Sesquiterpenes from Rhizomes of *Cyperus rotundus* with Cytotoxic Activities on Human Cancer Cells *in vitro*

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Two new sesquiterpenes, cyperusol A_3 (**1**) and 3β-hydroxycyperenoic acid (**2**), along with three known sesquiterpenes, britanlin E (**3**), 1β,4α-dihydroxyeudesm-11-ene (**4**), and 11,12-dihydroxyeudesm-4-en-3-one (**5**), were isolated from the AcOEt-soluble fraction of rhizomes of *Cyperus rotundus* L. The structures of **1** and **2** were elucidated by physical and spectroscopic methods (^{1}H- and ^{13}C-NMR, 2D-NMR, and MS). All of the isolates, **1**–**5**, were evaluated for their cytotoxic activities against human ovarian cancer cells (A2780) and endometrial adenocarcinoma cells (*Ishikawa*) using MTT assays.

Introduction. – Rhizomes of *Cyperus rotundus* L. (Cyperaceae) have been used in traditional Chinese medicine as estrogenic and anti-inflammatory agent for the treatment of women's diseases and also for the treatment of stomach ache, bowel disorders, and menstrual disorders [1]. Previous phytochemical investigations on *C. rotundus* have resulted in the isolation of a series of sesquiterpenes possessing diverse skeletons [2–6], as well as sesquiterpene alkaloids, triterpenes, sterols, flavonoids, and stilbenes [6–9]. The extract of rhizomes of *C. rotundus* has shown a broad range of biological activities, such as antidiabetic activity [10] and acetylcholinesterase inhibition [11]. In addition, this herb is used in the treatment of gynecological diseases such as uterine fibroids, ovarian cysts, and uterine and cervical cancer in China [12]. A recent study has demonstrated that the polyherbal Chinese formulation Sojucktang containing rhizomes of *C. rotundus* induced apoptosis in human endometrial cancer cells [13]. In a study which used neuroblastoma neuro-2a cells for screening of plants with antitumor activities from 374 natural and plant sources, the EtOH extract of rhizomes of *C. rotundus* showed a moderate cell growth inhibitory effect [14]. However, compounds with antitumor effects in the rhizomes of *C. rotundus* are poorly understood. As a part of our ongoing project to search for novel and plant-derived antitumor agents, an AcOEt-soluble fraction from rhizomes of *C. rotundus* was shown to exhibit significant cytotoxic activities against human ovarian cancer cells (A2780) and endometrial adenocarcinoma cells (*Ishikawa*) with observed IC_{50} values of 74.60 and 177.61 μg ml^{-1}, respectively, in a preliminary *in vitro* screening. To identify active compounds, repeated chromatography of the AcOEt-soluble fraction from rhizomes of *C. rotundus* was performed and led to the isolation of two new sesquiterpenes, **1** and **2**, together with three already known sesquiterpenes, **3**–**5** (*Fig. 1*). Thereafter, the above

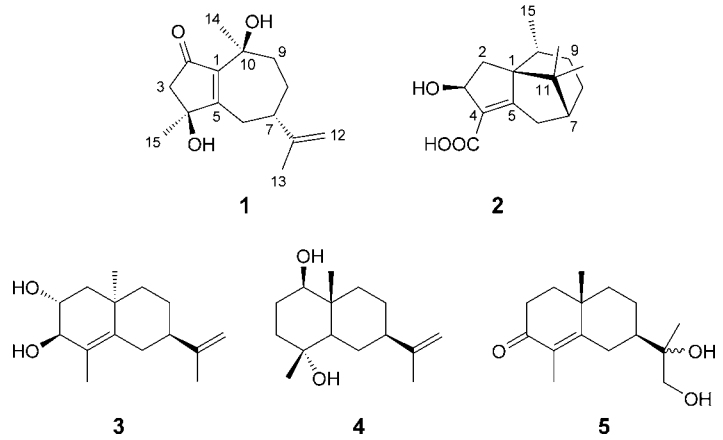

Fig. 1. *Structures of* **1**–**5** *isolated from rhizomes of* C. rotundus

mentioned compounds were evaluated for their cytotoxic activities against human ovarian cancer cells (A2780) and endometrial adenocarcinoma cells (*Ishikawa*) using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2*H*-tetrazolium bromide (MTT) assays. The structure elucidation of the new sesquiterpenes, **1** and **2**, and the biological evaluation of all isolates are described herein.

Results and Discussion. – *Isolation and Structure Elucidation.* Dried rhizomes of *C. rotundus* were extracted with 80% EtOH at room temperature by maceration to give an 80%-EtOH extract. The AcOEt-soluble fraction of the 80%-EtOH extract from rhizomes of *C. rotundus* was subjected to a series of column chromatography (silica gel, reversed-phase silica gel, *Sephadex LH-20*, silica-MPLC, and C_{18}-MPLC) to afford two new sesquiterpenes, cyperusol A_3 (**1**) and 3β-hydroxycyperenoic acid (**2**), along with three known sesquiterpenes, britanlin E (**3**), 1β,4α-dihydroxyeudesm-11-ene (**4**), and 11,12-dihydroxyeudesm-4-en-3-one (**5**).

Compound **1** was obtained as white amorphous powder. Its HR-DART-MS showed a *pseudo*-molecular-ion peak at *m*/*z* 233.1540 ($[M+H-H_2O]^+$, $C_{15}H_{21}O_2^+$; calc. 233.1536), indicating the molecular formula $C_{15}H_{22}O_3$. The IR spectrum of **1** showed absorption bands due to OH (3381 cm^{-1}), conjugated C=O, and olefinic CH_2 groups (1676, 1630, and 892 cm^{-1}). The ^{1}H-NMR spectrum of **1** (*Table 1*) showed the presence of three tertiary Me (δ(H) 1.72 (*dd*, *J* = 1.5, 1.0, Me(13)), 1.57 (*s*, Me(14)), and 1.52 (*s*, Me(15))) and an olefinic CH_2 group (4.81 (*dq*, *J* = 1.0, 1.0) and 4.77 (*dq*, *J* = 1.5, 1.5), both CH_2(12)). The ^{13}C-NMR spectrum of **1** (*Table 2*), in combination with a DEPT experiment, allowed the identification of 15 C-atoms: three Me, four CH_2, and one CH group, and six C_q-atoms, indicating one C=O (δ(C) 208.4) and four sp^2 C-atoms (175.7, 150.9, 143.2, and 109.6), and two sp^3 C_q-atoms linked to an O-atom (75.1 and 73.5). The connectivities of CH_2(6)/H–C(7), H–C(7)/CH_2(8), and CH_2(8)/CH_2(9) were revealed by ^{1}H,^{1}H-COSY experiments (*Fig. 2*), and the long-range correlations which are shown in *Fig. 2*, could be confirmed through analysis of the HMBC spectrum. These data suggested that **1** is a guaiane-type sesquiterpene with two OH groups at C(4) and

Table 1. *^{1}H-NMR Data* (500 MHz) *for* **1**[a])

Position	**1**[b])	Cyperusol A_1[b]) [15]	Cyperusol A_2[b]) [15]
3	3.02 (*d*, *J* = 18.0), 2.80 (*d*, *J* = 18.0)	2.98 (*d*, *J* = 18.0), 2.77 (*d*, *J* = 18.0)	2.93 (*d*, *J* = 18.0), 2.84 (*d*, *J* = 18.0)
6	3.12 (*ddd*, *J*= 16.0, 2.0, 2.0), 2.53 (*dd*, *J* = 16.0, 2.0)	3.01 (br. *d*, *J* ≈ 17), 2.68 (*dd*, *J* = 16.5, 11.3)	2.98 (*dd*, *J* = 16.4, 10.0), 2.78 (*dd*, *J* = 16.4, 2.8)
7	2.25 (*dddd*, *J*= 12.5, 10.5, 2.0, 2.0)	2.41 (*m*)	2.50 (*m*)
8	1.91–1.95 (*m*), 1.67–1.70 (*m*)	1.82 (*m*), 1.74 (*m*)	1.88 (*m*, 2 H)
9	2.03 (*ddd*, *J* = 14.0, 7.5, 2.0), 1.96 (*ddd*, *J* = 14.0, 11.5, 2.5)	2.31 (*ddd*, *J* = 13.4, 12.8, 6.1), 1.89 (*ddd*, *J* = 13.4, 4.6, 4.3)	2.27 (*ddd*, *J* = 13.2, 12.5, 6.1), 1.85 (*m*)
10	–	–	–
12	4.81 (*dq*, *J* = 1.0, 1.0), 4.77 (*dq*, *J* = 1.5, 1.5)	4.77 (br. *s*), 4.75 (br. *s*)	4.97 (br. *s*), 4.80 (br. *s*)
13	1.72 (*dd*, *J* = 1.5, 1.0)	1.69 (br. *s*)	1.72 (*s*)
14	1.57 (*s*)	1.59 (*s*)	1.70 (*s*)
15	1.52 (*s*)	1.47 (*s*)	1.57 (*s*)

[a]) The assignments were based on ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments. [b]) Measured in (D_5)pyridine.

Table 2. *^{13}C-NMR Data* (125 MHz) *for* **1**[a])

Position	**1**[b])	Cyperusol A_1[b]) [15]	cyperusol A_2[b]) [15]
1	143.2	143.7	143.5
2	208.4	207.6	206.9
3	52.7	52.7	52.7
4	75.1	75.0	75.4
5	175.7	177.0	176.3
6	32.4	28.5	29.8
7	46.1	43.8	44.3
8	30.6	28.0	27.9
9	41.5	37.6	37.9
10	73.5	71.6	71.8
11	150.9	150.2	150.3
12	109.6	109.9	110.4
13	20.4	19.9	20.2
14	28.3	27.5	27.8
15	26.7	26.6	26.9

[a]) The assignments were based on ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments. [b]) Measured in (D_5)pyridine.

C(10). The construction of **1** was identical with cyperusols A_1 and A_2 which were isolated from *Cyperus longus* [15] and which are epimers at C(4) ((4*R*) and (4*S*), resp.). However, we found that the 1D-NMR spectra (^{1}H- and ^{13}C-NMR) of **1** differ from those of cyperusols A_1 and A_2 in some points even though the NMR solvent used was the same (D_5)pyridine; *Tables 1* and *2*), indicating that **1** is a diastereoisomer of either cyperusol A_1 ((4*R*,7*R*,10*S*)) or cyperusol A_2 ((4*S*,7*R*,10*S*)). The relative configuration of **1** was confirmed by the observed correlations in the ROESY spectrum (*Fig. 3*).

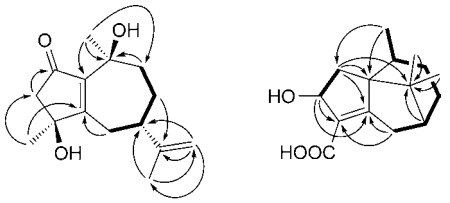

Fig. 2. *Key correlations observed in the* $^{1}H,^{1}H$*-COSY* (—) *and HMBC* (H → C) *spectra of* **1** *and* **2**

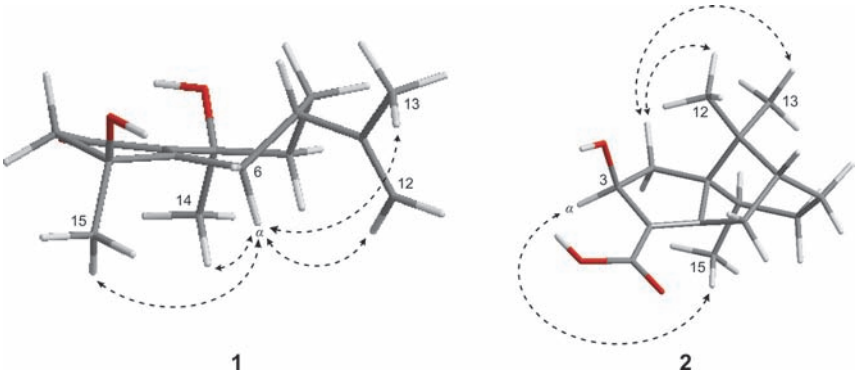

Fig. 3. *Key correlations observed in the ROESY spectra of* **1** *and NOESY spectra of* **2**

H_α–C(6) showed correlations with CH_2(12), Me(13), Me(14), and Me(15). The correlations CH_2(12)/Me(14) and CH_2(12)/Me(15) were revealed. H_β–C(6) also showed correlations with CH_2(12), Me(13), and Me(15) due to spatial distances within 4 Å, as supported by simulating with ChemDraw 3D Ultra (version 11.0), but no correlation with Me(14). In consideration of the ROESY correlations observed, we suggested that H_α–C(6), CH_2(12), Me(13), Me(14), and Me(15) are on the same side of the molecule, thus compound **1** is a diastereoisomer of cyperusol A_2 at C(7). Accordingly, the absolute configuration of **1** is proposed as (4*S*,7*S*,10*S*) on the basis of ROESY correlations and the known absolute configuration of cyperusol A_2. Also, these assignments were supported by the optical rotation of **1** ($[\alpha]_D^{23} = -126.9$ ($c =$ 0.025, MeOH)), which is different from cyperusols A_1 and A_2 ($[\alpha]_D^{23} = +12.5$ ($c = 0.5$, MeOH) and $[\alpha]_D^{23} = +32.1$ ($c = 0.3$, MeOH), resp. [15]). Thus, **1** was determined to be (4*S*,7*S*,10*S*)-4,10-dihydroxy-2-oxoguaia-1(5),11-diene (cyperusol A_3).

Compound **2** was obtained as viscous brownish oil. Its HR-DART-MS showed a *pseudo*-molecular-ion peak at *m*/*z* 233.1546 ($[M + H - H_2O]^+$, $C_{15}H_{21}O_2^+$; calc. 233.1536), indicating the molecular formula of $C_{15}H_{22}O_3$. The IR spectrum of **2** showed absorption bands due to OH (3403 cm^{-1}) and COOH (1686 cm^{-1}) groups. The ^{1}H-NMR spectrum of **2** (*Table 3*) revealed some signals common to patchoulane-type sesquiterpenes, namely the two geminal Me groups Me(12) and Me(13) (δ(H) 0.94 and 0.93, resp.), the Me group Me(15) at 0.74, appearing as a *doublet* ($J = 6.5$) and coupling with the H-atom of H–C(10) (1.97, *dq*, $J = 12.5, 6.5$). The ^{13}C-NMR and DEPT spectra

of **2** allowed the identification of 15 C-atoms: three Me, four CH_2, and three CH groups, and five C_q-atoms. The chemical shifts of the five C_q-atoms indicated one COOH (δ(C) 168.9), two aliphatic (65.4 and 42.2), and two sp^2 C-atoms (129.6 and 167.7; *Table 4*). The presence of a COOH group in **2** was supported by its HR-DART-MS spectrum which showed a fragment-ion peak at *m/z* 205.1590 ($[M+H-H_2O-CO]^+$). The H- and C-atom signals in the ^{1}H- and ^{13}C-NMR spectra of **2** exhibited strong similarities with those of cyperenoic acid, which was isolated from the same plant previously (*Tables 3* and *4*) [16]. Comparison of the ^{1}H- and ^{13}C-NMR spectra of **2** with those of cyperenoic acid readily indicated the presence of an additional OH group in **2** by observation of a CH–O H-atom at δ(H) 5.73 and a CH–O C-atom at δ(C) 81.0. Also, the IR spectrum of **2** evidenced the presence of the OH group (3403 cm^{-1}), which was not existent in cyperenoic acid [17]. These assignments

Table 3. *^{1}H-NMR Data* (500 MHz) *for* **2**[a])

Position	**2**[b])	Cyperenoic acid[c]) [16]
2	2.15 (*dd*, *J* = 13.0, 6.5),	1.76 (*ddd*, *J* = 13.0, 10.0, 10.0),
	1.92 (*dd*, *J* = 13.0, 6.5)	1.54 (*dd*, *J* = 13.0, 8.0)
3	5.73 (*dd*, *J* = 6.5, 6.5)	2.68 (*dd*, *J* = 14.5, 10.0), 2.79–2.84 (*m*)
6	3.09 (*dd*, *J* = 19.5, 6.0),	2.73 (*dd*, *J* = 19.0, 6.5),
	2.42 (*dd*, *J* = 19.0, 2.0)	2.26 (br. *d*, *J* = 19.0)
7	1.78 (*ddd*, *J* = 12.5, 6.0, 2.0)	1.96 (*ddd*, *J* = 6.5, 3.5, 2.0)
8	1.27 (*ddd*, *J* = 13.0, 6.0, 3.0),	1.88 (*dddd*, *J* = 13.0, 13.0, 6.5, 2.0),
	1.27 (*ddd*, *J* = 13.0, 13.0, 6.5)	1.36 (*ddd*, *J* = 13.0, 6.5, 3.5)
9	1.38 (*dt*, *J* = 13.5, 6.0),	1.53 (*dt*, *J* = 15.0, 6.5),
	1.00 (*ddd*, *J* = 13.5, 12.5, 6.0)	1.10 (*dddd*, *J* = 15.0, 13.0, 13.0, 6.5)
10	1.97 (*dq*, *J* = 12.5, 6.5)	2.06 (*dquint*, *J* = 13.0, 6.5)
12	0.94 (*s*)	0.99 (*s*)
13	0.93 (*s*)	0.82 (*s*)
14	–	–
15	0.74 (*d*, *J* = 6.5)	0.86 (*d*, *J* = 6.5)

[a]) The assignments were based on ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments. [b]) Measured in (D_5)pyridine. [c]) Measured in $CDCl_3$.

Table 4. *^{13}C-NMR Data* (125 MHz) *for* **2**[a])

Position	**2**[b])	Cyperenoic acid[c]) [16]	Position	**2**[b])	Cyperenoic acid[c]) [16]
1	65.4	68.2	9	28.8	27.9
2	38.3	25.7	10	35.6	35.9
3	81.0	36.3	11	42.2	41.7
4	129.6	123.1	12	19.8	19.3
5	167.7	173.1	13	26.9	26.2
6	31.9	31.3	14	168.9	170.6
7	47.6	48.2	15	18.8	17.9
8	27.6	26.9			

[a]) The assignments were based on ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments. [b]) Measured in (D_5)pyridine. [c]) Measured in $CDCl_3$.

were supported by 2D-NMR analyses. The COSY and HMB correlations (*Fig. 2*) confirmed the assignments of all H- and C-atom resonances, the location of the C=C bond and of the COOH group at C(4), and of the OH group at C(3). The absolute configuration at C(3) was proposed as (*S*) on the basis of the following NOESY correlations: H–C(3)/Me(15) and H–C(10)/Me(12,13), which are supported by further NOE correlations ((H–C(3)/H_α–C(2), H_α–C(2)/Me(15), H_β–C(2)/Me(12,13); *Fig. 3*), and on the basis of the known absolute configuration of patchoulane-type sesquiterpenes, such as cyperenoic acid, cyperene, and sugetriol triacetate, which are all major secondary metabolites of the rhizomes of *C. rotundus* [2][16][18]. Therefore, **2** was determined to be (3*S*)-3-hydroxypatchoulan-4-en-14-oic acid (3β-hydroxycyperenoic acid).

The known compounds were identified as britanlin E (**3**) [19], 1β,4α-dihydroxyeudesm-11-ene (**4**) [20], and 11,12-dihydroxyeudesm-4-en-3-one (**5**) [21] by comparison of their spectroscopic data with published values.

Bioactivities. Sesquiterpenes **1**–**5** obtained in this study were evaluated for their cytotoxic activities against human ovarian cancer cells (A2780) and endometrial adenocarcinoma cells (*Ishikawa*) using MTT assays. The effects of **1**–**5** were assessed using IC_{50} values (*Table 5*). Eudesmane sesquiterpene **5** exhibited the most potent cytotoxic activities against both A2780 and *Ishikawa* cells with observed IC_{50} values of 11.06 and 6.46 μM, respectively. Recently, several studies have focused on anticancer activities of sesquiterpenes, including eudesmane-type sesquiterpenes isolated from various plants such as *Kandelia candel* [22], *Solanum lyratum* [23], and *Litchi chinensis* [24]. Although in a recent investigation, **5** was found to exhibit significant inhibitory effects on NO production in lipopolysaccharide- and interferon-c-induced RAW264.7 murine macrophages [21], there are no reports concerning its antitumor effect. Whereas, the new guaiane sesquiterpene **1** showed moderate cytotoxic activity on *Ishikawa* cells with an observed IC_{50} value of 86.85 ± 0.41 μM, while **2**–**4** were not active on both A2780 and *Ishikawa* cell lines. It was reported that the essential oil of rhizomes of *C. rotundus* suppressed growth and proliferation along with increased apoptotic DNA fragmentation in L1210 murine lymphoblastic leukemia cell line [25]. In addition, luteolin, isolated from the AcOEt extracts of rhizomes of *C. rotundus*,

Table 5. *Cytotoxic Activities of* **1**–**5** *Isolated from* C. rotundus *on Human Ovarian Cancer Cells* (A2780) *and Endometrial Adenocarcinoma Cells* (*Ishikawa*)

Compound	IC_{50} Value [μM][a])	
	A2780	*Ishikawa*
1	> 100	86.85 ± 0.41
2	> 100	> 100
3	> 100	> 100
4	> 100	> 100
5	11.06 ± 0.25	6.46 ± 0.12
Cisplatin[b])	35.78 ± 0.55	18.52 ± 0.32

[a]) IC_{50} Value is defined as the concentration that results in a 50% decrease in the number of cells compared to that of control cultures. The values represent the means of the results from three independent experiments with similar patterns. [b]) Cisplatin was used as positive control.

significantly inhibited the proliferation of K562 cells (IC_{50} 25 μg ml^{-1}) [26]. However, antitumor effects of sesquiterpenes in the rhizomes of *C. rotundus* have not been investigated to date. In this study, we found an eudesmane sesquiterpene, 11,12-dihydroxyeudesm-4-en-3-one (**5**), as a cytotoxic constituent against human ovarian cancer cells (A2780) and endometrial adenocarcinoma cells (*Ishikawa*) in the AcOEt-soluble fraction from rhizomes of *C. rotundus*, which has been used for various gynecological diseases including cancer in traditional Chinese medicine. Based on these findings, we suggest that **5** should be considered as potential therapeutic and chemopreventive agent for gynecological cancer diseases, such as ovarian cancer and endometrial adenocarcinoma in humans. The *in vivo* antitumor effect of **5** and its detailed mechanism of action remain to be determined.

Conclusions. – In search of novel and plant-derived antitumor agents of *C. rotundus*, two new sesquiterpenes, cyperusol A_3 (**1**) and 3β-hydroxycyperenoic acid (**2**), along with three known sesquiterpenes, britanlin E (**3**), 1β,4α-dihydroxyeudesm-11-ene (**4**), and 11,12-dihydroxyeudesm-4-en-3-one (**5**), were isolated from the AcOEt-soluble fraction of rhizomes of *C. rotundus*. Sesquiterpenes **1**–**5** were evaluated for their cytotoxic activities against human ovarian cancer (A2780) and endometrial adenocarcinoma (*Ishikawa*) cell lines, and **5** showed the most potent cytotoxic activity with observed IC_{50} values of 11.06 and 6.46 μM, respectively. We suggest that the most potent metabolite, **5**, seems to be worthy of additional biological tests to evaluate its potential as therapeutic agent for gynecological cancer diseases.

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Experimental Part

General. Thin layer chromatography (TLC): silica gel *60 F_{254}* (SiO_2; *Merck*) and RP-18 *F_{254S}* plates (*Merck*); visualized by UV light and by spraying with 20% H_2SO_4 reagent (*Aldrich*) followed by heating at 110° for 5–10 min. Column chromatography (CC): SiO_2 *60A* (70–230 or 230–400 mesh ASTM; *Merck*), *Sephadex LH-20* (*Amersham Pharmacia Biotech*), and reversed-phase SiO_2 (*ODS-A* 12 nm *S-150*; *YMC Co.*). MPLC (Combi Flash Rf): *RediSep* SiO_2 (12 g, 40 g; *Teledyne Isco*) and *RediSep-C_{18}* columns (13 g, 13 g Gold, 26 g; *Teledyne Isco*). HPLC: *Gilson Gastorr BG-34* degasser, *Gilson 321* pump, *Gilson UV/VIS-155* detector, *YMC-Pack ODS-A* column (250 × 200 mm i.d., 5 μm). Optical rotations: *Jasco P-2000* polarimeter using a 10-cm microcell. UV Spectra: *Shimadzu UV-1650PC* spectrophotometer; λ_{max} (log ε) in nm. IR Spectra (ATR): *Varian 640-IR*; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Varian 500* and *400 MHz* FT-NMR spectrometer; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. HR-MS: *AccuTOF-single-reflectron time-of-flight* mass spectrometer (*Jeol, Ltd.*) equipped with a DART ion source (*IonSense*); in *m*/*z*.

Plant Material. The rhizomes of *C. rotundus* L. were obtained from a domestic Korean market (Kyungdong Crude Drugs Market, Seoul, South Korea) in June 2011. The origin of the herbal material was identified by *D. S. J.* and a voucher specimen (CYRO1–2011) was deposited in the Laboratory of Natural Product Medicine, College of Pharmacy, Kyung Hee University.

Extraction and Isolation. The dried and milled plant material (2.8 kg) was extracted three times with 10 l of 80% EtOH at r.t. by maceration. The extracts were combined and concentrated *in vacuo* at 40° to

give an 80%-EtOH extract (399 g). A portion of the 80%-EtOH extract (392 g) was suspended in H_2O (2 l) and successively extracted with hexane (3×2 l), AcOEt (3×2 l), and BuOH (3×2 l) to give hexane- (45.8 g), AcOEt- (23.5 g), BuOH- (52.4 g), and H_2O-soluble extracts (270.3 g), resp. The AcOEt-soluble extract (15.0 g) was subjected to CC (SiO_2 (70–230 mesh); 6.5×41 cm; CH_2Cl_2/MeOH 99:1 to 0:1) to give 17 fractions, *Frs. 1–17*. *Fr. 5* (710 mg) was further subjected to CC (*Sephadex LH-20*; CH_2Cl_2/MeOH 1:1) to give five subfractions, *Frs. 5.1–5.5*. *Fr. 5.3* (220 mg) was submitted to MPLC (*RediSep* SiO_2 (40 g); hexane/^{i}PrOH 90:10 to 80:20) to give six subfractions, *Frs. 5.3.1–5.3.6*. *Fr. 5.3.2* (110 mg) was further submitted to MPLC (*RediSep-C_{18}* (13 g Gold); MeOH/H_2O 50:50 to 70:30) to yield **3** (7.9 mg). *Fr. 6* (790 mg) was subjected to CC (*Sephadex LH-20*; CH_2Cl_2/MeOH 1:1) to give three subfractions, *Frs. 6.1–6.3*. *Fr. 6.2* (420 mg) was submitted to MPLC (*RediSep* SiO_2 (40 g); hexane/^{i}PrOH 100:0 to 90:10) to give ten subfractions, *Frs. 6.2.1–6.2.10*. *Fr. 6.2.2* (23 mg) was further submitted to MPLC (*RediSep-C_{18}* (13 g); MeOH/H_2O 30:70 to 70:30) to yield **1** (6.2 mg). *Fr. 6.2.5* (50 mg) was purified by MPLC (*RediSep-C_{18}* (26 g); MeOH/H_2O 35:65 to 60:40) to yield **4** (27.9 mg). *Fr. 7* (1.34 g) was subjected to CC (*Sephadex LH-20*; CH_2Cl_2/MeOH 1:1) to give four subfractions, *Frs. 7.1–7.4*. *Fr. 7.3* (819 mg) was subjected to CC (SiO_2 (230–400 mesh); 4×24.5 cm; hexane/AcOEt/MeOH 5:4:1) to give eight subfractions, *Frs. 7.3.1–7.3.8*. *Fr. 7.3.4* (222 mg) was submitted to MPLC (*RediSep-C_{18}* (26 g); MeOH/H_2O 20:80 to 35:65) to give seven subfractions, *Frs. 7.3.4.1–7.3.4.7*. *Fr. 7.3.4.5* (38.3 mg) was separated by prep. HPLC (*YMC-Pack ODS-A*; MeCN/H_2O (0.1% formic acid) 25:75 to 30:70, 5.5 and 4.5 ml min^{-1}) two times, repeatedly. *Fr. 7.3.4.5.2* (t_R 38 and 51 min; 11.6 mg) was further separated by prep. HPLC (*YMC-Pack ODS-A*; MeOH/H_2O (0.1% formic acid) 60:40 to 65:35, 5 ml min^{-1}) to yield **5** (t_R 22 min; 6.9 mg). *Fr. 13* (1.09 g) was subjected to CC (SiO_2 (230–400 mesh); 3.8×28 cm; CH_2Cl_2/MeOH/H_2O 8.5:1.5:0.1) to give seven subfractions, *Frs. 13.1–13.7*. *Fr. 13.2* (239 mg) was subjected to CC (*Sephadex LH-20*; CH_2Cl_2/MeOH 1:1) to give four subfractions, *Frs. 13.2.1–13.2.4*. *Fr. 13.2.2* (70 mg) was submitted to MPLC (*RediSep* SiO_2 (12 g); CH_2Cl_2/MeOH/H_2O 0:9:1 to 10:81:9) to give two subfractions, *Frs. 13.2.2.1* and *13.2.2.2*. *Fr. 13.2.2.1* (22.5 mg) was further submitted to MPLC (*RediSep-C_{18}* (26 g); MeOH/H_2O 45:55 to 63:37) to yield **2** (6.9 mg).

Cyperusol A_3 (=(*4*S,*7*S,*10*S)*-4,10-Dihydroxy-2-oxoguaia-1(5),11-diene*, *(3*S,*5*S,*8*S)*-3,4,5,6,7,8-Hexahydro-3,8-dihydroxy-3,8-dimethyl-5-(prop-1-en-2-yl)azulen-1(2*H)*-one*; **1**). White amorphous powder. $[\alpha]_D^{23} = -126.9$ ($c = 0.025$, MeOH). UV (MeOH): 237 (2.57). IR: 3381, 1676, 1630, 1370, 1284, 892. ^{1}H- and ^{13}C-NMR ((D$_5$)pyridine): *Tables 1* and *2*. HR-DART-MS: 233.1540 ($[M + H - H_2O]^+$, $C_{15}H_{21}O_2^+$; calc. 233.1536).

3β-Hydroxycyperenoic Acid (=(*3*S)*-3-Hydroxypatchoulan-4-en-14-oic Acid*, *(2*S,*3a*R,*4*R,*7*R)*-2,4,5,6,7,8-Hexahydro-2-hydroxy-4,9,9-trimethyl-3*H*-3a,7-methanoazulene-1-carboxylic Acid*; **2**). Viscous brownish oil. $[\alpha]_D^{24} = +13.4$ ($c = 0.06$, MeOH). UV (MeOH): 233 (2.47). IR: 3403, 2923, 1686, 1459, 1249, 1076, 1029. ^{1}H- and ^{13}C-NMR ((D$_5$)pyridine): *Tables 3* and *4*. HR-DART-MS: 233.1546 ($[M + H - H_2O]^+$, $C_{15}H_{21}O_2^+$; calc. 233.1536), 205.1590 ($[M + H - H_2O - CO]^+$).

Cytotoxicity Assay. The human ovarian cancer cells (A2780) and endometrial adenocarcinoma cells (*Ishikawa*) were originally from American-type culture collection. Cells were cultured in RPMI (*Life Technologies Inc.*, USA) supplemented with 5% fetal bovine serum (*Life Technologies Inc.*, USA), penicillin (100 U ml^{-1}; *Life Technologies Inc.*, USA), and streptomycin sulfate (100 µg ml^{-1}; *Life Technologies Inc.*, USA). Cytotoxic activities were assessed by MTT assays. Briefly, the cells ($5 \cdot 10^4$) were seeded in each well containing 50 µl of RPMI medium in a 96-well plate. After 24 h, various concentrations of compounds isolated from *C. rotundus* and cisplatin were added. After 48 h, 50 µl of MTT (5 mg ml^{-1} stock soln.; *Molecular Probes Inc.*, USA) were added, and the plates were incubated for additional 4 h. The medium was discarded and formazan blue that formed in the cells was dissolved in 50 µl of DMSO. The optical density was measured at 540 nm by microplate spectrophotometer (*SpectraMax*; *Molecular Devices*, CA).

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