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Sterols Isolated from *Nuruk* (*Rhizopus oryzae* KSD-815) Inhibit the Migration of Cancer Cells

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An activity-guided fractionation method was used to isolate anticancer components from *Nuruk* (*Rhizopus oryzae* KSD-815:KSD-815). Dried powder of KSD-815 was extracted with 80% methanol and partitioned successively using *n*-hexane, ethyl acetate, *n*-butanol, and water. The *n*-hexane and *n*-butanol fractions showed a strong antimigratory effect on human cancer cells. Both of these fractions were subjected to separation and purification procedures using silica gel, octadecyl silica gel, and Sephadex LH-20 column chromatographies to afford four purified compounds. These were identified as ergosterol peroxide (1), stigmast-5-en-3β,7β-diol (2), ergosta-7,22-dien-3β,5α,6β,9α-tetraol (3), and daucosterol (4), respectively, by spectroscopic methods such as nuclear magnetic resonance spectrometry, mass spectrometry, and infrared spectroscopy, and comparison with those in the literature. Compounds 1–4 were isolated from KSD-815 for the first time. Compounds 1 and 4 inhibited the migration of MDA-MB-231 cells at concentrations lower than 20 μM.

Keywords: *Nuruk*, *Rhizopus oryzae* KSD-815, sterol, antimigratory effect, MDA-MB-231, SK-HEP-1

Nuruk is the traditional Korean *koji* used to make fermented foods including alcoholic beverages. It consists of various grains and many kinds of microorganisms such as fungi, yeast, and some bacteria [2, 15]. Grains are ground to a paste, moistened, and then naturally inoculated by airborne microorganisms. The fermentation process is performed by *Bifidobacterium* sp., *Lactobacillus* sp., *Aspergillus* sp., *Absidia* sp., and *Rhizopus* sp. [3, 12, 14]. These microbes have high saccharogenic and dextrinogenic activities that transform foods and sugars to alcohol and lactic acid.

However, the isolation of chemical components from *nuruk* has rarely been reported. We isolated *Rhizopus oryzae* KSD-815 [4] from the alcohol-fermenting *nuruk* used to manufacture traditional alcoholic beverages. Alcohol extracts from *nuruk* were evaluated for several pharmacological activities and shown to exhibit some anticancer activity such as cytotoxicity and antimigratory effects on some cancer cells. A continuing activity-guided fractionation for the extracts led to isolation of active components. This paper describes the isolation and identification of four sterols from *Rhizopus oryzae* KSD-815, and the evaluation of these sterols for antimigratory effect on cancer cells.

Nuruk (*Rhizopus oryzae* KSD-815) was obtained from Kooksoondang Brewery Co. Ltd. and identified by Dr. Woo-Chang Sin [4], Research Laboratory of Kooksoondang Brewery, Sungnam, Korea. A voucher specimen (KHU060425) was reserved at the Laboratory of Natural Products Chemistry, KyungHee University, Yongin, Korea. Dried and powdered *Rhizopus oryzae* KSD-815 (8 kg) was extracted with 80% aqueous methanol (MeOH, 45 l×3) at room temperature. The extracts were partitioned using ethyl acetate (EtOAc, 4 l×3), *n*-BuOH (3.5 l×3), and H_2O (4 l), successively. The EtOAc extracts (231 g) were further partitioned between 80% MeOH (1 l×3) and *n*-hexane (1 l). The *n*-hexane extracts (RoHe, 135 g) were applied to a silica gel (SiO_2, Kiesel gel 60; Merck, Darmstadt, Germany) column chromatography (c.c.) and eluted with *n*-hexane-EtOAc (10:1→7:1→5:1→1:1) with monitoring by thin-layer chromatography (TLC, Kieselgel 60 F_{254}, RP-18 F_{254S}; Merck, Darmstadt, Germany) to provide 16 fractions (RoHe1 to RoHe16). Subfraction RoHe11 [392 mg, V_e/V_t (elution volume/total volume) 0.21–0.25] was applied to an octadecyl silica gel (ODS, LiChroprep RP-18; Merck, Darmstadt, Germany) c.c. and

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eluted with acetonitrile–H_2O (2:1) to afford 8 fractions (RoHe11-1 to RoHe11-8), and subfraction RoHe11-8-4 (77 mg, V_e/V_t 0.15–0.40) was further purified by ODS c.c. using acetonitrile–H_2O (3:1) as the eluting solution to give compound **1** [18.9 mg, V_e/V_t 0.70–0.80, TLC (RP-18 F_{254}) R_f 0.50 in acetonitrile–H_2O (5:1)]. Subfraction RoHe14 (192 mg, V_e/V_t 0.15–0.40) was subjected to SiO_2 c.c. and eluted with $CHCl_3$–MeOH (20:1) to afford 11 fractions (RoHe14-1 to RoHe14-11) and, ultimately, compound **2** [27 mg, V_e/V_t 0.27–0.41, TLC (SiO_2 F_{254}) R_f 0.70 in *n*-hexane-EtOAc (1:1)]. The *n*-BuOH extracts (RoB, 90 g) were applied to SiO_2 c.c. and eluted with $CHCl_3$–MeOH–H_2O (12:3:1) to provide 13 fractions (RoB1 to RoB13). Subfraction RoB2 (770 mg, V_e/V_t 0.15–0.40) was subjected to further SiO_2 c.c. and eluted with $CHCl_3$–MeOH–H_2O (16:3:1) to give 9 fractions (RoB2-1 to RoB2-9). Subfraction RoB–2-5 (20 mg, V_e/V_t 0.55-0.65) was applied to a Sephadex LH-20 column (Amersham Pharmacia Biotech, Uppsala, Sweden, 70% MeOH, 500 ml) to ultimately produce compound **3** [8 mg, V_e/V_t 0.55–0.65, TLC (RP-18 F_{254}) R_f 0.70 in MeOH–H_2O (1:1)]. Subfraction RoB2-7 (181 mg, V_e/V_t 0.70–0.78) was applied to ODS c.c. and eluted with MeOH–H_2O (2:1) to afford 5 fractions (RoB2-7-1 to RoB2-7-5) and to ultimately produce compound **4** [63 mg, V_e/V_t 0.30–0.45, TLC (RP-18 F_{254}) R_f 0.55 in MeOH–H_2O (4:1)].

Compound **1** (Ergosterol peroxide): colorless needles ($CHCl_3$); m.p. 181–183°C; $[\alpha]^{25}_D$=–31.7° (*c*=0.4, $CHCl_3$); EIMS *m/z* 428 $[M]^+$, 410 $[M-H_2O]^+$, 396, 377, 363, 251, 107, 69; IR_υ (KBr, cm^{-1}) 3,400, 1,459 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ) 6.48 (1H, d, *J*=8.8 Hz, H-7), 6.22 (1H, d, *J*=8.8 Hz, H-6), 5.20 (1H, dd, *J*=15.6, 8.0 Hz, H-22), 5.11 (1H, dd, *J*=15.6, 7.6 Hz, H-23), 3.95 (1H, m, H-3), 0.98 (3H, d, *J*=6.8 Hz, H-21), 0.88 (3H, d, *J*=6.8 Hz, H-28), 0.87 (3H, s, H-19), 0.81 (3H, d, *J*=6.8 Hz, H-26), 0.79 (3H, d, *J*=6.4 Hz, H-27), 0.79 (3H, s, H-18); ^{13}C NMR (100 MHz, $CDCl_3$, δ) see Table 1.

Compound **2** (Stigmast-5-en-3β,7β,-diol): colorless needles ($CHCl_3$); m.p. 157–158°C; $[\alpha]^{25}_D$=–15.8° (*c*=0.3, $CHCl_3$); EIMS *m/z* 430 $[M]^+$, 412 $[M-H_2O]^+$, 394 $[M-2H_2O]^+$, 289, 271, 253; IR_υ (KBr, cm^{-1}) 3,420, 1,660, 840 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ); 5.57 (1H, d, *J*=5.2 Hz, H-6), 3.83 (1H, br s, H-7), 3.55 (1H, br dd, *J*=4.4, 11.6 Hz, H-3), 0.97 (3H, s, H-19), 0.90 (3H, d, *J*=6.4 Hz, H-21), 0.82 (3H, t, *J*=7.6 Hz, H-29), 0.81 (3H, d, *J*=7.6 Hz, H-27), 0.78 (3H d, *J*=7.6, H-26), 0.66 (3H, s, H-18); ^{13}C NMR (100 MHz, $CDCl_3$, δ) see Table 1.

Compound **3** (Ergosta-7,22-dien-3β,5α,6β,9α-tetraol): White powder (MeOH); m.p. 272°C; $[\alpha]^{25}_D$=–33.7° (*c*=0.4, MeOH); EIMS *m/z* 446 $[M]^+$, 428 $[M-H_2O]^+$, 410 $[M-2H_2O]^+$, 392 $[M-3H_2O]^+$, 318, 300, 285; IR_υ (KBr, cm^{-1}) 3,367, 1,690, 1,260, 835; ^1H NMR (400 MHz, C_5D_5N, δ) 5.74 (1H, d, *J*=5.0, H-7), 5.25 (1H, dd, *J*=16.4, 8.0 Hz, H-22), 5.18 (1H, dd, *J*=16.4, 7.6 Hz, H-23), 4.40 (1H, br dd, 5.6, 12.8, Hz, H-3), 4.35 (1H, d, *J*=5.0 Hz, H-6), 3.05 (1H, dd, *J*=12.8, 13.6 Hz, H-4ax), 2.50(1H, dd, 13.6, 5.6 Hz, H-4eq), 1.53 (3H, s, H-19), 1.04 (3H, d, *J*=6.8 Hz, H-21), 0.93 (3H, d, *J*=6.8 Hz, H-28), 0.84 (3H, d, *J*=6.4 Hz, H-27), 0.83 (3H, d, *J*=6.8 Hz, H-26), 0.64 (3H, s, H-18); ^{13}C NMR (100 MHz, C_5D_5N, δ) see Table 1.

Compound **4** (Daucosterol): colorless crystals (MeOH-H_2O); m.p. 285–288°C; $[\alpha]^{25}_D$=–43.7° (*c*=0.3, pyridine); pos. FABMS *m/z* 577 $[M+H]^+$; IR_υ (KBr, cm^{-1}) 3,320, 3,030, 2,937, 1,640; ^1H NMR (400 MHz, C_5D_5N, δ) 5.34 (1H, br. d, *J*=4.8 Hz, H-6), 5.05 (1H, d, *J*=7.6 Hz, H-1'), 3.59 (1H, m, H-3), 1.00 (3H, d, *J*=6.8 Hz, H-21), 0.93 (3H, s, H-19), 0.90 (3H, d, *J*=7.2 Hz, H-26), 0.88 (3H, d, *J*=7.6 Hz, H-27), 0.85 (3H, t, *J*=7.6 Hz, H-29), 0.66 (3H, s, H-18); ^{13}C NMR (100 MHz, C_5D_5N, δ) see Table 1.

Table 1. ^{13}C NMR data of compounds **1, 2** (in chloroform-d_1) and **3, 4** (in pyridine-d_5).

Carbon No.	Compound **1**	Compound **2**	Compound **3**	Compound **4**
1	37.0	37.1	29.1	37.5
2	30.2	31.4	32.4	30.3
3	66.4	71.3	67.3	78.1
4	34.7	42.2	42.1	40.0
5	79.4	146.1	74.3	140.8
6	135.3	123.7	73.8	121.9
7	130.6	65.3	120.4	32.2
8	82.1	37.0	141.5	32.1
9	51.1	42.0	76.1	50.4
10	37.0	37.5	41.3	37.0
11	20.7	20.7	28.5	21.4
12	39.4	39.2	36.0	39.4
13	44.6	42.1	44.4	42.5
14	51.7	49.4	51.3	56.9
15	28.7	23.1	23.5	24.6
16	23.5	28.3	28.7	28.6
17	56.2	55.6	56.3	56.3
18	12.9	12.0	12.6	12.1
19	18.2	18.3	22.4	19.5
20	39.8	36.0	40.7	36.5
21	20.9	18.8	21.4	19.1
22	132.2	34.0	136.1	34.3
23	135.1	26.1	132.0	26.4
24	42.8	45.8	43.1	46.1
25	33.1	29.2	33.3	30.1
26	20.0	19.8	19.8	19.3
27	19.7	19.1	20.1	20.1
28	17.6	23.1	17.9	23.5
29		11.7		12.3
1'				102.5
2'				75.4
3'				78.6
4'				71.7
5'				78.5
6'				62.9

The MeOH extract of *Rhizopus oryzae* KSD-815 was found to be cytotoxic to some human cancer cells in a preliminary experiment (data not shown). The MeOH extract was pooled in H_2O and extracted with *n*-hexane, EtOAc, and *n*-BuOH. Repeated silica gel, ODS, and Sephadex LH-20 column chromatographies for the *n*-hexane and *n*-BuOH fractions supplied four sterols. The structures of the isolated compounds (**1–4**) were identified using the spectroscopic and physicochemical data. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were taken on a Varian Unity Inova AS 400 FT-NMR spectrometer (IL, U.S.A.). Electronic ionization mass spectrometry (EIMS) and fast atom bombardment mass spectrometry (FABMS) data were recorded on a JEOL JMSAX 505-WA (Tokyo, Japan) and infrared (IR) spectra were run on a Perkin Elmer Spectrum One FT–IR spectrometer (MA, U.S.A.). Compound **1** was isolated as colorless needles from $CHCl_3$, and exhibited a molecular ion peak ($[M]^+$) at *m/z* 428 in the EIMS spectrum. This compound is freely soluble in chloroform. IR absorption bands at 3,400 and 1,459 cm^{-1} are characteristics of hydroxyl and olefin groups, respectively. The NMR spectra of compound **1** showed signals typical of ergostane-sterols, which led to its identification as ergosterol peroxide, a well-known sterol in fungi. This was confirmed by comparison of IR, mass spectrometry, and ^{1}H and ^{13}C NMR data with those of an authentic sample and the values reported in the literature [9]. Compound **2**, also isolated as colorless needles from $CHCl_3$, exhibited a molecular ion peak ($[M]^+$) at *m/z* 430 in the EIMS spectrum. The IR absorption bands at 3,420 and 1,660 cm^{-1} are characteristic of hydroxyl and olefin groups, respectively. The ^{1}H NMR spectrum showed two singlet methyl signals at δ 0.66 (H-18) and 0.97 (H-19); three doublet methyl signals at δ 0.90 (*J*=6.4 Hz, H-21), 0.81 (*J*=7.6 Hz, H-27), and 0.78 (*J*=7.6 Hz, H-26); and a triplet methyl signal at 0.82 (*J*=7.6 Hz, H-29). Furthermore, the presence of one olefin proton at δ 5.57 (*J*=5.2 Hz, H-6) indicates that this compound is a stigmast-6-en type sterol. Two oxygenated methine proton signals were also observed at δ 3.83 (br s, H-7) and 3.55 (br dd, *J*=4.4, 11.6 Hz, H-3). The ^{13}C NMR and distortionless enhancement by polarization transfer (DEPT) spectra of compound **2** also showed resonances 29 carbons composed of 6 methyls, 10 methylenes, 10 methines, and 3 quaternary carbons, which confirm compound **2** as a stigmastane steroid. Among them, an olefin quaternary (δ_C 146.1, C-5), an olefin methine (δ_C 123.7, C-6), and two oxygenated methine (δ_C 71.3, C-3; δ_C 65.3, C-7) carbon signals were detected. The carbons with hydroxyl groups were determined to be C-3 and C-7 by heteronuclear multiple bonding connectivity (HMBC). That is, an oxygenated methine proton signal at δ 3.83 correlated with a pair of olefin carbons at δ 146.1 (C-5) and at δ 123.7 (C-6), and with a methine carbon signal at δ 37.0 (C-8) in the HMBC spectrum, indicating that the oxygenated methine carbon was C-7. Cross peaks observed between an oxygenated methine proton signal at δ 3.55 and two methylene carbon signals at δ_C 31.4 (C-2) and at δ_C 42.2 (C-4) showed the oxygenated methine carbon to be C-3. The configuration of both hydroxyls was identified as *b* from the chemical shifts (C-3: δ_C 71.3; H-3: δ 3.55; C-7: δ_C 65.3; H-7: δ 3.83) and coupling constants (H-3: br dd, *J*=4.4, 11.6 Hz; H-7: br s) of the corresponding carbon and proton signals. In the high magnetic field region, six methyl signals were observed at δ_C 19.8 (C-26), 19.1 (C-27), 18.8 (C-21), 18.3 (C-19), 12.0 (C-17), and 11.7 (C-29). Therefore, compound **2** was identified as stigmast-5-en-3β,7β-diol; this was confirmed by comparison with spectroscopic data from the literature [5]. Compound **3** was obtained as a white powder from MeOH. The IR spectrum of this compound was similar to that of compound **1**, with a hydroxyl group at 3,367 cm^{-1} and a double bond at 1,690 cm^{-1}. The EIMS spectrum showed a molecular ion peak ($[M]^+$) at *m/z* 446. The proton signals for six methyl groups at δ 1.53 (s, H-19), 1.04 (d, *J*=6.8 Hz, H-29), 0.93 (d, *J*=6.8 Hz, H-28), 0.84 (d, *J*=6.4 Hz, H-27), 0.83 (d, *J*=6.8 Hz, H-26), and 0.64 (s, H-18) in the ^{1}H NMR spectrum indicated that compound **3** had an ergostane sterol moiety. Two oxygenated methine proton signals were observed at δ 4.40 (1H, m, H-3) and δ 4.35 (1H, d, *J*=5.6 Hz, H-6). Three olefin methine proton signals were observed at 5.74 (1H, d, *J*=5.0 Hz, H-7), δ

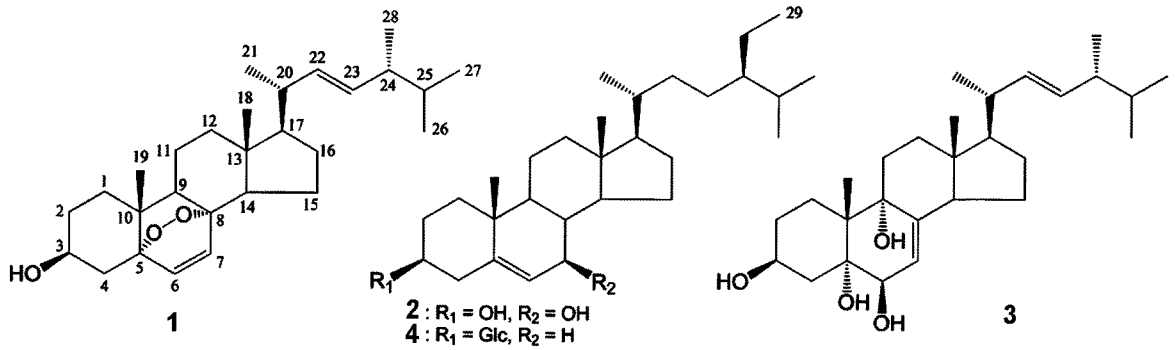

Fig. 1. Chemical structures of compounds **1–4** isolated from the *nuruk* (*Rhizopus oryzae* KSD-815).

5.25 (1H, dd, J=16.4, 8.0 Hz, H-22), and 5.18 (1H, dd, J=16.4, 7.6 Hz, H-23), the last two of which were due to an aliphatic chain double bond with a *trans*-configuration on the basis of the coupling constant (J=16.4 Hz) between two olefin protons. The ^{13}C NMR spectrum showed 28 carbon signals, which included one olefin quaternary carbon (δ_C 141.5), three olefin methine carbons (δ_C 120.4, 132.0, 136.1), two oxygenated quaternary carbons (δ_C 74.3, 76.1), two oxygenated methine carbons (δ_C 67.3, 73.8), and six methyl carbons (δ_C 12.6, 17.9, 19.8, 20.1, 22.4, 21.4). The location of each functional group was determined on the basis of heteronuclear single quantum correlation (HSQC) and HMBC. Two oxygenated methine carbon signals at δ_C 67.3 and 73.8 showed cross peaks with the proton signals at δ 4.40 (H-3) and 4.35 (H-6) in the HSQC spectrum. Three olefin methine carbon signals at 120.4, 132.0, and 136.1 were assigned to C-7, C-23, and C-22, respectively, because of their ^1J-correlations with their equivalent proton signals at δ 5.74 (H-7), 5.18 (H-23), and 5.25 (H-22) in the HSQC spectrum. One oxygenated quaternary carbon signal at δ_C 74.3 was assigned to C-5 because it correlated with an oxygenated methine proton signal (δ 4.35, H-6) through ^2J and with an olefin methine proton signal (δ 5.74, H-7) and a methyl proton signal (δ 1.53, H-19) through ^3J in the HMBC spectrum. Cross peaks observed for another oxygenated quaternary carbon signal at δ_C 76.1 with H-19 and H-7 through ^3J in the HMBC spectrum showed this oxygenated quaternary carbon to be C-9. An olefin quaternary carbon signal at δ_C 141.5 correlated with H-6 (^3J) as well as H-7 (^2J) in the HMBC spectrum, making it attributable to C-8. Thus, compound **3** was identified as ergosta-7,22-dien-3,5,6,9-tetraol. Its stereochemical characteristics were determined as 3β,5α,6β,9α-tetraol and 22-*trans*-ene by comparison of the spectroscopic and physicochemical data with those in the literature [16]. This finding is particularly valuable because naturally occurring steroids containing tetra-alcohols have rarely been observed. Compound **4** was isolated as a colorless crystal from MeOH–H_2O and exhibited a pseudomolecular ion peak $[M+H]^+$ at m/z 577 in the positive FABMS spectrum. IR absorption bands at 3,320 and 1,640 cm^{-1} were characteristic of hydroxyl and aromatic groups, respectively. The NMR spectra of compound **4** were identical to those of daucosterol, a well-known phytosterol glycoside. This was confirmed by comparison of the spectroscopic data for this sample including IR, mass spectrum, and ^1H and ^{13}C NMR data with those of an authentic sample and values reported in the literature [7].

To investigate the effect of the compounds on MDA-MB-231 and SK-HEP-1 cell migration, cancer cell motility was performed by wound-healing assay with a slight modification of the method of Albrecht-Buehler [1]. MDA-MB-231 human breast cancer cells and SK-HEP-1 human hepatocarcinoma cells were cultured in Dulbecco's modified Eagle's minimum essential medium (DMEM; Sigma, MO, U.S.A.) supplemented with 10% fetal bovine serum (FBS), penicillin (100 units/ml), and streptomycin (100 units/ml), which were purchased from Gibco-Invitrogen (NE, U.S.A.). All cell lines were incubated at 37°C in a humidified incubator at 5% CO_2. Confluent monolayers were scraped with a sterile 200-μl tip to create a scratch wound. After incubation with each compound for 24 h, the cells that migrated to the denuded zone were counted using a microscope. The cytotoxicity of each compound was first assayed on the human breast cancer cell line MDA-MB-231 and the human hepatocarcinoma cell line SK-HEP-1. Cell viability was measured in a dose-dependent manner by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay [10]. Cells treated with compounds showed no significant changes in viability compared with the no-treatment control cells, indicating that the compounds are not toxic to MDA-MB-231 and SK-HEP-1 cells at these doses. In the following experiments, each compound was evaluated for its inhibitory effect on the migration of cancer cells at various concentrations. As shown in Fig. 2A and 2B, compounds **1** and **2** inhibited the migration of and

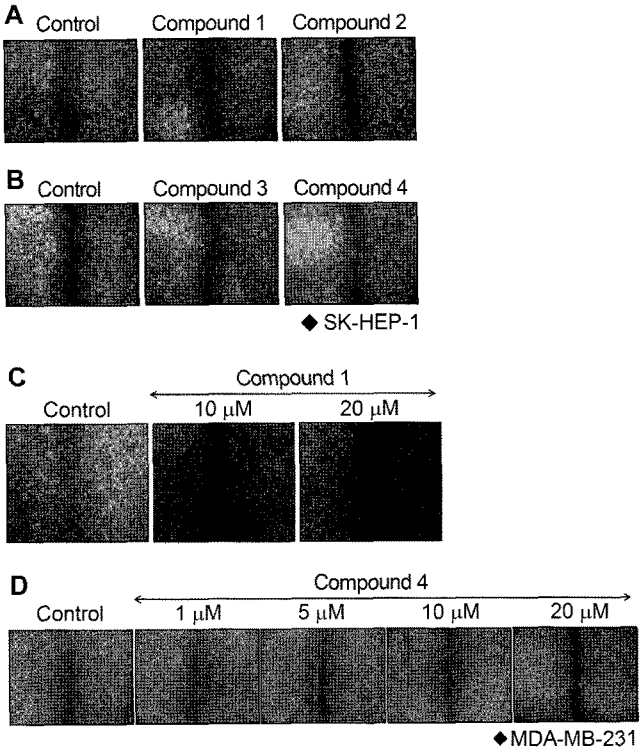

Fig. 2. Effect of each compound on the motility of SK-HEP-1 and MDA-MB-231 cells.

SK-HEP-1 or MDA-MB-231 cell monolayers were scratched with a sterile 200-μl tip and the cells were treated with each compound. **A** and **B**. SK-HEP-1 cells treated with 20 μM of compounds for 24 h, respectively. **C**. MDA-MB-231 cells treated with 10 or 20 μM of compound **1** for 24 h. **D**. MDA-MB-231 cells treated with 1, 5, 10, or 20 μM of compound **4** for 24 h.

SK-HEP-1 cells to some degree at the concentration of 20 μM. In particular, compounds **1** and **4** suppressed cell migration in MDA-MB-231 cells after 24 h incubation in a dose-dependent manner (Fig. 2C and 2D).

All compounds were isolated for the first time from *Rhizopus oryzae* KSD-815. Ergostane sterol, a C28 sterol, usually occurs in fungi or mushrooms, whereas stigmastane sterol, a C29 sterol, is found mainly in plants. The terminology "ergostane" was derived from the name of ergot, a fungal sclerotium found in some grains. Daucosterol (compound **4**), a β-sitosterol glucoside, must therefore come from the wheat flour used as culture media for *nuruk*, *Rhizopus oryzae* KSD-815. However, the other sterols, compounds **1–3**, have never been reported as components of wheat. Even though ergostane sterols from some plants have been reported [17], their presence was shown to be due to fungal contamination [11]. Compounds **1–3** were therefore concluded to be synthesized through oxidation or carbon-deletion of wheat sterols by *nuruk*, *Rhizopus oryzae* KSD-815. Metastasis is a multistep process that includes detachment of the tumor cells from their original location in the extracellular matrix (ECM) after degradation of ECM components, followed by their migration [8]. α-Chaconine, the main steroidal glycoalkaloid in potato sprouts, suppressed migration through inactivation of a PI3K/Akt pathway with downregulation of NF-κB in lung adenocarcinoma cell line A549 [13]. In this study, we demonstrated that steroid compounds from *Rhizopus oryzae* KSD-815 have antimigratory activity in MDA-MB-231 and SK-HEP-1 cells. Antimigratory effect in SK-HEP-1 cells was observed after 24 h in the presence of 20 μM of compounds **1** and **2**. Compounds **1** and **4** at 20 μM significantly inhibited MDA-MB-231 cell migration after 24 h. The migratory ability of cells can be increased further by the controlled degradation of extracellular matrix components by matrix metalloproteinases and serine proteases [6]. Further studies are required to determine which proteins mediate the antimigratory effects of compounds **1** and **4** on cancer cells; these compounds may be new anticancer drug candidates.

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