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KUKOAMINE B, A SPERMINE ALKALOID FROM *LYCIUM CHINENSE*

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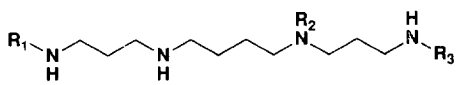
Key Word Index—*Lycium chinense*; Solanaceae; root bark; kukoamine B; spermine alkaloid, NMR.

Abstract—A new spermine alkaloid, kukoamine B, was isolated from the root bark of *Lycium chinense* and its structure was characterized by a combination of spectroscopic analyses and chemical degradation. Kukoamine B consists of two dihydrocaffeoyl moieties and a spermine unit.

INTRODUCTION

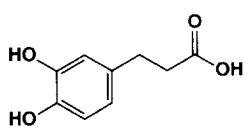
Dried root bark of *Lycium chinense* is used in oriental medicine as a tonic and is reported to exhibit hypotensive, hypoglycaemic, antipyretic and anti-stress ulcer activity in experimental animals [1, 2]. Kukoamine A (**1**) [1] was isolated as a hypotensive principle and (*S*)-9-hydroxy-*E*-10, *Z*-12-octadecadienoic (α-dimorhecolic) and (*S*)-9-hydroxy-*E*-10,*Z*-12,*Z*-15-octadecatrienoic acids showed inhibitory activity against angiotensin I-converting enzyme (ACE) [3]. Betaine and linoleic acid [4], β-sitosterol glucoside [5] and aurantiamide acetate (lyciumamide) [6, 7] were also isolated from this plant material. Kukoamine A (**1**) has been synthesized by two groups independently [8, 9]. In the present work, we describe the isolation and structural determination of kukoamine B (**2**), a new spermine alkaloid from *L. chinense*.



1 $R_1 = R_3$ = dihydrocaffeoyl, R_2 = H

2 $R_1 = R_2$ = dihydrocaffeoyl, R_3 = H

4 $R_1 = R_2 = R_3$ = H



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RESULTS AND DISCUSSION

The crude drug (10 kg) was extracted with *n*-hexane, methanol, 50% aq. methanol and water, successively. The 50% aq. methanol extract was dissolved in water and subjected to Diaion HP-20 column chromatography, followed by repeated chromatography on Sephadex LH-20, to give a new spermine alkaloid, kukoamine B (**2**).

Kukoamine B (**2**) was obtained as a pale yellow powder soluble in water and partially soluble in methanol. It showed positive colour reactions with $FeCl_3$ solution (black), Dragendorff's reagent (vermilion) and ninhydrin reagent (purple). Kukoamine B (**2**) showed a similar UV spectrum to that of kukoamine A (**1**) [1] and in the FAB-mass spectrum a $[M + 1]^+$ *m/z* 531 was observed which is the same as that of kukoamine A. Kukoamine A (**1**) possesses a symmetrical chemical structure and, in the ^{13}C NMR spectrum, 14 carbon signals were observed [1] against the molecular formula $C_{28}H_{42}N_4O_6$. On the other hand, in the ^{13}C NMR spectrum of kukoamine B (**2**), 28 signals including 14 methylenes, six methines and eight quarternary carbons were observed. In the ^1H NMR spectrum of this compound, signals attributed to 28 aliphatic hydrogens were observed, other than six aromatic hydrogens. Among these signals, all CH signals and six quarternary signals are attributed to those of two phenolic aromatic rings, the two quarternary signals (δ_C 175.7 and 176.0) to those of carbonyl carbons. The number of hydrogens attached to carbons is 34 from the results of the ^{13}C NMR spectrum; this is the same as kukoamine A (**1**). The 1,2,4-trisubstitution pattern of the two phenolic aromatic rings was deduced by a combination of ^1H and ^{13}C NMR data and it was thought that this compound consisted of two dihydrocaffeic acid (**3**) units and one spermine (**4**). Thus, the total number of hydrogens attached to oxygens and nitrogens was estimated to be eight and the molecular formula and M_r were deduced to be $C_{28}H_{42}N_4O_6$ and 530, respectively. When kukoamine B was hydrolysed with 6 M HCl at 115°, dihydrocaffeic acid (**3**) and the HCl salt of spermine (**4**) were obtained.

From the ^1H–^1H COSY spectrum of kukoamine B (**2**), connections of 10 methylene signals attributed to the spermine unit ($2\times$ $-CH_2-CH_2-CH_2-$ and $1\times$ $-CH_2-CH_2-CH_2-CH_2-$) were elucidated and, from the ^1H–^{13}C COSY spectrum, correlations as shown in Fig. 1 were deduced. In the ^1H NMR spectrum, methylene signals attached to the –NHCOR group appeared at lower field (δ_H 3.33) [1] and three such signals (δ_H 2.94, 2.97 and 3.14) were observed in kukoamine B (**2**). A multiplet methylene signal (δ_H 1.44) was found to be coupled to two triplet methylene signals at δ_H 2.94 and 2.31; these three signals were considered to be part of the spermine moiety. Another set of three methylenes (δ_H 3.14, 1.58 and 2.57) and a set of four methylene signals (δ_H 2.51, 1.23, 1.13 and 2.97) have been assigned in a similar way and correlations between δ_H 2.31 and δ_C 46.8 (δ_H 2.51) and δ_H 3.14 and δ_C 47.3 (δ_H 2.97) (Fig. 1) suggested the position of these three sets of methylenes. From these observations, δ_H 2.97 and 3.14 were attributed to the C-9 and C-11 methylene moieties and these two groups and a methylene (δ_H 2.94) were attached to the amide moiety (Table 1). Accordingly, it was concluded that the two dihydrocaffeoyl moieties were attached to the N-1 and N-10 positions of the spermine unit and the structure of kukoamine B was established as **2**. The occurrence of ^1H–^{13}C long-range couplings between δ_H 2.94 and δ_C 176.0 and δ_H 3.14 and δ_C 175.7 (Fig. 1) supported this conclusion. Because long range couplings between δ_H 2.57 and δ_C 176.0, δ_H 2.31 and δ_C 132.7, δ_H 2.59 and δ_C 175.7 and δ_H 2.45 and δ_C 133.3 were observed, the former two sets of signals were assigned to the dihydrocaffeoyl unit attached to N-1, the latter two to the dihydrocaffeoyl moiety attached to the N-10 position of the spermine moiety. Other signals were assigned as shown in Fig. 1 and Table 1. Because no correlation was observed between the phenolic carbons and hydrogens except for C-1′ and C-1″, part of the ^{13}C and ^1H NMR spectral data for the two phenylpropanoid moieties (H-2′–H-6′ and H-2″–H-6″) could not be distinguished.

Table 1. ^1H and ^{13}C NMR spectral data of kukoamine B (**2**)*

C	δ_C	δ_H	
2	35.3 *t*	2.94	2H, *t* (*J* = 7 Hz)
3	25.2 *t*	1.44	2H, *m*
4	44.2 *t*	2.31	2H, *t* (*J* = 7 Hz)
6	46.8 *t*	2.51	2H, *t* (*J* = 8 Hz)
7	22.5 *t*	1.23	2H, *m*
8	24.7 *t*	1.13	2H, *m*
9	47.3 *t*	2.97	2H, *t* (*J* = 8 Hz)
11	42.3 *t*	3.14	2H, *t* (*J* = 7 Hz)
12	24.6 *t*	1.58	2H, *m*
13	36.5 *t*	2.57	2H, *t* (*J* = 7 Hz)
1′	132.7 *s*	—	
2′	115.9 *d*	6.51	1H, *d* (*J* = 2 Hz)
3′	143.6 *s*	—	
4′	142.0 *s*	—	
5′	116.0 *d*	6.58	1H, *d* (*J* = 7 Hz)
6′	120.4 *d*	6.43	1H, *dd* (*J* = 7, 2 Hz)
7′	30.1 *t*	2.57	2H, *t* (*J* = 7 Hz)
8′	36.6 *t*	2.31	2H, *t* (*J* = 7 Hz)
9′	176.0 *s*	—	
1″	133.3 *s*	—	
2″	116.1 *d*	6.53	1H, *d* (*J* = 2 Hz)
3″	143.7 *s*	—	
4″	142.1 *s*	—	
5″	116.1 *d*	6.60	1H, *d* (*J* = 7 Hz)
6″	120.6 *d*	6.44	1H, *dd* (*J* = 7, 2 Hz)
7″	30.4 *t*	2.59	2H, *t* (*J* = 7 Hz)
8″	33.8 *t*	2.45	2H, *t* (*J* = 7 Hz)
9″	175.7 *s*	—	

*Assignments of H-2′–H-6′ and H-2″–H-6″ may be interchanged.

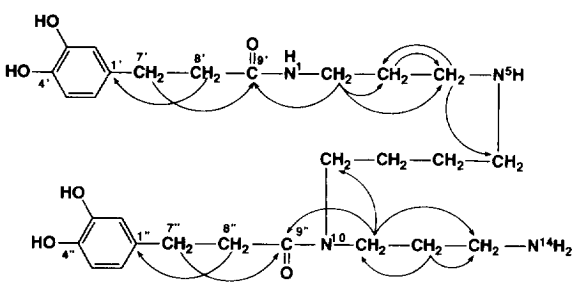

Fig. 1. ^{13}C–^1H correlations in the long-range C–H COSY spectrum of kukoamine B (**2**).

EXPERIMENTAL

General. ^1H NMR (300 and 500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded using TMS as int. standard and chemical shifts are recorded in δ units. Wakogel C-200 (Wako Pure Chemical Ind. Ltd), Diaion HP-20 (Nippon Rensui Co.), Dowex 50W × 8 (200–400 mesh) (Dow Chemical Co.) or Sephadex LH-20 (Pharmacia) were used for CC and DC-Fertigplatten Kieselgel 60 F_{254} (0.25 mm thick, Merck) was used for prep. TLC. DC-Alufolien Kieselgel 60 F_{254} (0.2 mm thick, Merck) and DC-Alufolien Cellulose F_{254} (0.2 mm thick, Merck) were used for TLC analyses.

Plant material. Dried and chopped root bark of *Lycium chinense* Miller was purchased from Kinokuniya Co. (Tokyo, Japan, Lot. No. M9IJ0G02A). A voucher specimen is deposited in the herbarium of this department.

Extraction and isolation. Dried and chopped root-bark of *Lycium chinense* (10 kg) was extracted with *n*-hexane (× 2), MeOH (× 3), MeOH–H_2O (1:1, × 2 times) and H_2O (× 1), to afford *n*-hexane (11 g), MeOH (200 g), 50% aq. MeOH (445 g) and H_2O (134 g) extracts. Part of the 50% aq. MeOH extract (191 g) was dissolved in H_2O and passed through a column of Diaion HP-20 (2.4 kg). The column was washed with H_2O to give frs I (39.3 g), II (76.4 g) and III (1.6 g). The column was then eluated with aq. MeOH to give fr. IV (65.5 g). Fr. III was chromatograhed repeatedly on a Sephadex LH-20 column to afford kukoamine B (120.7 mg) as a pale yellow powder. UV λ_{max}^{MeOH}: 281 nm. IR ν_{max}^{KBr}: 3300, 1603, 1522, 1384, 1281,

1112, 825 cm^{-1}. FAB-MS: *m/z* 531 $[M + 1]^+$, 515, 367, 307, 293, 277, 251, 215, 201, 197, 185, 165, 147, 131, 123, 115, 105, 93. ^1H and ^{13}C NMR (D_2O): Table 1.

Acid hydrolysis. Kukoamine **B** (22.8 mg) was dissolved in 6 M HCl (1 ml) in a sealed tube and heated for 15 hr at 115°. The reaction product was diluted with H_2O (10 ml) and extracted with Et_2O (2 × 10 ml). The combined Et_2O-soluble portions were washed with H_2O (10 ml) and concd *in vacuo* to give a gummy residue (8.5 mg) which was purified by prep. TLC ($CHCl_3$–MeOH, 4:1, R_f 0.54) to give dihydrocaffeic acid (**3**). Identification of this compound was achieved by NMR and TLC comparison with an authentic sample [1]. The H_2O-soluble portion (17.4 mg) was passed through a column of Dowex 50W × 8 (H^+ form) and the column eluted with 5% HCl to give spermine (**4**) as the HCl salt. Identification of **4** was made by TLC comparison with an authentic sample (8% aq. NaCl–HOAc, 100:1, R_f 0.65) [1].

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