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New Patchoulane-Type Sesquiterpenes from the Rhizomes of *Cyperus rotundus*

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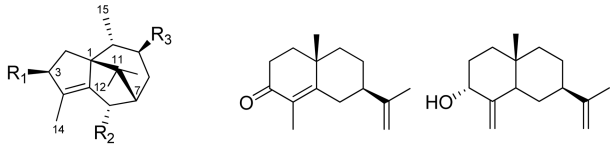
Cyperus rotundus L. (Cyperaceae) is a cosmopolitan sedge seen in tropical, subtropical and temperate regions all over the world. The rhizomes of *C. rotundus* have been used in traditional Chinese medicine as an estrogenic and anti-inflammatory agent for the treatment of women's diseases and also used for treatment of stomach ache, bowel disorders, and menstrual disorders.[1] The extract of the rhizomes of *C. rotundus* has been showed a broad range of biological activities, such as anti-diabetic activity,[2] acetylcholinesterase inhibitory activity,[3] and plant growth inhibitory activity,[4] as well as inhibition of nitric oxide and superoxide production.[5] Previous phytochemical investigations on *C. rotundus* have resulted in the isolation of a series of sesquiterpenes possessing diverse skeletons[6-8] as well as sesquiterpene alkaloids, triterpenes, sterols, and flavonoids.[9-11] Repeated chromatography of the *n*-hexane-soluble fraction from the rhizomes of *C. rotundus* led to the isolation and characterization of two new patchoulane-type sesquiterpenes (**1** and **3**), along with three known patchoulane- (**2**, **4** and **5**) and two known eudesman-type sesquiterpenes (**6** and **7**). The structures of **1**-**3** were determined by spectroscopic data interpretation, particularly by extensive 1D and 2D NMR studies. To our knowledge, this is the first report on the NMR assignment of **2**. The structures of other known compounds were identified to be sugetriol triacetate (**4**),[6,12] cyperotunone (**5**), α-cyperone (**6**),[12] and isocyperol (**7**)[13] by physical (mp, $[\alpha]_D$) and spectroscopic data (^{1}H-NMR, ^{13}C-NMR, 2D NMR, and MS) measurement and by comparison with published values.

Compound **1** was obtained as a colorless oil. Its HR-DART-MS gave a pseudo-molecular ion peak at *m/z*



1 R_1 = H, R_2 = R_3 = OAc
2 R_1 = R_3 = H, R_2= OAc
3 R_1= R_3 = H, R_2 = OH
4 R_1= R_2 = R_3 = OAc
5 R_1 = O, R_2 = R_3 = H

6 **7**

Figure 1. Structures of **1**-**7** isolated from the rhizomes of *C. rotundus*.

Table 1. ^{1}H-NMR Spectral Data for **1**-**3** (in $CDCl_3$)[a]

Position	δ_H (*J* in Hz)		
	1	**2**	**3**
2	1.66 overlap[b]	1.67 ddd (13.0, 10.0, 9.5)	1.65 ddd (13.0, 10.0, 9.5)
	1.54 dd (13.0, 6.5)	1.47 dd (13.0. 8.0)	1.46 dd (13.0, 8.0)
3	2.71 m		
	2.26 dd (16.5, 9.5)	2.69 dt (15.5, 8.5)	2.69 dt (16.5, 7.5)
		2.22 dd (16.5, 10.0)	2.20 dd (16.0, 10.0)
6	5.49 br d (6.0)	5.55 br d (6.0)	4.67 br s
7	2.32 dt (6.0, 3.0)	2.19 overlap[b]	1.90 dt (6.0, 3.0)
8	1.85 ddd (13.5, 7.0, 3.5)	1.75 m	1.73 m
	1.65 overlap[b]	1.29 m	1.53 m
9	4.76 td (10.5, 7.0)	1.44 dd (13.0, 6.5)	1.17 m
		1.31 overlap[b]	1.15 m
10	2.12 dq (10.5, 6.5)	2.03 m	2.01 m
12	0.85 s	0.82 s	0.77 s
13	1.03 s	0.96 s	0.96 s
14	1.67 br s	1.70 br s	1.79 br s
15	0.94 d (6.5)	0.85 d (6.5)	0.82 d (6.5)
6-COOCH_3	2.03 s	2.09 s	
9-COOCH_3	2.17 s	-	

[a]The assignments were based on COSY, HMQC, and HMBC experiments. [b]Overlapping with other signals.

Table 2. ^{13}C-NMR Spectral Data for **1-3** (in $CDCl_3$)[a]

Position	δ_C		
	1	**2**	**3**
1	64.1	64.9	65.0
2	26.9	27.0	27.2
3	42.3	42.3	42.3
4	138.0	137.0	137.0
5	138.7	139.7	144.2
6	71.0	71.5	68.7
7	51.7	51.9	53.6
8	28.4	22.8	21.8
9	75.1	28.4	29.2
10	40.6	35.5	35.4
11	41.4	41.5	41.4
12	26.8	26.2	26.1
13	20.3	20.2	20.4
14	14.2	14.3	14.2
15	14.0	18.1	18.1
6-COO*CH*$_3$	21.3	21.2	-
9-COO*CH*$_3$	21.0	-	-
6-*C*OOCH$_3$	171.0	171.2	-
9-*C*OOCH$_3$	171.5	-	-

[a]The assignments were based on COSY, HMQC, and HMBC experiments.

319.1880 $[M–H]^+$ (calcd for $C_{19}H_{27}O_4$: 319.1909), indicated a molecular formula of $C_{19}H_{28}O_4$. The ^{13}C-NMR and DEPT spectra of **1** allowed the identification of 19 carbon atoms: six methyl groups, three methylene groups, four methine groups, and six quaternary carbon atoms. The chemical shifts of the latter indicated two ester carbonyl (δ 171.5 and 170.0), two aliphatic (δ 64.1 and 41.4), and two sp^2 carbons (δ 138.7 and 138.0). The ^{1}H-NMR spectrum of **1** showed the presence of two secondary acetoxyl groups which were indicated by two acetoxyl methyls [δ 2.17 (3H, s) and δ 2.03 (3H, s)] and two hydrogens on carbons bearing the acetoxyls [δ 5.49 (1H, d, J = 6.0 Hz) and δ 4.76 (1H, td, J = 10.5 and 7.0 Hz)]. The presence of two acetoxyl groups in **1** was also supported by HR-DART-MS spectrum of **1** which showed two fragment ion peaks at *m/z* 261.1840 $[M–Ac]^+$ and 201.1654 $[M–2Ac]^+$. Compound **1** has six unsaturation degrees, two acetoxyl groups, and one double bond, therefore the presence of three rings was concluded. Further examination of the ^{1}H-NMR spectrum of **1** revealed some signals common to patchoulane-type sesquiterpenes, namely the two geminal methyl groups H-12 and H-13 (δ 0.85 and 1.03, respectively; each 3H, s), the methyl group H-15 at δ 0.94, appearing as a doublet (3H, J = 6.5 Hz) and coupling with the methine proton H-10 (δ 2.12, 1H, dq, J = 10.5 and 6.5 Hz), and the vinyl methyl group H-14 at δ 1.67, appearing as a broad singlet. Comparison of the above with data in the literature[4,6,12] suggested that **1** was a cyperene-related compound with patchoulane skeletone bearing two acetoxyl groups. These assignments were supported by 2D NMR techniques. The COSY and HMBC correlations (Figure 2) confirmed the assignments of all proton and carbon resonances and the location of the double bond (C-4) and two acetoxyl groups (C-6 and C-9). The absolute configuration at C-6 and C-9 was proposed as both *S* on the basis of NOESY correlations (Figure 3) from δ_H 5.49 (H-6) to δ_H 0.85 (H-12) and δ_H 4.76 (H-9) to δ_H 0.94 (H-15) and on the basis of sugetriol triacetate (**4**) and cyperene of known absolute configuration,[6,14,15] which are of major components of the rhizomes of *C. rotundus*. Thus, **1** was determined to be (6*S*,9*S*)-patchoulan-4-ene-6,9-diol diacetate {(6*S*,9*S*)-6,9-diacetoxy cyperene}.

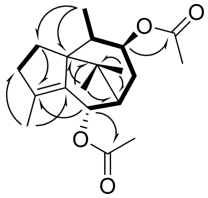
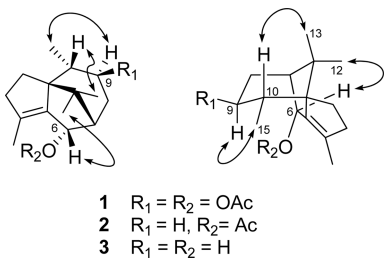


Figure 2. Selected correlations observed in the COSY (——) and HMBC (→) spectra of **1**.

Compound **2** was also obtained as a colorless oil. Its HR-DART-MS gave a pseudo-molecular ion peak at *m/z* 261.1841 $[M–H]^+$ (calcd for $C_{17}H_{25}O_2$: 261.1855), indicated a molecular formula of $C_{17}H_{26}O_2$. The proton and carbon signals in the ^{1}H- and ^{13}C-NMR spectra of **2** were very similar to those of **1** (Table 1). However, preliminary inspection of the ^{1}H-NMR spectrum of **2** revealed the absence of an oxymethine proton at δ_H 4.76 and an acetoxyl methyl at δ_H 2.17, which are of characteristics of compound **1**, and the presence of a methylene group (δ_H 2.30 and 2.00). Moreover, comparison of the ^{13}C-NMR data for **1** and **2** indicated that the oxymethine carbon (δ_C 75.1) and the acetoxyl carbons at C-9 (δ_C 171.5 and 21.0) of **1** were replaced by a methylene carbon (δ_C 28.4) in **2**. The presence of an acetoxyl groups in **2** was also supported by HR-DART-MS spectrum of **2** which showed a fragment ion peak at *m/z* 203.1805 $[M–Ac]^+$. The position of the acetoxyl group was confirmed as occurring at C-9 using the HMBC NMR technique. Thus, **2** was elucidated as a 9-deacetoxyl analogue of **1**, (6*S*)-patchoulan-4-ene-6-ol acetate {(6*S*)-6-acetoxy cyperene}. The absolute conformation of **2** was proposed in a similar manner to that of **1** (Figure 3). Although patchoulenyl acetate was isolated from *C. rotundus* previously and identified by Mass and IR,[15] the position and configuration of the acetoxyl group and full NMR assignment of **2** have not been reported to



1 $R_1 = R_2 = OAc$
2 $R_1 = H$, $R_2 = Ac$
3 $R_1 = R_2 = H$

Figure 3. Selected correlations observed in the NOESY (↔) NMR spectra of **1-3**.

date.

Compound **3** was obtained as a colorless oil. Its molecular formula was proposed as $C_{15}H_{24}O$ from a pseudo-molecular ion peak at m/z = 203.1800 $[MH–H_2O]^+$ (calcd for $C_{15}H_{23}$: 203.1800) in the HR-DART-MS, indicating the presence of a secondary hydroxyl group in **3**. The ^{1}H- and ^{13}C-NMR spectra of **3** exhibited strong similarities with those of **2** (Table 1). The inspection of the ^{1}H- and ^{13}C-NMR spectra of **3** readily indicated the absent of an acetoxyl methyl at δ_H 2.09 and δ_C 21.2 of **2**. It was also observed that both the oxymethine proton at δ_H 5.55 and the oxymethine carbon at δ_C 71.5 in the ^{1}H- and ^{13}C-NMR spectra of **2** were shifted to upfield in the those of **3** (δ_H 4.67 and δ_C 68.7) due to deacetylation. Thus, compound **3** was elucidated as a deacetyl analogue of **2**, (6*S*)-patchoulan-4-ene-6-ol {(6*S*)-cyperene-6-ol}. The absolute conformation of **3** was also proposed in a similar manner to those of **1** and **2** (Figure 3).

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher-Johns melting point apparatus without correction. Optical rotations were measured on a Jasco P-2000 polarimeter, using a 10-cm microcell. HR-Mass spectra were obtained using an AccuTOF-single-reflectron time-off light mass spectrometer (Jeol Ltd, Tokyo, Japan) equipped with a DART ion source (IonSense, Saugus, MA, USA). NMR spectra were obtained using a Varian 500 MHz NMR spectrometer using TMS as an internal standard and chemical shifts are expressed as δ values. IR spectra were obtained using a Varian 640-IR. TLC analyses was performed on Kieselgel 60 F_{254} (Merck) plates (silica gel, 0.25 mm layer thickness); compounds were visualized by dipping plates into 20% (v/v) H_2SO_4 reagent (Aldrich) and then heated at 110 °C for 5-10 min. Silica gel (Merck 60A, 70-230 or 230-400 mesh ASTM), Sephadex LH-20 (Amersham Pharmacia Biotech), and reversed-phase silica gel (YMC Co., ODS-A 12 nm S-150 μm) were used for column chromatography. All solvents used for the chromatographic separations were distilled before use.

Plant Material. The rhizomes of *Cyperus 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 one of the authors (D.S.J.) and a voucher specimen (CYRO1-2011) was deposited in the Lab. of Natural Product Medicine, College of Pharmacy, Kyung Hee University.

Extraction and Isolation. The dried and milled plant material (2.8 kg) was extracted with 10 L of 80% EtOH three times by maceration. The extracts were combined and concentrated in vacuo at 40 °C to give a 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 *n*-hexane (3 × 2 L), EtOAc (3 × 2 L), and BuOH (3 × 2 L) to give *n*-hexane- (45.8 g), EtOAc- (23.5 g), BuOH- (52.4 g), and water-soluble extracts (270.3 g), respectively. The *n*-hexane-soluble extract (44 g) was chromatographed over silica gel (70-230 mesh, ϕ 6.0 × 44 cm) as stationary phase with a *n*-hexane-EtOAc gradient (from 1:0 to 1:1 v/v; final stage, MeOH 100%) as mobile phase to afford 18 pooled fractions (H01~H18). Fraction H3 [eluted with *n*-hexane-EtOAc (19:1 v/v); 3.44 g] was further fractionated using silica gel column chromatography (CC) (230-400 mesh, ϕ 3.6 × 26 cm, *n*-hexane-CH_2Cl_2 = 9:1 → 85:15 v/v) to give compound **2** (30 mg). Compounds **3** (4.5 mg) and **6** (660 mg) were obtained from fraction H5 [eluted with *n*-hexane-EtOAc (19:1 v/v); 2.62 g] through silica gel CC (230-400 mesh, ϕ 3.6 × 30 cm, *n*-hexane-CH_2Cl_2 = 3:1 → 6:4 v/v). Fraction H6 [eluted with *n*-hexane-EtOAc (19:1 v/v); 2.80 g] was chromatographed over silica gel (230-400 mesh, ϕ 3.6 × 27 cm) as stationary phase with a *n*-hexane-EtOAc gradient (*n*-hexane-CH_2Cl_2 = 1:1 v/v; final stage, MeOH 100%) as mobile phase to afford 7 subfractions (H6-1~H6-7). Compounds **1** (111 mg) and **5** (147 mg) were purified by reversed-phase CC (YMC gel 150 μm, ϕ 2.6×21 cm) with MeOH-H_2O mixture (4:1 v/v) from fractions H6-7 and H6-6, respectively. Fraction H7 [eluted with *n*-hexane-EtOAc (19:1 v/v); 2.08 g] was further fractionated using a Sephadex column (ϕ 3.6 × 72 cm) with CH_2Cl_2-MeOH mixture (1:1 v/v), yielding compound **7** (60 mg). Compound **4** (640 mg) was purified by recrystallization (*n*-hexane) from fraction H9 [eluted with *n*-hexane-EtOAc (9:1 v/v); 4.33 g].

(6*S*,9*S*)-Patchoulan-4-ene-6,9-diol Diacetate (1): Colorless oil. $[\alpha]_D^{25}$: +102.5° (*c* 0.051, $CHCl_3$); IR (ATR) ν_{max} cm^{-1}: 2974, 1738, 1589, 1374, 1247, 1031; ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) data, see Table 1; HR-DART-MS m/z = 319.1880 $[M–H]^+$ (calcd for $C_{19}H_{27}O_4$: 319.1909), 261.1840 $[M–Ac]^+$, 201.1654 $[M–2Ac]^+$.

(6*S*)-Patchoulan-4-ene-6-ol Acetate (2): Colorless oil. $[\alpha]_D^{25}$: +19.8° (*c* 0.071, $CHCl_3$); IR (ATR) ν_{max} cm^{-1}: 2935, 1730, 1519, 1215, 1027, 754; ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) data, see Table 1; HR-DART-MS m/z = 261.1841 $[M–H]^+$ (calcd for $C_{17}H_{25}O_2$: 261.1855), 203.1805 $[M–Ac]^+$.

(6*S*)-Patchoulan-4-ene-6-ol (3): Colorless solid; $[\alpha]_D^{25}$: +28.2° (*c* 0.021, $CHCl_3$); IR (ATR) ν_{max} cm^{-1}: 3490-3280, 2928, 1519, 1216, 1014, 760; ^{1}H-NMR ($CDCl_3$, 500 MHz) and ^{13}C-NMR ($CDCl_3$, 125 MHz) data, see Table 1; HR-DART-MS m/z = 203.1800 $[MH–H_2O]^+$ (calcd for $C_{15}H_{23}$: 203.1800).

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Supporting Information. The spectral data of compounds **1-3** are available on request from the correspondence author.

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