Rotundines A−C, Three Novel Sesquiterpene Alkaloids from *Cyperus rotundus*

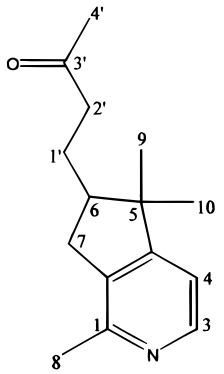
Sei-Joon Jeong,[†] Tomofumi Miyamoto,[†] Masanori Inagaki,[†] Youn-Chul Kim,[‡] and Ryuichi Higuchi*[,†]

Graduate School of Pharmaceutical Sciences, Kyushu University, Fukuoka, 812-8582, Japan, and College of Pharmacy, Wonkwang University, Iksan, 570-749, Korea

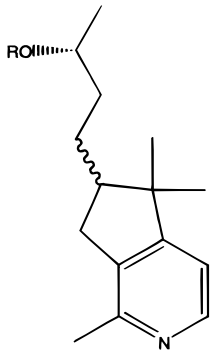
Received November 24, 1999

Rotundines A (**1**), B (**2**), and C (**3**), three novel sesquiterpene alkaloids with an unprecedented carbon skeleton, were isolated from the rhizomes of *Cyperus rotundus*. The structures of **1**−**3** were elucidated by spectral and chemical methods.

The rhizomes of *Cyperus rotundus* Linn. (Cyperaceae) have been used as an analgesic in traditional Chinese medicine.[1] Phytochemical research on this plant has led to the identification of several essential-oil components.[2,3] As part of our ongoing search for new alkaloids from medicinal plants, we have isolated three sesquiterpene alkaloids from the rhizomes of *C. rotundus*. Herein, we describe the isolation and structure determination of these alkaloids, rotundines A−C (**1**−**3**), which are based on a new sesquiterpene skeleton. To the best of our knowledge, this is the first report of alkaloids from this plant.



1



2 R=H
3 (6-*epi*-**2**)
2a, **3a** R=(*S*)-MTPA
2b, **3b** R=(*R*)-MTPA

Rotundine A (**1**) was obtained as a colorless oil and responded positively to Dragendorff's reagent. Its molecular formula was determined as $C_{15}H_{21}NO$ by HRFABMS ([M + H]$^+$ 232.1705, calcd 232.1701). The UV maximum at 260 nm and ^{1}H NMR signals at δ 7.22 (1H, d, J = 5.7 Hz) and 8.51 (1H, d, J = 5.7 Hz) indicated the presence of a pyridine moiety. All ^{13}C NMR resonances of the pyridine moiety could not be observed in $CDCl_3$, but on the addition of DCl, the quaternary carbons (C-1, C-4a, C-7a) and the methyl carbon of the pyridine moiety could be assigned. An IR band (1713 cm^{-1}, C=O) and ^{13}C NMR signals [δ 208.1 (s), 30.0 (q)] suggested the presence of an acetyl moiety. Furthermore, the ^{1}H and ^{13}C NMR spectra of **1** (Tables 1 and 2) indicated the existence of the remaining functional groups. Analysis of the HMBC spectrum, as well as the ^{1}H−^{1}H COSY and HSQC data (Figure 1), suggested a structure as either a cyclopenta[*c*]pyridine or cyclopenta-[*b*]pyridine derivative. In the NOESY experiment of **1** (Figure 1), the H-8 methyl proton showed NOEs with the H-7 methylene protons, and the H-4 proton with the H-9 and H-10 methyl protons. Based on these results, the structure of rotundine A (**1**) was assigned as 1,5,5-trimethyl-6-(3′-oxobutyl)-6,7-dihydro-5*H*-cyclopenta[*c*]pyridine.

Table 1. ^{1}H NMR Data for Compounds **1**−**3** (600 MHz, in $CDCl_3$ + TFA)[a]

proton	**1**[b]	**2**	**3**
3	8.51 d (5.7)	8.60 d (5.3)	8.59 d (5.3)
4	7.22 d (5.7)	7.35 d (5.3)	7.35 d (5.3)
6	2.01 m	2.14 m	2.13 m
7	2.50 dd (10.1, 16.0)	2.58 dd (10.7, 16.3)	2.56 dd (10.0, 16.3)
	3.01 dd (7.8, 16.0)	3.11 dd (7.6, 16.2)	3.11 dd (7.8, 16.3)
8	2.64 s	2.72 s	2.72 s
9	1.03 s	1.05 s	1.05 s
10	1.36 s	1.38 s	1.39 s
1′	1.61 m	1.40−1.59 m	1.40 m
	1.93 m		1.81 m
2′	2.54 m	1.40−1.59 m	1.49 m
	2.59 m		1.61 m
3′		3.86 m	3.86 m
4′	2.18 s	1.25 d (6.2)	1.25 d (6.2)

[a] J values (in Hz) are given in parentheses. [b] Recorded at 500 MHz.

Table 2. ^{13}C NMR Data for Compounds **1**−**3** (125 MHz, in $CDCl_3$ + TFA)[a]

carbon	**1**	**2**	**3**
1	148.8 s[b]	149.3 s	149.3 s
3	144.7 d	140.7 d	140.7 d
4	116.1 d	117.8 d	117.8 d
4a	171.9 s[b]	171.6 s	171.6 s
5	46.6 s	47.5 s	47.5 s
6	49.9 d	50.5 d	50.5 d
7	34.0 t	33.7 t	33.8 t
7a	139.7 s[b]	139.7 s	139.7 s
8	17.2 q[b]	17.5 q	17.5 q
9	22.9 q	22.7 q	22.7 q
10	25.9 q	23.9 q	23.9 q
1′	23.1 t	25.1 t	25.4 t
2′	42.3 t	37.8 t	38.0 t
3′	208.1 d	67.9 d	68.2 d
4′	30.0 q	25.6 q	25.6 q

[a] Multiplicities deduced by DEPT experiments. [b] These signals were measured in $CDCl_3$ + DCl.

Rotundine B (**2**) was obtained as colorless oil. Its molecular formula was deduced as $C_{15}H_{23}NO$ by HRFABMS ([M

* To whom correspondence should be addressed. Tel.: +81-92-642-6635. Fax: +81-92-642-6639. E-mail: higuchi@shokka.phar.kyushu-u.ac.jp.
[†] Kyushu University.
[‡] Wonkwang University.

10.1021/np990588r CCC: $19.00
Published on Web 04/06/2000

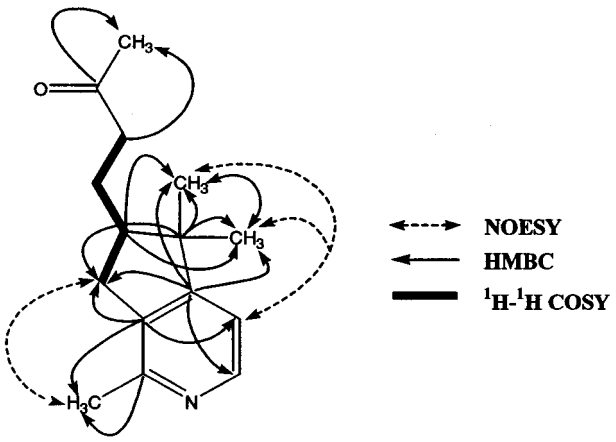


Figure 1. Significant 2D correlations of rotundine A (**1**).

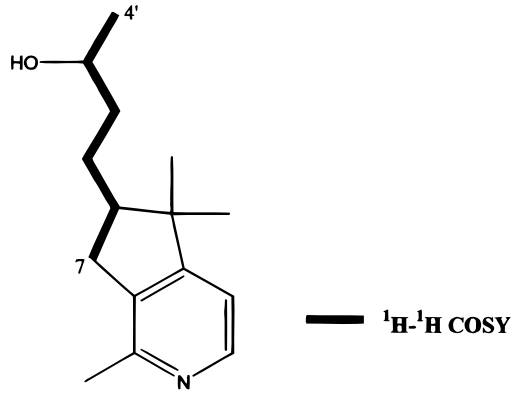


Figure 2. ^{1}H−^{1}H COSY correlations of rotundines B (**2**) and C (**3**).

+ H]$^+$ 234.1858, calcd 234.1893). The ^{1}H NMR spectrum of **2** was quite similar to that of **1** except for the signals ascribable to an oxygenated methine proton at δ 3.80 (1H, m, H-3′) and methyl protons at δ 1.25 (3H, d, J = 6.2 Hz, H-4′). The ^{1}H−^{1}H COSY spectrum revealed correlations from the H-7 methylene protons (δ 2.58, 3.11) to the H-4′ methyl protons (Figure 2). These data suggested a structure in which the ketone group of **1** was replaced by a secondary alcohol group. Interpretation of the HMBC, HSQC, and NOESY NMR spectra enabled its structure to be established as 1,5,5-trimethyl-6-(3′-hydroxybutyl)-6,7-dihydro-5*H*-cyclopenta[*c*]pyridine. The absolute configuration at C-3′ was established using the modified Mosher's method.[4] Determination of $\Delta\delta_H$ (δ_S−δ_R) data of the (*S*)-α-methoxy-α-(trifluoromethyl)phenylacetic acid (MTPA) and (*R*)-MTPA esters, **2a** and **2b**, indicated that the absolute configuration of C-3′ was *R*.

Rotundine C (**3**), $C_{15}H_{23}NO$, closely resembled **2** in its ^{1}H and ^{13}C NMR spectra except for differences of the chemical shifts at the H-1′ and H-2′ protons and the C-1′, C-2′, and C-3′ ^{13}C NMR signals. Therefore, compound **3** was presumed to be a stereoisomer of **2**. Analysis of the $\Delta\delta_H$ (δ_S−δ_R) data of the (*S*)-MTPA and (*R*)-MTPA esters, **3a** and **3b**, indicated that the absolute configuration of C-3′ was *R*, the same as **2**. From the data obtained, it may be assumed that **3** is an epimer of **2** at the C-6 position.

The absolute configuration at C-6 of rotundines A, B, and C (**1**−**3**) has not yet been determined, although these compounds have an unprecedented sesquiterpene skeleton. Although monoterpene and sesquiterpene alkaloids containing a pyridine skeleton have been isolated from natural sources,[5,6] sesquiterpene alkaloids with a cyclopenta[*c*]-pyridine have not been reported previously.

Experimental Section

General Experimental Procedures. Optical rotations were recorded on a JASCO DIP-370 digital polarimeter. UV spectra were recorded on a JASCO U-best30 spectrophotometer. IR spectra were recorded on a JASCO FT/IR-410 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on Varian Unity 500 (500 MHz) and 600 (600 MHz) spectrometers. The chemical shifts are reported in parts per million relative to the solvent ($CDCl_3$, δ_H 7.24, δ_C 77.0). FABMS and HRFABMS were recorded on a JEOL SX-102 spectrometer.

Plant Material. Rhizomes of *C. rotundus* were purchased from Uchida Wakanyaku Co., Ltd. (Tokyo, Japan) in March 1999. A voucher specimen (lot. 242118) is deposited in the herbarium of the Department of Natural Products Chemistry, Graduate School of Pharmaceutical Sciences, Kyushu University, Japan.

Extraction and Isolation. Dried rhizomes (3 kg) of *C. rotundus* were extracted three times with MeOH for 48 h at room temperature. The MeOH extract (210 g) was dissolved in 3% HCl (1 L) and filtered, and the filtrate was extracted with 5% MeOH/$CHCl_3$. The 3% HCl layer was then basified with NH_4OH solution to pH 9, and the mixture was extracted with 5% MeOH/$CHCl_3$. The crude alkaloid fraction (520 mg) was chromatographed on Sephadex LH-20 (MeOH, 750 mL) to obtain four fractions (A−D). Fraction B (100 mg), which responded positively to Dragendorff's reagent, was chromatographed on Si gel ($CHCl_3$/MeOH/Et_2NH, 50:0:1−30:1:1) to yield four fractions (B1−B4). Fraction B2 (13 mg) was chromatographed by preparative TLC (Si gel 60, $CHCl_3$/MeOH/Et_2NH, 15:1:0.1) to yield compound **1** (2.2 mg). Fraction C (43 mg) was chromatographed using HPLC on Cosmosil 5C18-AR-II with CH_3CN/0.1% TFA (5:95−95:5, UV 254 nm, 3 mL/min) to obtain compounds **2** (3.1 mg) and **3** (3.3 mg).

Rotundine A (1): colorless oil; $[\alpha]^{27}_D$ −12.3° (*c* 0.31, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 260 (3.15) nm; IR ($CHCl_3$) ν_{max} 3007, 2963, 2933, 1713, 1596, 1455 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS *m/z* 232 [M + H]$^+$ (52), 207 (18), 176 (10), 154 (47), 136 (55), 109 (30), 95 (53), 69 (87), 57 (100); HRFABMS *m/z* 232.1701 (calcd for $C_{15}H_{22}NO$ [M + H]$^+$, 232.1705).

Rotundine B (2): colorless oil; $[\alpha]^{25}_D$ −14.7° (*c* 0.15, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 264 (3.40) nm; IR ($CHCl_3$) ν_{max} 3019, 2961, 2928, 2857, 1671 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS *m/z* 234 [M + H]$^+$ (62), 175 (8), 154 (46), 136 (38), 109 (25), 95 (41), 59 (100); HRFABMS *m/z* 234.1861 (calcd for $C_{15}H_{24}NO$ [M + H]$^+$, 234.1858).

(*S*)-MTPA Ester (2a) of 2. Rotundine B (**2**, 0.5 mg) was dissolved in CH_2Cl_2 (0.15 mL) and treated with (*S*)-(−)-MTPA (1.89 mg); 1,3-dicyclohexylcarbodiimide (1.52 mg); and 4-(dimethylamino)pyridine (0.79 mg). The mixture was left to stand for 72 h at room temperature, with the resulting mixture purified by preparative TLC (Si gel 60, $CHCl_3$/MeOH, 10:1) to give the (*S*)-MTPA ester (0.7 mg): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.27 (1H, d, J = 5.0 Hz, H-3), 6.87 (1H, d, J = 5.0 Hz, H-4), 5.21 (1H, m, H-3′), 2.86 (1H, m, H-7), 2.42 (3H, m, H-8), 2.28 (1H, m, H-7), 1.87 (1H, m, H-6), 1.38 (3H, d, J = 6.4 Hz, H-4′), 1.22 (3H, s, H-10), 0.84 (3H, s, H-9).

(*R*)-MTPA Ester (2b) of 2. Rotundine B (**2**, 0.4 mg) was esterified with (*R*)-(+)-MTPA according to the same procedure described for **2a**, to yield **2b** (0.6 mg): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.29 (1H, d, J = 5.0 Hz, H-3), 6.89 (1H d, J = 5.0 Hz, H-4), 5.20 (1H, m, H-3′), 2.93 (1H, m, H-7), 2.44 (3H, s, H-8), 2.38 (1H, m, H-7), 1.93 (1H, m, H-6), 1.31 (3H, d, J = 6.4 Hz, H-4′), 1.25 (3H, s, H-10), 0.90 (3H, s, H-9).

Rotundine C (3): colorless oil; $[\alpha]^{25}_D$ −10.2° (*c* 0.11, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 262 (3.48) nm; IR ($CHCl_3$) ν_{max} 3008, 2969, 2931, 2859, 1671 cm^{-1}; ^{1}H NMR, see Table 1; ^{13}C NMR, see Table 2; FABMS *m/z* 234 [M + H]$^+$ (100), 207 (10), 154 (46), 136 (47), 107 (16), 91 (20), 73 (31), 59 (36); HRFABMS *m/z* 234.1893 (calcd for $C_{15}H_{24}NO$ [M + H]$^+$, 234.1858).

(*S*)-MTPA Ester (3a) of 3. Rotundine C (**3**, 1.0 mg) was dissolved in dry pyridine (0.1 mL) and treated with (*R*)-(−)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) (10 μL). The mixture was left to stand at room temperature

for 3 h, with the resulting mixture being purified by preparative TLC (Si gel 60, $CHCl_3$/MeOH, 10:1) to give the (*S*)-MTPA ester (0.7 mg): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.27 (1H, J = 5.0 Hz, H-3), 6.87 (1H, d, J = 5.0 Hz, H-4), 5.20 (1H, m, H-3′), 2.86 (1H, m, H-7), 2.42 (3H, s, H-8), 2.28 (1H, m, H-7), 1.89 (1H, m, H-6), 1.77 (1H, m, H-1′), 1.38 (3H, d, J = 6.4 Hz, H-4′), 1.22 (3H, s, H-10), 0.84 (3H, s, H-9).

(*R*)-MTPA Ester (3b) of 3. Rotundine C (**3**, 1.1 mg) was esterified with (*S*)-(+)-MTPA-Cl according to the same procedure described for **3a**, to yield **3b** (0.7 mg): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.29 (1H, J = 5.0 Hz, H-3), 6.89 (1H, J = 5.0 Hz, H-4), 5.19 (1H, m, H-3′), 2.93 (1H, m, H-7), 2.43 (3H, s, H-8), 2.38 (1H, m, H-7), 1.95 (1H, m, H-6), 1.85 (1H, m, H-1′), 1.31 (3H, d, J = 6.4 Hz, H-4′), 1.25 (3H, s, H-10), 0.90 (3H, s, H-9).

References and Notes

(1) Jiangsu New Medical College. *Dictionary of Chinese Crude Drugs*; Shanghai Scientific and Technologic Publisher: Tokyo, 1985; p 750.
(2) Hikino, H.; Aota, K.; Takemoto, T. *Chem. Pharm. Bull.* **1965**, *13*, 628−630.
(3) Hikino, H.; Aota, K.; Kuwano, D.; Takemoto, T. *Tetrahedron Lett.* **1969**, *32*, 2741−2742.
(4) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092−4096.
(5) Sakan, T.; Fujino, A.; Murai, F.; Butsugan, Y.; Suzui, A. *Bull. Chem. Soc. Jpn.* **1959**, *32*, 315−316.
(6) Büchi, G.; Goldman, I. M.; Mayo, D. W. *J. Am. Chem. Soc.* **1966**, *88*, 3109−3113.

NP990588R