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Sterol Ferulates, Sterols, and 5-Alk(en)ylresorcinols from Wheat, Rye, and Corn Bran Oils and Their Inhibitory Effects on Epstein−Barr Virus Activation

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Sterol ferulate, free sterol, and 5-alk(en)ylresorcinol constituents of wheat, rye, and corn bran oils were studied. Among the sterol ferulates, one novel compound, 24-methylenecholestanol ferulate (**7**), along with six known compounds, namely, 24-methylcholestanol ferulate (**1**), 24-methylcholesterol ferulate (**2**), 2-methyllathosterol ferulate (**3**), stigmastanol ferulate (**4**), sitosterol ferulate (**5**), and schottenol ferulate (**6**), were isolated and characterized. Five known free sterols, namely, 24-methylcholesterol (**8**), stigmastanol (**9**), sitosterol (**10**), schottenol (**11**), and stigmasterol (**12**), were isolated and identified. 5-Alk(en)ylresorcinols were found in wheat and rye bran oils but not in corn bran oil. Of these, one new compound, 5-*n*-(2′-oxo-14′-*Z*-heneicosenyl) resorcinol (**19**), and seven known compounds, namely, 5-*n*-heptadecyl- (**13**), 5-*n*-nonadecyl- (**14**), 5-*n*-heneicosyl- (**15**), 5-*n*-tricosyl- (**16**), 5-*n*-pentacosyl- (**17**), 5-*n*-(14′-*Z*-nonadecenyl)- (**18**), and 5-*n*-(2′-oxoheneicosyl)-resorcinols (**20**), were isolated and characterized. These compounds were evaluated with respect to their inhibitory effects on the induction of Epstein−Barr virus early antigen (EBV-EA) by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in Raji cells, which is known to be a primary screening test for antitumor promoters. Four compounds, **1**, **2**, **4**, and **11**, showed potent inhibitory effects on EBV-EA induction.

KEYWORDS: Wheat bran; rye bran; corn bran; sterol ferulate; sterol; 5-alk(en)ylresorcinol; antitumor promoter; Epstein−Barr virus early antigen

INTRODUCTION

In the course of our search for potential antitumor promoters (cancer chemopreventive agents) from edible plants and fungi and from crude herbal drugs (*1*), we have found that various triterpene alcohol ferulates and sterol ferulates from rice bran oil exhibited activity in an in vivo primary screening assay for antitumor promoters by inhibiting the inflammatory ear edema induced by 12-*O*-tetradecanoylphorbol-13-acetate (TPA) in mice (*2*) and on tumor promotion in two-stage carcinogenesis in mouse skin initiated by 7,12-dimethylbenz[*a*]anthracene and promoted by TPA (*3*). In continuing our study on the constituents of the bran oils of Gramineae plants, we have investigated wheat, rye, and corn bran oils and the inhibitory effects of their constituents on Epstein−Barr virus early antigen (EBV-EA) activation induced by TPA as a primary screening for antitumor promoters.

MATERIALS AND METHODS

Crystallizations were performed in methanol (MeOH), and melting points measured are uncorrected. UV spectra obtained on a Shimadzu UV-2200 spectrometer, and IR spectra on a JASCO IR-300 IR spectrometer, were recorded in ethanol, $CHCl_3$, and KBr disks, respectively. Nuclear magnetic resonance (NMR) spectra were recorded with a JEOL LA-500 spectrometer at 500 MHz (^{1}H NMR) and 125 MHz (^{13}C NMR) in $CDCl_3$ with tetramethylsilane (TMS; ^{1}H NMR) and $CDCl_3$ at δ 77.0 (^{13}C NMR) as internal standard. Electron-impact mass spectra (EIMS) and high-resolution EIMS (HREIMS) were recorded on a JEOL JMS-GC mass spectrometer (70 eV) using a direct inlet system.

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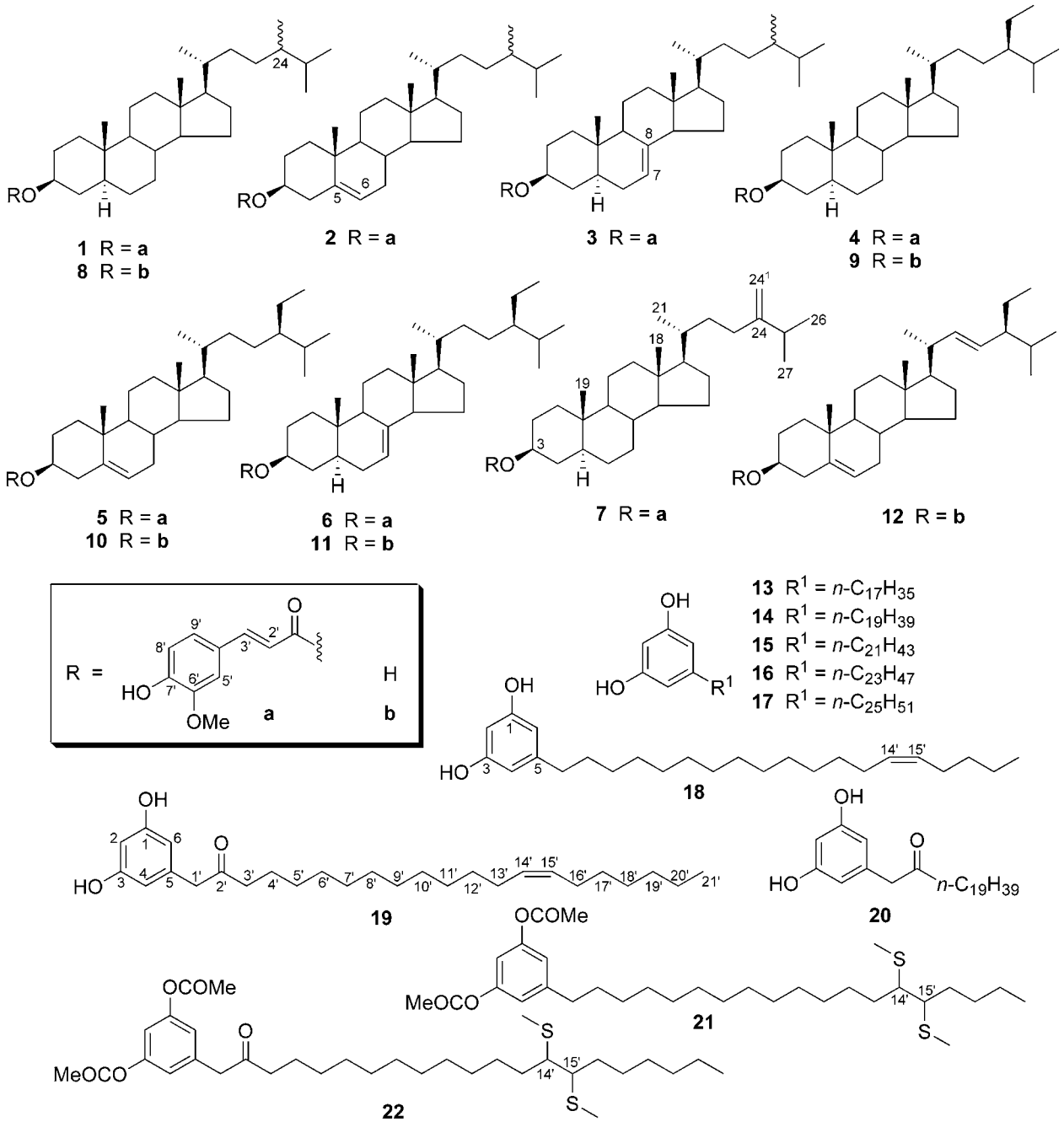

Figure 1. Structures of sterol ferulates, sterols, and 5-alk(en)ylresorcinols in this study.

Thin-layer chromatography (TLC) on 10 × 10 cm silica gel 60G (Merck) was developed using $CHCl_3$/acetone (49:1, v/v) (TLC I) and *n*-hexane/ethyl acetate (EtOAc) (6:1, v/v) (TLC II). Silica gel 60, 220−400 mesh (Merck), was used for open column chromatography. Reversed-phase preparative high-performance liquid chromatography (HPLC) was carried out on a 25 cm × 10 mm i.d. C_{18} silica column at 25 °C using either an ERC-ODS-2352 column (ERC Co., Ltd., Tokyo, Japan), with CH_3CN/*n*-BuOH/acetic acid (AcOH) (47:2:1, v/v/v) as mobile phase at 5.0 mL/min (HPLC I), or a Pegasil ODS II column (Senshu Scientific Co., Ltd., Tokyo, Japan), with MeOH (HPLC II) and MeOH/H_2O (49:1, v/v) (HPLC III) as mobile phase at 3.0 mL/min. Relative retention times (t_{RR}) of compounds were expressed relative to stigmastanol ferulate (**4**) [retention time (t_R) 49 min], cholesterol (cholest-5-en-3β-ol; t_R 30 min), and 5-*n*-heptadecylresorcinol (**13**) (t_R 10 min) in HPLC I, II, and III, respectively. A refractive index detector was used for reversed-phase HPLC.

Gas−liquid chromatography (GLC) for sterols was performed on a Shimadzu GC-14B instrument (Shimadzu, Co., Kyoto, Japan) using a 30 m × 0.3 mm i.d. DB-17 fused-silica capillary column (J&W Scientific, Inc., Folsom, CA) at a column temperature of 275 °C and nitrogen as a carrier gas at 60 mL/min (split ratio 60:1). Cholesterol (t_R 9 min) was the standard for the determination of t_{RR} of sterols in GLC.

Chemicals and Materials. *n*-Hexane-extracted crude oils of wheat bran, rye bran (midds), and corn bran were donated by Riken Vitamin Co. (Tokyo, Japan). Eight compounds, (24*R*,*S*)-24-methylcholesterol ferulate (**2**), stigmastanol ferulate (**4**), sitosterol ferulate (**5**), (24*R*,*S*)-24-methylcholesterol (**8**), stigmastanol (**9**), sitosterol (**10**), schottenol (**11**), and stigmasterol (**12**), were used as reference compounds (*2*) (**Figure 1**). TPA was purchased from ChemSyn Laboratories (Lenexa, KS). The cell culture reagents, *n*-butyric acid, and other reagents were purchased from Nacalai Tesque, Inc. (Kyoto, Japan).

Extraction and Isolation. *Wheat Bran Oil.* Wheat bran oil (30 g) was chromatographed on a silica gel (400 g) column with a stepwise gradient of *n*-hexane/ethyl acetate (EtOAc) [1:0 (3.0 L), 19:1 (4.2 L), 9:1 (2.1 L), 6:1 (2.4 L), and 4:1 (1.8 L)] as eluant. *n*-Hexane/EtOAc (9:1) eluted fractions A (R_f value 0.64 on TLC I; 1.66 g), *n*-hexane/EtOAc (6:1) eluted fraction B (R_f 0.37; 2.69 g), and *n*-hexane/EtOAc

(4:1) eluted fraction C (R_f 0.20; 5.28 g) were obtained. Repeated column chromatography on a silica gel of individual fractions eventually afforded purified fractions A (115 mg; sterol ferulate fraction), B (210 mg; sterol fraction), and C [572 mg; 5-alk(en)ylresorcinol fraction]. Fraction A was subjected to HPLC I, which yielded six compounds, namely, 24-methylcholestanol ferulate (**1**; t_{RR} 0.85), **2** (0.68), 24-methyllathosterol ferulate (**3**; 0.56), **4** (1.00), **5** (0.81), and schottenol ferulate (**6**; 0.76). Fraction B, upon HPLC II, afforded isolated five sterols, namely, **8** (t_{RR} 1.17 in HPLC; t_{RR} 1.27 in GLC), **9** (1.30; 1.55), **12** (1.06; 1.37), and a mixture of **10** (1.21; 1.54) and **11** (1.21; 1.82). Separation of **10** (R_f 0.25) and **11** (R_f 0.19) was performed by TLC II. HPLC III of fraction C gave six 5-alk(en)ylresorcinols, namely, 5-*n*-heptadecyl- (**13**; t_{RR} 1.00), 5-*n*-nonadecyl- (**14**; 1.45), 5-*n*-heneicosyl- (**15**; 2.08), 5-*n*-tricosyl- (**16**; 2.96), 5-*n*-pentacosyl- (**17**; 4.17), and 5-*n*-(14′-*Z*-nonadecenyl)resorcinols (**18**; 1.06).

Rye Bran Oil. Using the same fractionation procedure as above, rye bran oil (30 g) yielded purified fractions A (248 mg), B (733 mg), and C (1.14 g). Six compounds, **1**−**6**, from fraction A; five compounds, **8**−**12**, from fraction B; and seven compounds, **13**−**17**, 5-*n*-(2′-keto-14′-*Z*-heneicosenyl)resorcinol (**19**), and 5-*n*-(2′-ketoheneicosenyl)-resorcinol (**20**), from fraction C, were isolated by HPLC.

Corn Bran Oil. Corn bran oil (30 g) was fractionated as above to yield purified fractions A (252 mg) and B (823 mg). The presence of 5-alk(en)ylresorcinols (fraction C) was not confirmed in the corn bran oil. Upon HPLC, fraction A yielded seven sterol ferulates, **1**−**6** and 24-methylenecholestanol ferulate (**7**), and fraction B afforded five sterols, **8**−**12**.

Identification and Characterization. Identification of three sterol ferulates, **2**, **4**, and **5**, and five sterols, **8**−**12**, was performed by MS and ^{1}H NMR comparison with reference compounds. Compounds **2** and **8** from all three bran oils were mixtures of C-24 stereoisomers, and their 24*R*:24*S* isomeric ratios were estimated to be ca. 2:1 on the basis of the ^{1}H NMR data (*4*). Identification of three sterol ferulates, **1**, **3**, and **6**, was performed by MS and ^{1}H NMR comparison with relevant compounds in the literature (*2*, *4*), and characterization of a new ferulate, **7**, was done on the basis of spectroscopic methods. Identification of seven known 5-alk(en)ylresorcinols, **13**−**17** (*5*), **18** (*6*), and **20** (*7*), and characterization of one new 5-alkenylresorcinol, **19**, also were undertaken on the basis of spectroscopic methods. Unsaturation at C-14′ for compounds **18** and **19** was confirmed by analysis of the MS fragmentation of their dimethyl disulfide adducts (*8*).

Preparation of Dimethyl Disulfide Adducts of 5-Alkenylresorcinols 18 and 19. 5-Alkenylresorcinol **18** (or **19**) was acetylated with acetic anhydride-pyridine at room temperature overnight to give the **18**-diacetate (or **19**-diacetate). A solution of **18**-diacetate (or **19**-diacetate) (3 mg) in dimethyl disulfide (1 mL) containing I_2 (13 mg) in a stoppered glass vial was stirred for 30 min at 35 °C (*8*). A 30% (w/v) aqueous $NaHCO_3$ solution was then added to the reaction mixture until the color of I_2 disappeared, and the mixture was extracted with *n*-hexane/diethyl ether (1:1, v/v) to give a bis(methylthio)alkylresorcinol diacetate (**21**) (or **22**).

The spectroscopic and spectrometric data for four sterol ferulates, **1**, **3**, **6**, and **7**, eight 5-alk(en)ylresorcinols, **13**−**20**, and two dimethyl disulfide adducts, **21** and **22**, as well as the melting point for **7**, are given below.

*(24R,S)-24-Methylcholestanol ferulate (**1**).* EIMS *m*/*z* 578 (M^+). ^{1}H NMR: H-3 (δ_H 4.83, m), H-18 (0.66, s), H-19 (0.85, s), H-21 (24*R*: 0.90, d, J = 6.6 Hz; 24*S*: 0.91, d, J = 6.6 Hz), H-26 (0.84, d, J = 7.1 Hz), H-27 (24*R*: 0.80, d, J = 7.1 Hz; 24*S*: 0.79, d, J = 7.1 Hz), H-24^1 (24*R*: 0.78, d, J = 6.8 Hz; 24*S*: 0.77, d, J = 6.6 Hz), H-2′ (6.27, d, J = 15.9 Hz), H-3′ (7.60, d, J = 16.1 Hz), H-5′ (7.03, d, J = 1.9 Hz), OMe-6′ (3.92, s), OH-7′ (5.83, s), H-8′ (6.91, d, J = 8.1 Hz), H-9′ (7.06, dd, J = 1.9, 8.4 Hz). The 24*R*:24*S* isomeric ratios of **1** from all of the three bran oils were estimated to be ca. 2:1 based on the intensity for the relevant ^{1}H NMR signals.

*(24R,S)-24-Methyllathosterol ferulate (**3**):* HREIMS, *m*/*z* M^+ 576.4178 calcd for $C_{38}H_{56}O_4$, found 576.4183; ^{1}H NMR, H-3 (δ_H 4.83, m), H-7 (5.16, m), H-18 (0.54, s), H-19 (0.85, s), H-21 (24*R*: 0.92, d, J = 6.6 Hz; 24*S*: 0.93, d, J = 6.3 Hz), H-26 (0.85, d, J = 7.1 Hz), H-27 (24*R*: 0.81, d, J = 6.8 Hz; 24*S*: 0.79, d, J = 7.1 Hz), H-24^1 (24*R*: 0.78, d, J = 6.6 Hz; 24*S*: 0.79, d, J = 6.6 Hz). The ^{1}H NMR signals for the feruroyl moiety were essentially the same as those of compound **1**. The 24*R*:24*S* isomeric ratios of **3** from all three bran oils were estimated to be ca. 2:1 from the ^{1}H NMR data.

*Schottenol ferulate (**6**):* HREIMS, *m*/*z* M^+ calcd for $C_{39}H_{58}O_4$ 590.4341, found 590.4335; ^{1}H NMR, H-3 (δ_H 4.83, m), H-7 (5.16, m), H-18 (0.54, s), H-19 (0.84, s), H-21 (0.93, d, J = 6.1 Hz), H-26 (0.82, d, J = 7.1 Hz), H-27 (0.84, d, J = 7.3 Hz), H-24^2 (0.85, t, J = 7.3 Hz). The ^{1}H NMR signals for the feruroyl moiety were essentially the same as those of compound **1**.

*24-Methylenecholestanol ferulate (**7**):* fine needles; mp 128−129 °C; UV, λ_{max} (nm) 243, 293, 318; IR, ν_{max} (cm^{-1}) 3420 (OH), 1707, 1268 (OBz), 886 (>C=CH_2), 815 (C=CH); EIMS, *m*/*z* (%) 576 (M^+, 69), 561 (2), 449 (4), 394 (14), 367 (3), 298 (7), 256 (5), 194 (100), 177 (41); HREIMS, *m*/*z* M^+ calcd for $C_{38}H_{56}O_4$ 576.4183, found 576.4178; ^{1}H NMR, H-3 (δ_H 4.82, m), H-18 (0.66, s), H-19 (0.85, s), H-21 (0.94, d, J = 6.4 Hz), H-25 (2.23, sept, J = 6.8 Hz), H-26 (1.02, d, J = 6.8 Hz), H-27 (1.03, d, J = 6.8 Hz), H-24^1 (4.66 and 4.71, each br s). The ^{1}H NMR signals for the feruroyl moiety were essentially the same as those of compound **1**.

*5-n-Heptadecylresorcinol (**13**):* EIMS, *m*/*z* (%) 348 (M^+, 20), 306 (1), 278 (2), 264 (2), 222 (1), 208 (3), 180 (2), 166 (8), 137 (14), 124 (100), 123 (28); HREIMS, *m*/*z* M^+ calcd for $C_{23}H_{40}O_2$ 348.3032, found 348.3028; ^{1}H NMR, OH-1 (δ_H 4.70, s), H-2 (6.17, s), OH-3 (4.70, s), H-4 (6.24, s), H-6 (6.24, s), H-1′ (2.48, t, J = 7.6 Hz), H-2′ (1.57, s), H-3′H-16′ (1.26, s), H-17′ (0.88, t, J = 6.0 Hz).

*5-n-Nonadecylresorcinol (**14**):* EIMS, *m*/*z* (%) 376 (M^+, 40), 292 (2), 250 (2), 208 (4), 180 (2), 166 (12), 137 (24), 124 (100), 123 (51); HREIMS, *m*/*z* M^+ calcd for $C_{25}H_{44}O_2$ 376.3344, found 376.3341; ^{1}H NMR, essentially the same as those of compound **13**.

*5-n-Heneicosyresorcinol (**15**):* EIMS, *m*/*z* (%) 404 (M^+, 53), 362 (3), 264 (5), 208 (4), 180 (3), 166 (13), 137 (20), 124 (100), 123 (43); HREIMS, *m*/*z* M^+ calcd for $C_{27}H_{48}O_2$ 404.3657, found 404.3654; ^{1}H NMR, essentially the same as those of compound **13**.

*5-n-Tricosylresorcinol (**16**):* EIMS, *m*/*z* (%) 432 (M^+, 35), 376 (1), 292 (1), 250 (2), 208 (3), 180 (2), 166 (9), 152 (2), 137 (11), 124 (100), 123 (25); HREIMS, *m*/*z* M^+ calcd for $C_{29}H_{52}O_2$ 432.3977, found 432.3967; ^{1}H NMR, essentially the same as those of compound **13**.

*5-n-Pentadecylresorcinol (**17**):* EIMS, *m*/*z* (%) 460 (M^+, 27), 376 (2), 292 (1), 250 (2), 208 (3), 180 (2), 166 (9), 152 (2), 137 (11), 124 (100), 123 (25); HREIMS, *m*/*z* M^+ calcd for $C_{31}H_{56}O_2$ 460.4281, found 460.4280; ^{1}H NMR, essentially the same as those of compound **13**.

*5-n-14′-(Z)-Nonadecenylresorcinol (**18**):* EIMS, *m*/*z* (%) 374 (M^+, 11), 278 (3), 166 (6), 137 (10), 124 (100), 123 (18); HREIMS, *m*/*z* M^+ calcd for $C_{25}H_{42}O_2$ 374.3187, found 374.3185; ^{1}H NMR, OH-1 (δ_H 4.74, m), H-2 (6.17, br s), OH-3 (4.74, m), H-4 (6.24, s), H-6 (6.24, s), H-1′ (2.48, t, J = 7.6 Hz), H-2′ (1.57, s), H-3′H-12′ (1.26, s), H-13′ (2.02, br s), H-14′ and H-15′ (5.35, ddd, J = 4.4, 10.0, 11.2 Hz), H-16′ (2.02, br s), H-17′ and H-18′ (1.26, s), H-19′ (0.90, t, J = 6.0 Hz).

*5-n-(2′-Oxo-14′-(Z)-heneicosenyl)resorcinol (**19**):* fine needles; mp 68−70 °C; IR, ν_{max} (cm^{-1}) 3424 (OH), 1701 (C=O), 837 (C=CH); EIMS, *m*/*z* (%) 416 (M^+, 6), 294 (3), 293 (23), 267 (83), 166 (11), 137 (6), 124 (100), 123 (42); HREIMS, *m*/*z* M^+ calcd for $C_{27}H_{44}O_3$ 416.3293, found 416.3290; ^{13}C and ^{1}H NMR, C-1 [δ_C 157.1; δ_H 4.87, s (OH)], C-2 [101.7; 6.26, s] C-3 [157.1; 4.87, s (OH)], C-4 [109.1; 6.26, s], C-5 [137.0], C-6 [109.1; 6.26, s], C-1′ [49.9; 3.55, s], C-2′ [209.2], C-3′ [42.1; 2.48, t, J = 7.0 Hz], C-4′ [23.5; 1.58], C-5′C-12′ [29.0−29.8; 1.25, br s], C-13′ [27.2; 2.04], C-14′ and C-15′ [129.9; 5.35], C-16′ [27.2; 2.04], C-17′ and C-18′ [29.0−29.8; 1.25, br s], C-19′ [31.9; 1.25], C-20′ [22.7; 1.25], C-21′ [14.1; 0.88, t, J = 7.0 Hz].

*5-n-(2′-Oxoheneicosyl)resorcinol (**20**):* EIMS, *m*/*z* (%) 418 (M^+, 42), 321 (14), 295 (100), 166 (11), 137 (8), 124 (93), 123 (41); HREIMS, *m*/*z* M^+ calcd for $C_{27}H_{46}O_3$ 418.3449, found 418.3447; ^{1}H NMR, OH-1 (δ_H 4.87, m), H-2 (6.26, br s), OH-3 (4.87, m), H-4 and H-6 (6.26, s), H-1′ (3.55, s), H-3′ (2.48, t, J = 7.3 Hz), H-4′ (1.58, br s), H-5′C-20′ (1.25, s), H-21′ (0.88, t, J = 6.0 Hz). In the NOESY spectrum, this compound showed a significant NOE correlation between H-4 (and H-6) and H-1′.

*5-n-14′,15′-Bis(methylthio)nonadecanylresorcinol diacetate (**21**):* EIMS, *m*/*z* (%) 552 (M^+, 15; $C_{31}H_{52}O_4S_2^+$), 435 (100; $C_{25}H_{39}O_4S^+$), 416 (17), 393 (22), 351 (15), 303 (9), 117 (14; $C_6H_{13}S^+$).

Table 1. Yields of Sterol Ferulate, Sterol, and 5-Alk(en)ylresorcinol Fractions and Isolated Compounds from Wheat, Rye, and Corn Bran Oils

		yield (mg) from 10 g of oil material		
	compound	wheat bran oil	rye bran oil	corn bran oil
sterol ferulate		38[a]	83[a]	84[a]
1	(24*R*,*S*)-24-methylcholestanol ferulate	4	25	10
2	(24*R*,*S*)-24-methylcholesterol ferulate	3	8	5
3	(24*R*,*S*)-24-methyllathosterol ferulate	1	1	0.2
4	stigmastanol ferulate	8	21	36
5	sitosterol ferulate	3	3	3
6	schottenol ferulate	1	1	1
7	24-methylenecholestanol ferulate			0.5
sterol		70[a]	244[a]	274[a]
8	(24*R*,*S*)-24-methylcholesterol	10	46	23
9	stigmastanol	5	19	6
10	sitosterol	24	82	118
11	schottenol	2	11	4
12	stigmasterol	2	8	23
5-alk(en)ylresorcinol		191[a]	380[a]	
13	5-*n*-heptadecylresorcinol	3	40	
14	5-*n*-nonadecylresorcinol	40	60	
15	5-*n*-heneicosylresorcinol	62	51	
16	5-*n*-tricosylresorcinol	14	59	
17	5-*n*-pentacosylresorcinol	5	36	
18	5-*n*-(14′-(*Z*)-nonadecenyl)resorcinol	0.5		
19	5-*n*-(2′-keto-14′-(*Z*)-heneicosenyl)resorcinol		1	
20	5-*n*-(2′-ketoheneicosyl)resorcinol		0.3	

[a] Yield of the fraction from each oil.

*5-n-[2′-Oxo-14′,15′-bis(methylthio)heneicosanyl]resorcinol diacetate (**22**):* EIMS, *m*/*z* (%) 594 (M^+, 17; $C_{33}H_{54}O_5S_2^+$), 477 (70), 449 (100; $C_{25}H_{37}O_5S^+$), 421 (60), 293 (31), 267 (16), 166 (18), 145 (15; $C_8H_{17}S^+$).

In Vitro EBV-EA Activation Experiment. The inhibition of EBV-EA activation was assayed using Raji cells (EBV genome-carrying human lymphoblastoid cells; nonproducer type), cultivated in 10% fetal bovine serum (FBS) RPMI-1640 medium (Sigma, St. Louis, MO). The indicator cells (Raji cells; 1×10^6 cells/mL) were incubated in 1 mL of the medium containing 4 mM *n*-butyric acid as an inducer, 32 pM TPA [20 ng/mL in dimethyl sulfoxide (DMSO)], and a known amount (32, 16, 3.2, or 0.32 nmol) of the test compound at 37 °C in a CO_2 incubator. After 48 h, the cell suspensions were centrifuged at 1000 rpm for 10 min, and the supernatant was removed. The activated cells were stained with high-titer EBV-EA-positive sera from nasopharyngeal carcinoma patients, and the conventional indirect immunofluorescence technique was employed for detection. In each assay, at least 500 cells were counted and the experiments were repeated three times. The average extent of EA induction was determined and compared with that on positive control experiments in which the cells were treated with *n*-butyric acid plus TPA, where the extent of EA induction was ordinarily $>\sim$40%. The viability of treated Raji cells was assayed by the Trypan Blue staining method (*9*).

RESULTS AND DISCUSSION

Sterol ferulate, free sterol, and 5-alk(en)ylresorcinol constituents of wheat, rye, and corn bran oils were investigated in this study. Whereas wheat and rye bran oils contained all of the compound groups mentioned above, 5-alk(en)ylresorcinols were not found in corn bran oil. Seven sterol ferulates (**1**−**7**), five sterols (**8**−**12**), and eight 5-alk(en)ylresorcinols (**13**−**20**), among which **7** and **19** were new naturally occurring compounds, were isolated and characterized in this study. **Table 1** shows the yields of sterol ferulate, sterol, and 5-alk(en)ylresorcinol fractions and individual compounds from the oil materials.

Sterol Ferulates. Characterization of a new sterol ferulate, **7**, was undertaken by UV, MS, and ^{1}H NMR comparison with those of reference sterol ferulates and with the literature data for the relevant compounds (*2*−*4*). Three 24-methylsterol ferulates, **1**−**3**, from all three Gramineae bran oils were found to be mixtures of 24*R* and 24S stereoisomers, with the 24*R* isomer predominating. The coexistence of both stereoisomers at C-24 of 24-methylsterols, although as the feruloyl esters in this case, is consistent with the observations for most of the other higher plant materials investigated (*2*, *4*). Two saturated sterol ferulates, **1** and **4**, were found to be present as the major sterol ferulates in the three bran oils investigated in this study (**Table 1**). This is consistent with previous observation in wheat (*10*, *11*), rye (*10*, *11*), and corn bran oils (*10*, *12*−*14*).

Sterols. The presence of **8** as the C-24 epimeric mixture and the predominance of **10** in the free sterol fractions from the three Gramineae bran oils (**Table 1**) are consistent with those of most of the higher plants (*4*).

5-Alk(en)ylresorcinols. Characterization of a new 5-alkenylresorcinol, **19**, was undertaken by comparison of the MS and ^{1}H NMR spectra with those of relevant 5-*n*-[2′-oxoalk(en)yl]-resorcinols (*7*). The ^{13}C DEPT NMR, ^{1}H−^{1}H correlation spectroscopy (COSY), ^{1}H-detected multiple coherence (HMQC), and heteronuclear multiple-bond correlation (HMBC) experiments, done for the signal assignments of **19**, supported the proposed structure. A phase-sensitive nuclear Overhauser enhancement and exchange spectroscopy (NOESY) experiment on **19** exhibited a significant NOE correlation between H-4 (and H-6) (δ_H 6.26) and H-1′ (δ_H 3.55), which is consistent with the presence of an oxo group at C-2′. The presence of a double bond at C-14′(15′) in **19** was confirmed by the MS analysis of its dimethyl disulfide adduct **22**. Compound **22** showed prominent fragment ions at *m*/*z* 449 ($C_{25}H_{37}O_5S^+$) and 145 ($C_8H_{17}S^+$) due to the cleavage at C-14′(15′). Compound **18** also was confirmed to possess a double bond at C-14′(15′) by preparation of its dimethyl disulfide adduct **21**, which exhibited prominent fragment ions in the MS at *m*/*z* 435 ($C_{25}H_{39}O_4S^+$) and 117 ($C_6H_{13}S^+$) arising from the cleavage at C-14′(15′). Among the eight 5-alk(en)ylresorcinols characterized, three compounds, **14**−**16**, were found to be present as the major 5-alk(en)-ylresorcinol constituents in both of wheat and rye bran oils. Several investigations have so far been done for the 5-alk(en)-ylresorcinols in wheat (*7*, *13*−*15*) and rye grains (*6*, *13*−*18*).

Table 2. Percentage of EBV-EA Induction in the Presence of Compounds **1**–**20** with Respect to a Positive Control (100%)[a]

	concentration (mol ratio/TPA)			
compound	1000	500	100	10
sterol ferulate				
1	4.9 ± 0.5 (70)	35.7 ± 1.3	72.3 ± 2.1	92.6 ± 0.6
2	2.1 ± 0.3 (70)	33.6 ± 1.2	71.2 ± 1.9	90.8 ± 0.7
3	16.5 ± 0.6 (70)	57.3 ± 2.0	85.2 ± 2.4	100 ± 0.3
4	3.5 ± 0.3 (60)	34.1 ± 1.5	71.9 ± 2.0	91.2 ± 0.7
5	19.4 ± 1.1 (70)	60.2 ± 1.8	87.1 ± 2.2	100 ± 0.4
6	19.0 ± 0.8 (70)	59.1 ± 1.4	86.0 ± 2.3	100 ± 0.3
7	13.5 ± 0.7 (70)	49.0 ± 1.3	77.5 ± 2.1	100 ± 0.5
sterol				
8	14.9 ± 0.7 (60)	51.2 ± 2.0	80.2 ± 2.2	100 ± 0.3
9	11.5 ± 0.5 (60)	46.2 ± 1.8	78.0 ± 2.0	100 ± 0.4
10	15.7 ± 0.5 (70)	51.8 ± 2.1	84.9 ± 2.3	100 ± 0.2
11	1.1 ± 0.3 (70)	29.7 ± 1.4	73.3 ± 2.0	95.4 ± 0.5
12	13.0 ± 0.7 (70)	50.4 ± 1.9	83.2 ± 2.1	100 ± 0.2
resorcinol				
13	13.2 ± 0.6 (60)	47.6 ± 1.7	76.0 ± 1.8	100 ± 0.4
14	15.1 ± 0.7 (60)	49.7 ± 1.5	80.5 ± 2.1	100 ± 0.3
15	16.4 ± 0.5 (60)	51.0 ± 2.1	82.4 ± 2.3	100 ± 0.3
16	17.3 ± 0.7 (60)	52.6 ± 2.0	83.9 ± 2.0	100 ± 0.3
17	19.7 ± 0.7 (60)	55.6 ± 2.5	86.7 ± 2.6	100 ± 0.2
18	18.4 ± 0.9 (60)	51.3 ± 2.2	84.9 ± 2.0	100 ± 0.5
19	13.4 ± 0.5 (60)	50.1 ± 2.1	79.5 ± 2.0	100 ± 0.5
20	15.9 ± 0.7 (60)	51.8 ± 2.3	81.7 ± 2.1	100 ± 0.3
ref compound				
β-carotene	8.6 ± 0.3 (70)	34.2 ± 1.1	82.1 ± 2.1	100 ± 0.3

[a] Values represent relative percentages to the positive control value ($n = 3$, and ± SD). TPA (32 pmol, 20 ng) = 100%. Values in parentheses are viability percentages of Raji cell.

Inhibitory Effects on EBV-EA Induction. The inhibitory effects on the induction of EBV-EA induced by TPA were examined as a preliminary evaluation of the potential antitumor-promoting activities for 20 compounds, **1**−**20**, from the three Gramineae bran oils. The inhibitory effects (**Table 2**) were compared with those of a reference compound, β-carotene, a vitamin A precursor that has been studied extensively in cancer chemoprevention using animal models (*19*). Among the 30 compounds tested, 4 compounds, namely, three sterol ferulates, **1**, **2**, and **4**, and one sterol, **11**, exhibited potent inhibitory effects (95−99% inhibition at 1×10^3 mol ratio/TPA) on EBV-EA induction by TPA with preservation of the high viability (60−70% at 1×10^3 mol ratio/TPA) of the Raji cells. The inhibitory effects of the four compounds (**1**, **2**, **4**, and **11**) were almost equivalent with those of β-carotene. The inhibitory effects against EBV-EA activation have been demonstrated to be closely parallel to those against tumor promotion in vivo (*1*), and compounds **1**, **2**, **4**, and **11** are suggested to be valuable antitumor promoters (potential cancer chemopreventive agents). Using wheat, rye, and corn bran oils as food ingredients might be advantageous because their sterol and sterol ferulate constituents have, as shown in this study, possible cancer chemopreventive properties, in addition to serum total cholesterol lowering properties (*11*). Moreover, it might be worth noting that plant sterols (phytosterols), especially sitosterol (**10**), are suggested to have protective effect against the most common cancers in the developed countries (*20*) including colon (*21*), prostate (*22*), and breast (*23*). Eight 5-alk(en)ylresorcinols, **13**−**20**, exhibited only a moderate activity against EBV-EA induction, and it was observed that increasing the chain length resulted in decreasing activity as for the 5-*n*-alkylresorcinols **13**−**17**.

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LITERATURE CITED

(1) Akihisa, T.; Yasukawa, K. Antitumor-promoting and anti-inflammatory activities of triterpenoids and sterols from plants and fungi. In *Studies in Natural Products Chemistry, Vol. 25, Bioactive Natural Products (Part F)*; Atta-ur-Rahman, Ed.; Elsevier Science: Amsterdam, The Netherlands, 2001; pp 43−87.

(2) Akihisa, T.; Yasukawa, K.; Yamaura, M.; Ukiya, M.; Kimura, Y.; Shimizu, N.; Arai, K. Triterpene alcohol and sterol ferulates from rice bran and their anti-inflammatory effects. *J. Agric. Food Chem.* **2000**, *48*, 2313−2319.

(3) Yasukawa, K.; Akihisa, T.; Kimura, Y.; Tamura, T.; Takido, M. Inhibitory effect of cycloartenol ferulate, a component of rice bran, on tumor promotion in two-stage carcinogenesis in mouse skin. *Biol. Pharm. Bull.* **1998**, *21*, 1072−1078.

(4) Goad, L. J.; Akihisa, T. *Analysis of Sterols*; Blackie Academic and Professional: London, U.K., 1997.

(5) Cirigottis, K. A.; Cleaver, L.; Corrie, J. E. T.; Grasby, R. G.; Green, G. H.; Mock, J.; Nimgirawath, S.; Read, R. W.; Ritchie, E.; Taylor, W. C.; Vadasz, A.; Webb, W. R. G. Chemical studies of the Proteceae. VII. An examination of the woods of 17 species for 5-alk(en)ylresorcinol derivatives. *Aust. J. Chem.* **1974**, *27*, 345−355.

(6) Suzuki, Y.; Esumi, Y.; Uramoto, M.; Kono, Y.; Sakurai, A. Structural analyses of carbon chains in 5-alk(en)ylresorcinols of rye and wheat whole flour by tandem mass spectrometry. *Biosci., Biotechnol., Biochem.* **1997**, *61*, 480−486.

(7) Seitz, L. M. Identification of 5-(2-oxoalkyl)resorcinols and 5-(2-oxoalkenyl)resorcinols in wheat and rye grains. *J. Agric. Food Chem.* **1992**, *40*, 1541−1546.

(8) Shibahara, A.; Yamamoto, K.; Nakayama, T.; Kajimoto, G. Rapid determination of double bond position in monounsaturated fatty acids by GC-MS and its application to fatty acid analysis. *J. Jpn. Oil Chem. Soc.* **1985**, *34*, 618−625.

(9) Takaishi, Y.; Ujita, K.; Tokuda, H.; Nishino, H.; Iwashima, A.; Fujita, T. Inhibitory effects of dihydroagarofuran sesquiterpenes on Epstein−Barr virus activation. *Cancer Lett.* **1992**, *65*, 19−26.

(10) Seitz, L. M. Stanol and sterol esters of ferulic and *p*-coumaric acids in wheat, corn, rye, and triticale. *J. Agric. Food Chem.* **1989**, *37*, 662−667.

(11) Hakala, P.; Lampi, A.-M.; Ollilainen, V.; Werner, U.; Murkovic, M.; Wähälä, K.; Karkola, S.; Phronen, V. Steryl phenolic acid esters in cereals and their milling fractions. *J. Agric. Food Chem.* **2002**, *50*, 5300−5307.

(12) Norton, R. A. Isolation and identification of steryl cinnamic acid derivatives from corn bran. *Cereal Chem.* **1994**, *71*, 111−117.

(13) Norton, R. A. Quantitation of steryl ferulate and *p*-coumarate esters from corn and rice. *Lipids* **1995**, *30*, 269−274.

(14) Moreau, R. A.; Singh, V.; Eckhoff, S. R.; Powell, M. J.; Hicks, K. B.; Norton, R. A. *Cereal Chem.* **1999**, *76*, 449−451.

(15) Verdeal, K.; Lorenz, K. Alkylresorcinols in wheat, rye, and triticale. *Cereal Chem.* **1977**, *54*, 475−483.

(16) Gohil, S.; Pettersson, D.; Salomonsson, A.-C.; Åman, P. Analysis of alkyl- and alkenylresorcinols in triticale, wheat and rye. *J. Sci. Food Agric.* **1988**, *45*, 43−52.

(17) Kozubek, A. Isolation of 5-*n*-alkyl, 5-*n*-alkenyl- and 5-*n*-alkdienyl-resorcinol homologs from rye grains. *Acta Aliment. Pol.* **1985**, *9*, 185−198.

(18) Kozubek, A.; Tyman, J. H. P. Cereal grain 5-alk(en)ylresorcinolic lipids: mono and dienoic homologues are present in rye grains. *Chem. Phys. Lipids* **1995**, *78*, 29−35.

(19) Murakami, A.; Ohigashi, H.; Koshimizu, K. Anti-tumor promotion with food phytochemicals: A strategy for cancer chemoprevention. *Biosci., Biotechnol., Biochem.* **1999**, *60*, 1−8.

(20) Awad, A. B.; Fink, C. S. Phytosterols as anticancer dietary components: evidence and mechanism of action. *J. Nutr.* **2000**, *130*, 2127−2130.
(21) Raicht, R. F.; Cohen, B. I.; Fazzini, E.; Sarwal, A.; Takahashi, M. Protective effect of plant sterols against chemically-induced colon tumor in rats. *J. Cancer Res.* **1980**, *40*, 403−405.
(22) Awad, A. B.; Gan, Y.; Fink, C. S. Effect of β-sitosterol, a plant sterol, on growth, protein phosphatase 2A and phospholipids D in LNCaP cells. *Nutr. Cancer* **2000**, *36*, 74−78.
(23) Awad, A. B.; Down, A. C.; Fink, C. S. Inhibition of growth and stimulation of apoptosis by β-sitosterol treatment of MDA-MB-231 human breast cancer cells in culture. *Int. J. Mol. Med.* **2000**, *5*, 541−546.

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