New β-Carboline Alkaloids from a Chinese Medicinal Plant, *Arenaria kansuensis*. Structures of Arenarines A, B, C, and D

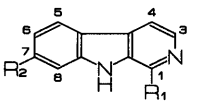
Feng-E WU,[a] Kazuo KOIKE,[a] Tamotsu NIKAIDO,[a] Yohko SAKAMOTO,[a] Taichi OHMOTO*,[a] and Keiji IKEDA[b]

School of Pharmaceutical Sciences, Toho University,[a] 2–2–1 Miyama, Funabashi, Chiba 274, Japan and Central Laboratories, Nippon Flour Mills Co., Ltd.,[b] 2114–2 Nurumizu, Atsugi, Kanagawa 243, Japan. *Received December 1, 1988*

Six β-carboline alkaloids were isolated from a Chinese medicinal plant, *Arenaria kansuensis*. The structures of four new alkaloids named arenarines A, B, C, and D were identified based on spectral and chemical evidence.

Keywords *Arenaria kansuensis*; arenarine A; arenarine B; arenarine C; arenarine D; Caryophyllaceae; β-carboline; alkaloid

The whole plants of *Arenaria kansuensis* MAXIM. (Chinese name: Xue ling zhi) (Caryophyllaceae), a very important Chinese folk medicine, have been used to treat influenza, lung inflammation, jaundice, and rheumatism.[1] However, no detailed studies have been reported on the chemical constituents of the plant. The present paper deals with the structure elucidation of four new β-carboline alkaloids named arenarines and the identification of two known β-carboline alkaloids.



	R_1	R_2
1:	$COCH_3$	H
2:	$COOCH_3$	H
3:	$COCH_2OCH_3$	H
4a:	$CH(OH)CH_2OCH_3$	H
4b:	$CH(OAc)CH_2OCH_3$	H
5:	$COCH_3$	OCH_3
6:	$COCH_3$	OH
7:	CH_3	OCH_3

Compounds **1** and **2** were identified as 1-acetyl-β-carboline[2] and 1-methoxycarbonyl-β-carboline,[3] respectively.

Structures of Arenarines A (3) and B (4a) Arenarines A (**3**) and B (**4a**) were determined to have the molecular formulae $C_{14}H_{12}N_2O_2$ and $C_{14}H_{14}N_2O_2$, respectively, by high-resolution mass spectrometry (HRMS). The ultraviolet (UV) absorptions of arenarines A (**3**) and B (**4a**) were characteristic of β-carboline type alkaloids.[2-4] The infrared (IR) spectra of **3** and **4a** showed an amino band at 3310 cm^{-1} and a conjugated carbonyl band at 1690 cm^{-1} for **3** and an amino band at 3300 cm^{-1} for **4a**. The proton magnetic resonance (^{1}H-NMR) spectra of the two alkaloids **3** and **4a** were similar. A pair of *ortho*-coupled signals [δ 8.49 (1H, d) and 8.18 (1H, dd) for **3** and δ 8.35 (1H, d) and 7.91 (1H, dd) for **4a**] were assigned to 3-H and 4-H. Four aromatic signals [δ 8.16 (1H, dddd), 7.35 (1H, ddd), 7.63 (1H, ddd), and 7.62 (1H, ddd) for **3** and δ 8.13 (1H, dddd), 7.28 (1H, ddd), 7.56 (1H, ddd), and 7.54 (1H, ddd) for **4a**] were assigned to 5-H to 8-H. The major differences between arenarines A (**3**) and B (**4a**) were in the spectral features arising from the C-1 functional group. In arenarine A (**3**), a methoxyl signal [^{1}H: δ 3.62 (3H, s) and ^{13}C:

TABLE I. ^{1}H-NMR Spectral Data for β-Carboline Alkaloids[a]

Proton	1	2	3	4a	4b	5	6	7
3-H	8.55 d (4.9)	8.60 d (5.0)	8.49 d (4.9)	8.35 d (5.0)	8.42 d (5.1)	8.49 d (4.9)	8.37 d (5.1)	8.31 d (5.5)
4-H	8.16 dd (4.9, 0.5)	8.16 dd (5.0, 0.5)	8.18 dd (4.9, 0.5)	7.91 dd (5.0, 0.5)	7.94 dd (5.1, 0.5)	8.03 d (4.9)	8.13 d (5.1)	7.71 d (5.5)
5-H	8.15 dddd (7.8, 1.3, 0.5, 0.5)	8.17 dddd (7.8, 1.3, 0.5, 0.5)	8.16 dddd (7.8, 1.3, 0.5, 0.5)	8.13 dddd (7.8, 1.3, 0.5, 0.5)	8.12 dddd (7.8, 1.3, 0.5, 0.5)	8.00 d (8.6)	8.00 d (8.6)	7.97 d (8.8)
6-H	7.34 ddd (7.8, 6.9, 1.4)	7.34 ddd (7.8, 6.9, 1.4)	7.35 ddd (7.8, 6.9, 1.3)	7.28 ddd (7.8, 7.0, 1.2)	7.28 ddd (7.8, 7.0, 1.2)	6.93 dd (8.6, 2.0)	6.81 dd (8.6, 2.0)	6.91 dd (8.8, 2.2)
7-H	7.61 ddd (7.9, 6.9, 1.3)	7.62 ddd (7.9, 6.9, 1.3)	7.63 ddd (8.1, 6.9, 1.3)	7.56 ddd (7.9, 7.0, 1.2)	7.57 ddd (7.9, 7.0, 1.2)			
8-H	7.59 ddd (7.9, 1.4, 0.5)	7.59 ddd (7.9, 1.4, 0.5)	7.62 ddd (8.1, 1.3, 0.5)	7.54 ddd (7.9, 1.2, 0.5)	7.55 ddd (7.9, 1.2, 0.5)	7.02 d (2.0)	7.05 d (2.0)	6.97 d (2.2)
9-H	10.30 s	9.92 s	10.22 s	9.63 s	9.13 s	10.23 s		8.06 s
1-$COCH_3$	2.90 s					2.89 s	2.79 s	
1-$COOCH_3$		4.13 s						
1′-H				5.38 t (6.1)	6.47 dd (7.0, 3.3)			
2′-H × 2			5.24 s	3.84, 3.88 (each dd, 9.4, 6.1)	4.07 dd (11.0, 3.3) 4.24 dd (11.0, 7.0)			
1′-$OCOCH_3$					2.18 s			
2′-OCH_3			3.62 s	3.49 s	3.43 s			
7-OCH_3						3.94 s		3.92 s
1-CH_3								2.80 s

a) Compounds **1**, **2**, **3**, **4a**, **4b**, **5**, and **7** in $CDCl_3$ and **6** in CD_3OD. Coupling constants in Hz.

δ 59.58], a methylene signal [^{1}H: δ 5.24 (2H, s) and ^{13}C: δ 75.0] and carbonyl carbon signal (δ 199.7) were observed and assigned to a $-COCH_2OCH_3$ group. In arenarine B (**4a**), a methoxyl signal [^{1}H: δ 3.49 (3H, s) and ^{13}C: δ 59.2], a nonequivalent methylene signal [^{1}H: δ 3.84 and 3.88 (each 1H, dd, J=9.4, 6.1 Hz) and ^{13}C: δ 72.2], and a methine signal [^{1}H: δ 5.38 (1H, t, J=6.1 Hz) and ^{13}C: δ 76.8] were observed. The methine proton at δ 5.38 was assigned to a proton on a hydroxy-bearing carbon because the signal shifted to δ 6.47 upon acetylation. These results suggested that arenarine B (**4a**) has a $-CH(OH)CH_2OCH_3$ group. On the basis of the above data, the structures of arenarines A and B were proposed to be formulae **3** and **4a** (except for the configuration at the C-1′ position of arenarine B), respectively.

Structures of Arenarines C (5) and D (6) Arenarines C (**5**) and D (**6**) were determined to have the molecular formulae $C_{14}H_{12}N_2O_2$ and $C_{13}H_{10}N_2O_2$, respectively, by HRMS. The UV spectra of **5** and **6** were similar to those of compounds **1**—**3**, which suggested that **5** and **6** have a carbonyl function at the C-1 position of a β-carboline skeleton. The IR absorption bands at 3370 and 1667 cm^{-1} for **5** and 3390 and 1675 cm^{-1} for **6** were assigned to amino and conjugated carbonyl groups, respectively. The ^{1}H-NMR spectra of the two alkaloids **5** and **6** were similar. A pair of *ortho*-coupled signals [δ 8.49 and 8.03 (each 1H, d) for **5** and δ 8.37 and 8.13 (each 1H, d) for **6**] were assigned to 3-H and 4-H. Three aromatic protons signals [δ 8.00 (1H, d, J=8.6 Hz), 6.93 (1H, dd, J=8.6, 2.0 Hz), and 7.02 (1H, d, J=2.0 Hz) for **5** and δ 8.00 (1H, d, J=8.6 Hz), 6.81 (1H, dd, J=8.6, 2.0 Hz) and 7.05 (1H, d, J=2.0 Hz) for **6**] were assigned to 5-H, 6-H, and 8-H by comparison with those of harmine (**7**). A three-proton singlet [δ 2.89 (3H, s) for **5** and δ 2.79 (3H, s) for **6**] was assigned to an acetyl group at the C-1 position. The major differences between arenarines C (**5**) and D (**6**) were in the features arising from the C-7 functional group. In arenarine C (**5**), a singlet signal at δ 3.94 (3H, s) was assigned to the methoxyl group at the C-7 position. Methylation of arenarine D (**6**) was carried out with diazomethane to afford arenarine C (**5**). On the basis of the above results, the structures of arenarines C and D were proposed to be formulae **5** and **6**.

This is the first report on the isolation and structure determination of β-carboline alkaloids from the genus *Arenaria* (Caryophyllaceae).

Experimental

All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. The UV and IR spectra were recorded with Hitachi 340 and Hitachi 260-30 spectrophotometers, respectively. The mass spectrum (MS) and HRMS were measured on JEOL JMS D-300 and JEOL DX-303 mass spectrometers, respectively. The ^{1}H- and ^{13}C-NMR spectra were measured with a JEOL JNM GX-400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometer. Chemical shifts are expressed in δ (ppm) downfield from tetramethylsilane as an internal standard, and coupling constants in herts (Hz). The following abbreviations are used: s=singlet, d=doublet, t=triplet, sh=shoulder. Column chromatography was carried out on silica gel (BWH-820 MH, Fuji Davison). Thin-layer chromatography (TLC) and preparative TLC were performed on silica gel (GF_{254}, Merck) and detection was done with Dragendorff reagent or by UV illumination. Harmine (7-methoxy-1-methyl-β-carboline, **7**) was purchased from Sigma Chemical Co., Ltd.

Extraction Dried whole plants (4 kg) of *Arenaria kansuensis* from the market in the city of Chengdu, China, were extracted with 48 l of methanol at room temperature for 72 h. The extract was evaporated to dryness, then the residue (93 g) was dispersed in water and shaken with ether, chloroform, ethyl acetate and *n*-butanol, successively.

Isolation The ether extract (39.4 g) was dissolved in ether and shaken with 5% $NaHCO_3$ and 5% NaOH succesively. The ether fraction (15.0 g) was chromatographed on silica gel to give 1-acetyl-β-carboline (**1**, 8 mg), arenarine A (**3**, 11 mg), and arenarine C (**5**, 5 mg). The chloroform extract (6.6 g) was chromatographed with silica gel to give 1-methoxycarbonyl-β-carboline (**2**, 2 mg), arenarine B (**4a**, 4 mg), and arenarine D (**6**, 11 mg).

1-Acetyl-β-carboline (1) Colorless needles (ethyl acetate), mp 212—213 °C (lit.[2] mp 204—205 °C). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 260 (3.45), 286 (3.64), 308 (3.24), 380 (3.24). IR ν_{max}^{KBr} cm^{-1}: 3390, 2198, 1675, 1620, 1490, 1205, 1160. MS *m/z* (%): 210 (M^+, 100), 182 (31), 168 (73). Compound **1** was shown to be identical with 1-acetyl-β-carboline by mixed melting point determination and comparisons of TLC behavior and IR spectra.

1-Methoxycarbonyl-β-carboline (2) Colorless needles (ethyl acetate), mp 162—163 °C (lit.[3] mp 163 °C). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 244 (3.62), 256 (3.62), 274 (3.68), 300 (3.42), 370 (3.17). IR ν_{max}^{KBr} cm^{-1}: 3390, 1682, 1628, 1465, 1312, 1210, 1175, 1072. ^{1}H-NMR: see Table I. MS *m/z* (%): 226 (M^+, 77), 194 (16), 168 (100), 157 (15). Compound **2** was shown to be identical with 1-methoxycarbonyl-β-carboline by mixed melting point determination and comparisons of TLC behavior and IR and ^{1}H-NMR spectra.

Arenarine A (1-Methoxymethylcarbonyl-β-carboline, 3) Yellow needles (ethyl acetate), mp 182—183 °C. UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 264 (3.71), 288 (3.91), 310 (3.52), 382 (3.52). IR ν_{max}^{KBr} cm^{-1}: 3310, 2930, 1690, 1630, 1205, 1125, 1025. ^{1}H-NMR: see Table I. ^{13}C-NMR ($CDCl_3$) δ: 29.4, 59.6, 75.0, 112.1, 119.4, 120.6, 121.0, 121.9, 129.5, 131.8, 134.4, 135.3, 138.2, 141.3, 199.7. MS *m/z* (%): 240 (M^+, 43), 225 (100), 210 (3), 197 (10), 182 (9), 167 (58). HRMS Calcd for $C_{14}H_{12}N_2O_2$ *m/z*: 240.0899. Found *m/z*: 240.0908.

Arenarine B (1-(2-Methoxy-1-hydroxyethyl)-β-carboline, 4a) Pale yellow needles (ethyl acetate), mp 157—158 °C. UV $\lambda_{max}^{CH_3OH}$ nm (log ε): 236 (4.11), 250 (sh, 3.93), 280 (sh, 3.61), 288 (3.78). IR ν_{max}^{KBr} cm^{-1}: 3300, 2940, 1636, 1500, 1440, 1244, 1136, 1100. ^{1}H-NMR: see Table I. ^{13}C-NMR ($CDCl_3$) δ: 59.2, 72.2, 76.8, 111.8, 114.1, 120.0, 121.3, 121.6, 128.6, 128.7, 129.0, 133.7, 137.0, 140.6. MS *m/z* (%): 242 (M^+, 20), 226 (11), 211 (9), 197 (100), 182 (5), 168 (24). HRMS Calcd for $C_{14}H_{14}N_2O_2$ *m/z*: 242.1055. Found *m/z*: 242.1055.

Acetylation of **4a**: Arenarine B (**4a**, 1.3 mg) was acetylated with Ac_2O (0.2 ml) in pyridine (0.2 ml) at room temperature for 48 h to give monoacetylarenarine B (1.5 mg) as pale yellow needles. IR ν_{max}^{KBr} cm^{-1}: 3380, 1740, 1630, 1235. MS *m/z* (%): 284 (M^+, 37), 241 (6), 225 (100), 197 (82), 168 (18). ^{1}H-NMR: see Table I.

Arenarine C (1-Acetyl-7-methoxy-β-carboline, 5) Colorless needles [*n*-hexane–ethyl acetate (1 : 1)], mp 190 °C (dec.). UV $\lambda_{max}^{CHCl_3}$ nm (log ε): 260 (4.10), 292 (4.30), 334 (3.94), 366 (3.91). IR ν_{max}^{KBr} cm^{-1}: 3370, 1662, 1630, 1570, 1460, 1260, 1165, 1150. ^{1}H-NMR: see Table I. ^{13}C-NMR ($CDCl_3$) δ: 25.0, 55.7, 98.2, 110.4, 114.3, 118.1, 122.8, 131.8, 135.6, 135.8, 138.5, 143.0, 161.7, 203.5. MS *m/z* (%): 240 (M^+, 100), 212 (33), 198 (63), 183 (14), 169 (6), 155 (13). HRMS Calcd for $C_{14}H_{12}N_2O_2$ *m/z*: 240.0899. Found *m/z*: 240.0922.

Arenarine D (1-Acetyl-7-hydroxy-β-carboline, 6) Pale yellow needles (ethyl acetate), mp 176—177 °C (dec.). UV $\lambda_{max}^{CH_3OH}$ nm (log ε): 260 (3.45), 288 (3.66), 330 (3.15), 370 (3.15). IR ν_{max}^{KBr} cm^{-1}: 3390, 2975, 1675, 1640, 1390, 1270, 1175. ^{1}H-NMR: see Table I. ^{13}C-NMR (CD_3OD) δ: 26.2, 98.7, 111.8, 114.7, 118.9, 123.7, 128.7, 133.9, 136.1, 136.5, 145.6, 160.9, 204.0. MS *m/z* (%): 266 (M^+, 100), 198 (37), 184 (92), 156 (15). HRMS Calcd for $C_{13}H_{10}N_2O_2$ *m/z*: 226.0742. Found *m/z*: 226.0742.

Methylation of **6**: Arenarine D (**6**, 2 mg) was dissolved in methanol and methylated with diazomethane–ether at room temperature for 48 h to give 7-*O*-methylarenarine D (2 mg), mp 190 °C. MS *m/z*: (240, M^+). IR λ_{max}^{KBr} cm^{-1}: 3370, 1662, 1630, 1260, 1150, which was shown to be identical with arenarine C (**5**) by mixed melting point determination and comparisons of TLC behavior and IR spectra.

References

1) Jiansu New Medicinal College, "Great Chinese Medicinal Plants Dictionary," Shanghai Science and Technique Publishing House, Shanghai, China, 1977, p. 2087.
2) T. Ohmoto and K. Koike, *Chem. Pharm. Bull.*, **30**, 1204 (1982).
3) T. Ohmoto and K. Koike, *Chem. Pharm. Bull.*, **32**, 3579 (1984).
4) T. Ohmoto and K. Koike, *Chem. Pharm. Bull.*, **31**, 3198 (1983); T. Ohmoto, K. Koike, T. Higuchi and K. Ikeda, *Chem. Pharm. Bull.*, **33**, 3356 (1985).