was aspirated, 100 μL DMSO was added to solubilize the formazan precipitate, and the plate was read at 595 nm on a microtitre plate reader 1 h after adding DMSO. The reading reflected the number of viable cells, and was expressed as% cell growth.

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