Novel Anthracene Derivatives Isolated from Rice Hulls of *Oryza sativa* and Their Growth Inhibitory Activity of Radish Seed

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Three new compounds orizaanthracenol (1-methoxyanthracen-2-ol, **1**), 1-hydroxy-7-((2*S*,3*R*,4*R*,5*S*)-2″,3″,4″-trihydroxy-5″-(hydroxymethyl)tetrahydro-2*H*-pyran-1-yloxy)anthracen-2-yl 3′,7′-dimethyloctanoate (**2**) and 1-hydroxy-7-((2*S*,3*R*,4*R*,5*S*)-2″,3″,4″-trihydroxy-5″-(hydroxymethyl)tetrahydro-2*H*-pyran-1-yloxy)anthracen-2-yl 3′,7′,11′,15′,19′-pentamethyltricosanoate (**3**) have been isolated and determined from the rice hulls of *Oryza sativa*. The structures of the compounds were elucidated using 1D and 2D NMR spectral methods, viz: ¹H-NMR, ¹³C-NMR, DEPT, COSY and HSQC, and HMBC aided by IR, EIMS and FAB-MS. These isolated compounds were tested in bioassay on germination of radish to detect their inhibitory activities, in which compound **1** exhibited the most inhibition at the concentration of 40 ppm was 50.96% of total dry weight. Furthermore, germination and growth of radish were reduced by 27 to 48%, and dry weight of shoot and root were arranged from 50 to 52%, whilst compounds **2** and **3** exhibited weak inhibition than **1**.

Key Words : *Oryza sativa* L., Rice hull compounds, Orizaanthracenol (1-methoxyanthracen-2-ol), 1-Hydroxy-7-((2*S*,3*R*,4*R*,5*S*)-2″,3″,4″-trihydroxy-5″-(hydroxymethyl)tetrahydro-2*H*-pyran-1-yloxy)anthracen-2-yl 3′,7′-dimethyloctanoate, 1-Hydroxy-7-((2*S*,3*R*,4*R*,5*S*)-2″,3″,4″-trihydroxy-5″-(hydroxymethyl)tetrahydro-2*H*-pyran-1-yloxy)anthracen-2-yl 3′,7′,11′,15′,19′-pentamethyltricosanoate

Introduction

In continuation of our study on rice hulls of *Oryza sativa* constituents, we reported new and known compounds of inhibitory and cytotoxic activities.[1-6] This papers deals with the isolation and characterization of three additional new compounds of anthracene derivatives **1-3** on the basis of using spectral data analysis and chemical reactions and inhibitory activity of compounds **1-3** against radish germination of shoot and root length.

Results and Discussion

Compound **1**, named orizaanthracenol, was obtained as yellow semi-soild compound from ethyl acetate extract. It responded for tests of phenolic compounds and had characteristic absorption bands in the IR spectrum for hydroxyl group at 3430 cm⁻¹ and aromatic ring at 1640, 1514 and 850 cm⁻¹. Its electron impact mass spectrum displayed important ion peaks at m/z 122 [C5, 6–C13, 14 fission]⁺, 107 [222–Me]⁺, 91 [122–OMe]⁺ and 76 [C10, 10a – C8a, 9 fission]⁺.

Fifteen peaks were observed in the ¹³C-NMR spectrum. Because the ¹³C peak at 56.44 ppm was assigned to be methoxyl carbon, aromatic compound composed of 14 carbons was suspected. In addition, IR data interpretation gives information about an existence of aromatic ring. A typical aromatic compound containing 14 carbons is anthracene, but its structure is symmetry so that its ¹³C-NMR

peaks should be 7 only. As mentioned before, because compound **1** includes methoxyl carbon, methoxyl anthracene can be expected. Its ¹³C-NMR spectrum exhibits carbon signals in the range *δ* 160.28–115.49 for anthracene carbons. The ¹H-NMR spectrum of **1** showed two 1H double doublets at *δ* 7.48 (*J* = 9.0, 2.4 Hz), and 6.75 (*J* = 8.52, 3.0 Hz), assigned to *ortho*- and *meta*- coupled H-8 and H-5, respectively. Two 1H doublets at *δ* 7.36 (*J* = 8.40 Hz), and 6.70 (*J* = 8.40 Hz), were accounted to *ortho*-coupled H–3 and H–4, respectively. Two 1H multiplets at *δ* 7.43 and 6.38 were accounted correspondingly to H–7 and H–6. The H–10 and H–9 protons appeared as a 2H broad signals at *δ* 6.78. A 3H sharp singlet at *δ* 3.31 as attributed to the methoxyl group. The multiplicity of these carbons were determined by DEPT experiments. In the COSY spectrum of **1**, correlations of aromatic protons were observed as H-3 with H-4, H-5 with H-6, H-7 and H-8, H-4 with H-3 and H-10 and H-9 with H-8 were observed. The HMBC spectrum of **1** displayed long-ranged correlations of H-3 with C-3, C-2, C-1 and C-4, H-10 with C-4, C-10a and C-5 and H-9 with C-9a, C-1, C-8a and C-8. The methoxyl protons exhibited correlation with C-1 and C-9a supporting the location of the methoxyl group at C-1. The direct correlations between protons and carbons were determined based on the interpretation of the HSQC spectrum. On the basis of the long-ranged correlations of HMBC, the structure of **1** has been elucidated as 1-methoxy-anthracen-2-ol.

Compound **2** named 1-hydroxy-7-((2*S*,3*R*,4*R*,5*S*)-2″,3″,4″-trihydroxy-5″-(hydroxymethyl)tetrahydro-2*H*-pyran-1-yloxy)-
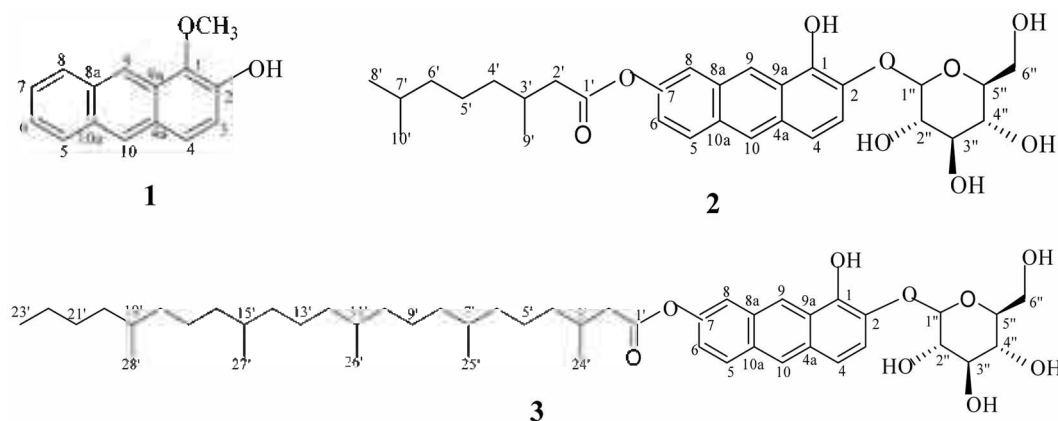
Figure 1. Structures and nomenclatures of compounds **1**, **2**, and **3**.

anthracen-2-yl 3',7'-dimethyloctanoate, was obtained as yellow semi-solid mass from ethyl acetate extract. It showed chemical tests of phenolic compounds and had IR absorptions bands for hydroxyl groups at 3420, 3390, and 3250 cm^{-1}, ester group at 1720 cm^{-1}, and aromatic ring at 1603, 1512 and 850 cm^{-1}. Its positive ion mode FAB-MS spectrum showed a molecular ion peak at m/z 543. The ion peaks at m/z 155 [C$_{10}$H$_{19}$O]$^{+}$, 163 [C$_6$H$_{11}$O5]$^{+}$, and 224 [M–155–163]$^{+}$.

The ^{13}C-NMR spectrum gives 14 peaks similar to those observed from compound **1**. Therefore, compound **2** contains anthracene moiety, but because methoxyl carbon is not found in the ^{13}C-NMR spectrum this compound does not have methoxyl group. Five ^{13}C peaks between 64 ppm and 76 ppm shows an existence of saccharide. Acid hydrolysis of **2** yielded D-glucose which was determined by a comparison of its authentic sample on TLC. From the interpretation of HSQC, the ^{1}H peaks attached directly to the ^{13}C peaks were determined. A 1H doublet at δ 6.70 Hz was accounted to

anomeric H–1". The other hydroxy methine and oxygenated methylene protons of the glucose moiety appeared in the range δ 3.87–3.34. The ^{1}H-NMR spectrum of **2** displayed three *ortho*-coupled doublets at δ 7.58 (J = 8.84 Hz), 7.45 (J = 8.82 Hz) and 6.74 (J = 8.84 Hz), all integrating for 1H each assigned to H–3, H–5 and H–4, respectively. Another 1H doublet at δ 7.51 (J = 1.8 Hz), was ascribed to *meta*-coupled H–8. A 1H double doublet at δ 7.37 with coupling interactions of 1.8 and 8.82 Hz was attributed to *ortho*-, *meta*-coupled H–6. Two broad signals at δ 6.77 and 6.63, integrated 1H each, were attributed to aromatic H–9 and H–10, respectively. In the ^{13}C-NMR spectrum, another 10 peaks except anthracene moiety and glucose were observed and they were attached directly to protons between 0.9 ppm and 2.3 ppm. Therefore, an existence of aliphatic chain was suspected. This chain contains three methyl groups observed at 14.41, 14.56, and 15.94 ppm which are attached to 1.28, 1.28, and 0.90 ppm, respectively. Four methylene carbons
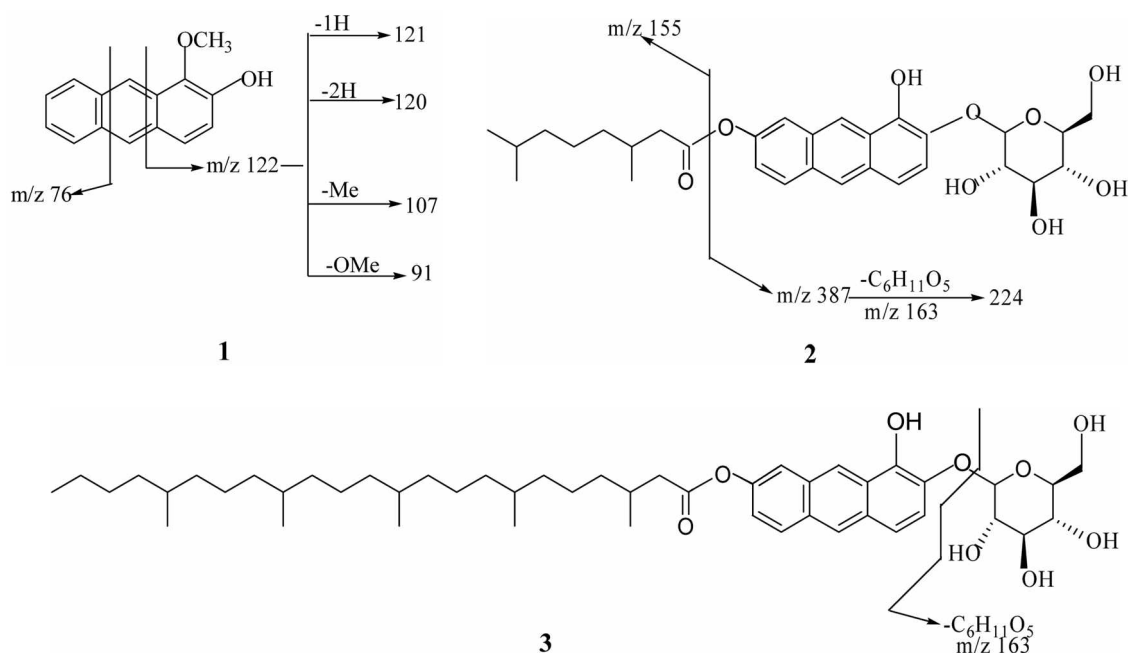

Figure 2. Fragmentation pattern of compounds **1**, **2**, and **3**.

were observed in this chain. The existence of the aliphatic chain was proved by the interpretation of FAB-MS (Fig. 2). The ^{13}C peak at 177.99 ppm shows a carboxyl group. From the HMBC spectrum, this carboxyl carbon is connected to a methylene group whose proton chemical shift is two 1H doublets at δ 2.28 ($J = 7.2$ Hz) and 2.26 ($J = 7.2$ Hz). Based on the interpretation of COSY, the partial structure of aliphatic chain was determined to be terpene moiety. In the HMBC spectrum, correlation of C-1' with H-2' and C-7' with H-8' were observed. Because the HMBC spectrum gave a long-ranged correlation between C-2 and anomeric proton of glucose, as shown in Figure 1, glucose is connected to 2-OH of anthracene. As a result, the structure of **2** has been elucidated as 1-hydroxy-7-((2S,3R,4R,5S)-2",3",4"-trihydroxy-5"-(hydroxymethyl)tetrahydro-2H-pyran-1-yloxy)anthracen-2-yl 3',7'-dimethyloctanoate .

Compound **3**, named 1-hydroxy-7-((2S,3R,4R,5S)-2",3", 4"-trihydroxy-5"-(hydroxymethyl)tetrahydro-2H-pyran-1-yloxy)anthracen-2-yl 3',7',11',15',19'-pentamethyltricosanoate, was obtained as a dark yellow gummy mass ethyl acetate extract. It gave positive tests of phenolic compounds and glycosides. Its mass spectrum displayed a molecular ion peak at m/z 795 ($C_{48}H_{75}O_9$) in FAB positive ion mode. Its electron impact mass spectrum showed important ion peaks at m/z 163 [$C_6H_{11}O_5$]$^+$, 179 [$C_6H_{11}O_6$]$^+$, 208 [$C_{14}H_8O_2$]$^+$, 223 [$C_{14}H_8O_3$]$^+$, 350 [$C_{28}H_{55}O$–57]$^+$, 335 [350–Me]$^+$, 318 [333–Me]$^+$, 303 [318–Me]$^+$, 290 [305–Me]$^+$, 275 [290–Me]$^+$, and 155 [$C_{11}H_{23}$]$^+$.

The ^{13}C-NMR spectrum of compound **3** showed more carbon peaks than that of compound **2**. Except 18 peaks, other ^{13}C peaks match with those of compound **2**. That is,

36.00 (C–9'), 35.07 (C–10'), 25.96 (C–11'), 34.13 (C–12'), 29.31 (C–13'), 29.35 (C–14'), 21.59 (C–15'), 29.68 (C–16'), 29.78 (C–17'), 27.37 (C–18'), 18.32 (C–19'), 25.49 (C–20'), 24.98 (C–21'), 22.73 (C–22'), 13.48 (C–23'), 21.40 (C–26'), 20.56 (C–27'), and 14.89 (C-28') were found. Their assignments were carried out according to the same manner as compound **2**. As a result, the structure of **3** has been elucidated as 1-hydroxy-7-((2S,3R,4R,5S)-2",3",4"-trihydroxy-5"-(hydroxymethyl)tetrahydro-2H-pyran-1-yloxy)anthracen-2-yl 3',7',11',15',19'-pentamethyltricosanoate.

Inhibitory effects of compounds on radish. The Table 1 showed the compound **1** was the most inhibition on radish growth. At the concentration of 40 ppm, compound **1** exhibited remarkably inhibition in total dry weight at 50.96%. Germination, root and shoot length were reduced by 27 to 48%, dry weight of shoot and root were about 50 to 52%, respectively. However, germination at the concentration of 10 ppm was slightly stimulated at 9.93% and less suppression in root and shoot length 10 to 12%. Compound **2** showed the suppression 40% in both total dry weight, root and shoot dry weight at the concentration of 40 ppm. At the concentrations of 30 and 10 ppm germination inhibition was 14.67% and 30.12%; 1.33% and 18.77 respectively in total dry weight and found less inhibition. Compound **3** showed the least suppression in all of dose concentrations, about 13% inhibition on germination and root length of radish and 33.46% in total dry weight at the concentration of 40 ppm. At the concentration of 10 ppm and 20 ppm compound **3** displayed a trivial suppression on root and shoot length of radish and slightly promoted root length at 17.09%. Overall, three compounds **1-3** at the concentrations of 10 ppm

Table 1. Inhibitory effects of compounds (**1-3**) at four concentrations (10, 20, 30 and 40 ppm) on seed germination, seedling length and dry weight of radish

Compounds	Conc. (ppm)	GP*	RL**	SL***	RDW****	SDW*****	TDW******
1	10	9.93	9.61	11.82	23.42	16.81	20.12
	20	6.67	14.75	21.94	38.37	31.00	34.68
	30	9.30	18.24	32.75	44.84	45.10	44.97
	40	26.67	29.18	47.58	50.70	51.23	50.96
CV (%)		99.20	13.68	6.86	6.45	1.70	3.58
LSD (0.05)		15.56	4.62	3.68	4.78	1.15	2.54
2	10	1.33	5.47	1.59	24.23	13.31	18.77
	20	10.67	9.78	11.48	30.90	22.78	26.84
	30	14.67	18.24	23.21	34.33	25.91	30.12
	40	18.00	23.54	28.03	39.99	40.18	40.08
CV (%)		48.23	13.06	13.28	5.95	5.31	4.92
LSD (0.05)		10.13	3.51	4.02	3.62	2.55	2.68
3	10	4.00	17.09	8.61	13.92	23.40	4.88
	20	5.33	1.82	11.94	29.88	25.66	18.77
	30	10.00	9.61	16.19	35.34	31.58	27.77
	40	13.00a	13.10	21.37	39.79	43.97	33.46
CV (%)		79.04	102.20	9.37	8.03	1.28	4.40
LSD (0.05)		12.15	3.58	2.56	4.49	0.75	2.52

*GP, germination percentage; **RL, root length; ***SL, shoot length; ****RDW, root dry weight; *****SDW, shoot dry weight; ******TSDW, total seedling dry weig

exhibited the negligible suppression on radish germination by 2 to 4%, and a little promoting in germination and shoot length However, increasing concentrations at 30 ppm and 40 ppm of test compounds showed greater inhibitory activity on radish growth. These are similar results to that of Nishimura[7] and Mitchell,[8] who observed that anthraquinoes, emodin, physcion, showed inhibitory activity against the seedling gowth of lettuce, green amaranth and timothy grass bioassay at 50 ppm and 100 ppm. However, we could not directly compare our results with the result of Nishimura[7] and Mitchell[8] because of different test concentrations and test species. It denotes that the toxic of all of these compounds were proportional with the concentrations and to be generally correlated with inhibitory effects on germination, growth of shoot root and total dry weight. A great amounts of anthraquinones were detected in dry fallen leaves more than 4 months after defoliation, these concentrations are able to suppress the plant seedlings.[7] Furthermore, presence of anthraquinone which is derivative from anthrancene naturally occurs in some plants has tended growth inhibitory activities and laxative effects.[7,8] Additionally, anthrancene combining to other polycyclic aromatic hydrocarbon is a persistant and toxic soil, environmental contaminant.[9,10] At present, worldwide the exploitation and utilization of allelopathy for controlling weeds are critically studied that could be either through directly using natural allelopathic interactions or by using the secondary compounds (allelochemicals) as natural herbicides.[11] Allelochemicals are present in all types of plant tissues and are released into soil rhizosphere by a variety of mechanisms, including decomposition of residues, volatilization and root exudation.[12] Furthermore, numerous of allelochemicals belonging to chemical classes of resorcinol, flavone, hydroxamic acid, phenols, phenolic acids, indoles, terpenic acid, fatty acid, terpenoid were identified and were responsible for allelopathic activity.[1,13,14] These tested compounds showed various both inhibition and slightly stimulation. Especially, the compound **1**, was first identified from rice hulls and exhibited greatest activity, and may be used for reducing weed interference and diminishing the dependency on synthetic agricultural chemicals, labor cost and environmental pollution concern. However, utilizing those compounds in practice, it needs to be further study and examine accurately how much (ppm) concentration can suppress weeds in natural condition and whether those concentrations may induce environmental contamination with their toxicity.

Experimental Section

Chemicals. All chemicals were used of analytical grade: hexane, ethyl acetate, methanol, ethanol, sulfuric acid, vanillin were purchased from Daejung Chemicals & Metals Co., Ltd, Korea. Precoated TLC plates (layer thickness 0.25 mm) and silica gel column chromatography (70-230 mesh ASTM) and LiChroprep RP-18 (40-63 μm) were from Merck (Darmstadt, Germany). Authentic samples of acid were purchased from Sigma-aldrich (USA).

Instruments. Melting points were determined on Electrochemical Eng. melting point apparatus. Optical rotation was measured on an AA-10 model polarimeter. Both ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were obtained with a Brucker Avance (DRX-600) spectrometer operating at 600 and 150 MHz, respectively. NMR spectra were obtained in deuterated methanol, using tetramethylsilane (TMS) as internal standard, with chemical shifts expressed in parts per million (δ) and coupling constants (J) in hertz. The ^1H NMR, ^{13}C NMR, DEPT, COSY, HSQC, and HMBC spectra were collected in MeOH-d₄ solution. For the ^1H NMR analyses, 8 transients were acquired with a 1 s relaxation delay using 64 K data points. The 90° pulse duration was of 15 μs, spectral width was 8,741 Hz. The ^{13}C NMR and DEPT spectra were obtained with a spectral width of 36,232 Hz using 64 K data points. Their 90° pulse duration was of 15 μs. All two-dimensional spectra were acquired with 1 K data points for t_2 increments and 256 for t_1 increments. The long-range coupling delay for HMBC was 50 ms. Prior to Fourier transformation, zero filling of 1 K and sine window functions were applied using XWIN-NMR (Bruker, Karlsruhe, Germany). EI-Mass spectra were recorded on a JEOL JMS-SX 102 A spectrometer and FABMS on a JEOL JMS-AX 505 WA, FABMS matrix is used metanitrobenzyl alcohol. IR spectra were recorded on a Thermo Mattson 60-AR spectrophotometer.

Plant material. The cultivar of *O. sativa* grown at Konkuk University experimental field in Korea, were harvested in October, 2002. The hulls from harvested plants were separated by milling machine and dried at room temperature (25 °C) for 7d, and then dried rice hulls were ground or powdered using a Wiley mill through a 40-mesh screen. The voucher specimen of hulls (No. KKU 121, Ilpumbyeo) has been deposited in the herbarium of our department.

Extraction of rice hulls. The powered hulls of *O. sativa* (10 kg) were immersed in MeOH (60 litre) for a week at room temperature and then concentrated in vacuo to yield extract (150 g). This material was suspended in water and extracted with EtOAc (1.5 litre) and *n*-BuOH (1.0 litre) successively to produce ethyl acetate (35 g) and butanol (19 g) extract.

Isolation of compounds from ethyl acetate extract. The entire ethyl acetate extract was subjected to normal phase column chromatography (CC) over silica gel (800 g, column 130 cm × 25 mm dia) to yield 40 fractions (each of 500 mL) with the following eluants: fraction 1 with *n*-hexane, fractions 2–5 with *n*-hexane : ethyl acetate (9 : 1), fractions 6–11 with *n*-hexane : ethyl acetate (8 : 2), fractions 12–15 with *n*-hexane : ethyl acetate (7 : 3), fractions 16–20 with *n*-hexane : ethyl acetate (1 : 1), fractions 21–22 with ethyl acetate, fractions 23–28 with ethyl acetate : methanol (9.5 : 0.5), fractions 29–30 with ethyl acetate : methanol (9 : 1), fractions 31–36 with ethyl acetate : methanol (7 : 3), and fractions 37–40 with methanol. All fractions were examined by TLC before mixing. From fractions 1, 2-5, 6, 11, 12 and 23 were subjected to CC and obtained known and new compounds, which already discussed in our previous

work.[1-6] The fractions 29–30 of 1st column from ethyl acetate extract was re-chromatographed over silica gel (150 g, column 60 cm × 10 mm dia) to yield five fractions with the following eluants: fraction 1 with chloroform, fraction 2–3 with $CHCl_3$:MeOH (9.8 : 0.2), fractions 4–5 with $CHCl_3$: MeOH (9.5 : 0.5) and fraction 6 with $CHCl_3$: MeOH (9 : 1). Fractions 4-5 showing same on reverse phase tlc with methanol/water (8 : 2), after mixing and purified by CC over Lichroprep RP-18 (ODS silica gel, 40-63 μm; 50 g; 40 cm × 10 mm dia) each fraction 50 mL) eluted sequentially with methanol/water with following eluants: fractions 1-2 with $MeOH:H_2O$ (2 : 8), fractions 3-4 with $MeOH:H_2O$ (4 : 6), fractions 5-6 with $MeOH:H_2O$ (6 : 4), fractions with 7-8 $MeOH:H_2O$ (8 : 2) and finally eluted with MeOH. Compound **1** (16 mg), was obtained from fractions 3 and compounds **2** (13 mg) and **3** (24 mg) were obtained from fractions 7-8 in small quantities.

Inhibitory activity determination method. Three isolated compounds (**1-3**) were used in this experiment to evaluate their inhibitory activities on radish seeds. Radish (*Raphanus sativus*) seeds were selected for bioassay, because the suppression imposed on radish is greater than on other plant and is much sensitive at low chemical concentrations. Empty and undeveloped radish seeds were discarded by floating in distilled water, and stored at −35 °C until used for bioassays. Three isolated compounds were dissolved in water at four doses concentrations at 10, 20, 30 and 40 ppm, respectively. 50 seeds of radish were placed on Petri dish (9 cm in diameter) lined with filter paper (Whatman No. 42) and added with 10 mL of diluted solvent of three compounds in each, respectively. Treatments with distilled water were used as the control. All Petri dishes were transferred into growth chamber under following condition: 25 °C, humidity of 75%, lighted time of 9.00–17.00. Germination, shoot and root length after 4 days were calculated for all treatments. After the germination percentage was determined, the seedlings were oven-dried at 65 °C for 4 h for dry weight determination. The inhibition percentage was as follows:

Inhibition percentage (%) =
[1–(sample extracts)/control] × 100

Statistical analysis. All recorded data were analyzed using SAS version 6.12 (SAS Institute, 1997).[15] All of the treatments were replicated thrice with completely randomized design. The pooled mean values were separated based on the least significant difference (LSD) at the 0.05 probability level.

1-Methoxyanthracen-2-ol (1). Yellow semi-solid; $[\alpha]_D^{25}$ +8.9 (*c* 0.12); IR (KBr) ν_{max}: 3430, 2950, 2845, 1640, 1605, 1514, 1384, 1250, 1180, 1115, 1030, 850 cm^{-1}; ^{1}H-NMR (600 MHz, MeOH-d$_4$): δ 7.48 (1H, dd, J = 9.0, 2.4 Hz, H–8), 7.43 (1H, m, H–7), 7.36 (1H, d, J = 8.4 Hz, H–3), 6.78 (2H, br s, H–10, H–9), 6.75 (1H, dd, J = 8.52, 3.0 Hz, H–5), 6.70 (1H, d, J = 8.40 Hz, H–4), 6.38 (1H, m, H–6), 3.31 (3H, s, OMe); ^{13}C-NMR (150 MHz, MeOH-d$_4$): δ 160.28 (C–1), 158.60 (C–2), 131.98 (C–3), 121.11 (C–4), 143.18 (C–4a),

116.75 (C–10), 128.34 (C–10a), 115.92 (C–5), 115.65 (C–6), 115.49 (C–7), 130.55 (C–8), 129.21 (C–8a), 116.75 (C–9), 148.35 (C–9a), 56.44 (OMe); EI-MS m/z (rel. int.) 224 [M]$^+$ ($C_{15}H_{12}O_2$) (not observed), 164 (23.6), 151 (18.5), 122 (8.0), 107 (7.6), 91 (17.0), 76 (5.5); FABMS (positive mode) m/z 225 [M+H]$^+$ ($C_{15}H_{13}O_2$).

1-Hydroxy-7-((2S,3R,4R,5S)-2'',3'',4''-trihydroxy-5''-(hydroxymethyl)tetrahydro-2H-pyran-1-yloxy)anthracen-2-yl 3',7'-dimethyloctanoate (2). Yellow semi-solid; $[\alpha]_D^{25}$ +18.9 (*c* 0.18); IR (KBr) ν_{max}: 3420, 3390, 3250, 2933, 2845, 1720, 1603, 1512, 1459, 1382, 1241, 1165, 1135, 850 cm^{-1}; ^{1}H-NMR (600 MHz, MeOH-d$_4$): δ 7.58 (1H, d, J = 8.84 Hz, H–3), 7.51 (1H, d, J = 1.8 Hz, H–8), 7.37 (1H, dd, J = 1.8, 8.82 Hz, H–6), 7.45 (1H, d, J = 8.82 Hz, H–5), 6.74 (1H, d, J = 8.84 Hz, H–4), 6.77 (1H, s, H–9), 6.63 (1H, s, H–10), 6.70 (1H, d, J = 7.2 Hz, H–1''), 3.87 (1H, m, H-5''), 3.84 (1H, d, 7.9 Hz, H–2''), 3.81 (1H, m, H–3''), 3.77 (1H, m, H–4''), 3.34 (2H, d, 4.3 Hz, hydroxy methylene H), 2.28 (1H, d, J = 7.2 Hz, H$_2$–2'a), 2.26 (1H, d, J = 7.2 Hz, H$_2$–2'b), 2.05 (1H, m, H–3'), 1.78 (1H, m, H–7'), 1.53 (2H, br s, H$_2$–4'), 1.38 (2H, m, H$_2$–5'), 1.29 (2H, m, H$_2$–6'), 1.28 (6H, br s, Me–8', Me–10'), 0.90 (3H, d, J = 6.0 Hz, Me–9'); ^{13}C-NMR (150 MHz, MeOH-d$_4$): δ 161.41 (C–1), 146.58 (C–2), 131.18 (C–3), 116.00 (C–4), 137.48 (C–4a), 115.18 (C–10), 133.40 (C–10a), 127.11 (C–5), 116.99 (C–6), 154.31 (C–7), 146.92 (C–8), 149.14 (C–8a), 111.84 (C–9), 147.12 (C–9a), 105.08 (C–1''), 75.41 (C–2''), 71.26 (C–3''), 66.61 (C–4''), 77.70 (C–5''), 64.19 (hydroxy methylene C), 177.99 (C–1'), 34.21 (C–2'), 56.52 (C–3'), 26.23(C–4'), 29.03 (C–5'), 23.84 (C–6'), 48.17 (C–7'), 14.56 (C–8'), 15.94 (C–9'), 14.41 (C–10'); EI-MS m/z: 542 [M]$^+$ (not observed), 379 [M– $C_6H_{11}O_5$]$^+$ (1.4), 378 (12.9), 334 (19.9), 318 (46.3), 289 (37.2), 271 (34.4), 259 (36.7), 224 (27.9), 203 (39.7), 185 (50.8), 163 (52.1), 155 (83.2), 135 (72.4), 109 (88.4), 95 (96.2), 81 (94.1), 55 (100); FABMS (positive ion mode) m/z 543 [M +H]$^+$ ($C_{30}H_{39}O_9$).

1-Hydroxy-7-((2S,3R,4R,5S)-2'',3'',4''-trihydroxy-5''-(hydroxymethyl)tetrahydro-2H-pyran-1-yloxy)anthracen-2-yl 3',7',11',15',19'-pentamethyltricosanoate (3). Dark yellow semi-solid; $[\alpha]_D^{25}$ +28.1 (*c* 0.23); IR ν_{max} (KBr): 3421, 3380, 3260, 2933, 2855, 1725, 1640, 1602, 1512, 1460, 1269, 1164, 1125, 1045 cm^{-1}; ^{1}H-NMR (600 MHz, MeOH-d$_4$): δ 7.36 (1H, d, J = 7.5 Hz, H-3), 6.98 (1H, d, J = 8.64 Hz, H–5), 6.93 (1H, d, J = 8.64 Hz, H–4), 6.87 (1H, br s, H–9), 6.84 (1H, br s, H–10), 6.78 (1H, dd, J = 3.04, 8.64 Hz, H–6), 6.76 (1H, d, J = 3.04 Hz, H–8), 5.02 (1H, d, J = 7.02 Hz, H–1''), 3.87 (1H, d, J = 8.04 Hz, H–2''), 3.79 (1H, m, H–3''), 3.81 (1H, m, H–4''), 3.84 (1H, m, H–5''), 3.34 (2H, br d, 4.6 Hz, hydroxy methylene H), 2.59 (1H, m, H-3'), 2.45 (1H, m, H–7'), 2.24 (1H, d, J = 7.2 Hz, H$_2$–2' a), 2.07 (1H, d, J = 7.2 Hz, H$_2$–2' b), 2.05 (1H, m, H–1'), 1.83 (1H, m, H–15'), 1.71 (1H, m, H–19'), 1.66 (2H, m, H$_2$–4'), 1.54 (4H, m, 2 x CH$_2$), 1.32 (2H, m, CH$_2$), 1.27 (14H, m, 7 x CH$_2$), 1.18 (2H, m, CH$_2$), 1.14 (2H, m, CH$_2$), 1.20 (2H, m, CH$_2$), 1.05 (2H, m, CH$_2$), 0.99 (3H, d, J = 6.0 Hz, Me–24'), 0.96 (3H, d, J = 6.1 Hz, Me–25'), 0.92 (3H, d, J = 6.2 Hz, Me–26'), 0.89 (3H, d, J = 7.28 Hz, Me–27'), 0.76 (3H, d, J =

6.1 Hz, Me–28'), 0.74 (3H, t, J = 7.38 Hz, Me–23'); ^{13}C-NMR (150 MHz, MeOH-d$_4$): δ 160.28 (C–1), 146.11 (C–2), 130.30 (C–3), 115.92 (C–4), 144.63 (C–4a), 114.93 (C–10), 132.74 (C–10a), 126.02 (C–5), 115.20 (C–6), 153.20 (C–7), 147.27 (C–8), 148.01 (C–8a), 110.74 (C–9), 147.70 (C–9a), 104.11 (C–1"), 73.04 (C–2"), 70.15 (C–3"), 65.51 (C–4"), 76.14 (C–5"), 63.09 (hydroxy methylene C), 176.92 (C–1'), 43.81 (C–2'), 33.14 (C–3'), 42.76 (C–4'), 39.48 (C–5'), 38.23 (C–6'), 31.69 (C–7'), 37.44 (C–8'), 36.00 (C–9'), 35.07 (C–10'), 25.96 (C–11'), 34.13 (C–12'), 29.31 (C–13'), 29.35 (C–14'), 21.59 (C–15'), 29.68 (C–16'), 29.78 (C–17'), 27.37 (C–18'), 18.32 (C–19'), 25.49 (C–20'), 24.98 (C–21'), 22.73 (C–22'), 13.48 (C–23'), 27.79 (C–24'), 21.82 (C–25'), 21.40 (C–26'), 20.56 (C–27'), 14.89 (C–28'); EI-MS m/z (rel. int.): 350 [C$_{28}$H$_{55}$O– 57]$^+$ (4.4), 334 (12.7), 318 (33.5), 289 (50.8), 275 (36.5), 203 (31.9), 185 (49.3), 179 (26.1), 167 (46.6), 163 (45.1), 155 (73.1), 121 (86.7), 109 (99.4), 95 (100), 81 (91.3), 69 (73.9), 55 (96.8); FAB-MS (positive mode) m/z 795 [M+H]$^+$ (C$_{48}$H$_{75}$O$_9$).

Acid hydrolysis of 2. Compound **2** (4 mg) was refluxed with 2 mL of 1 M hydrochloric acid : dioxane (1 : 1, v/v) in a water bath for 4 h. The reaction mixture was evaporated to dryness and partitioned between chloroform and water four times. The chloroform extract was concentrated and contained the aglycone portion whilst the water extract contained D-glucose (co-chromatographed on TLC with authentic sample).

Acid hydrolysis of 3. Compound **3** (5 mg), was refluxed with 1 mL of 1 M hydrochloric acid : dioxane (1 : 1, v/v) in a water bath for 4 h. The reaction mixture was evaporated to dryness and partitioned between chloroform and water four times. The chloroform extract was concentrated and contained the aglycone portion whilst the water extract contained D-glucose (co-chromatographed on TLC with authentic sample).

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