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Analysis of Alkaloids in the Seeds of *Zizyphus jujuba* by High Performance Liquid Chromatography

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Abstract □ A high performance liquid chromatographic method was developed for the separation and determination of seven alkaloids in "sanjoin" (the seeds of *Zizyphus jujuba*, Rhamnaceae), a plant with potent sedative activity. A reverse phase system of Lichrosorb RP-Select B column and 0.05 M potassium phosphate buffer (pH=3.5)-acetonitrile with gradient elution was employed. Two known alkaloids, juzirine and lysicamine, were newly isolated fom "sanjoin".

Keywords □ *Zizyphus jujuba*, Rhamnaceae, cyclopeptide alkaloid, determination of alkaloid, lysicamine, juzirine.

Sanjoin, the seed of *Zizyphus jujuba* (Rhamnaceae), has long been used as a folk medicine for the treatment of insomnia and nervous breakdown[1-3]. Our group[4-7] reported several alkaloids as the sedative principle of "sanjoin". The present study describes isolation and identification of two alkaloids which were not isolated in the previous work and HPLC determination of seven alkaloids in this herbal drug.

EXPERIMENTAL METHODS

Procedure for the determination of the alkaloids

Instruments: The instrument employed was a Hitachi (L-6200 Intelligent) pump (Hitachi, Japan) equipped with 20 μl loop injector model 7125 (Rheodyne, USA) connected to a Hitachi UV/VIS spectrophotometric detector and Phillips integrator (Phillips, Netherlands). A reverse phase column, Lichrosorb RP-Select B (5 μm, 150 mm×4.0 mm i.d., Merck) was used at room temperature.

Materials: Acetonitrile-HPLC grade was purchased from Merck Ltd. (Merck, Germany). Always distilled and deionized water was used for the experiment. Other chemicals used were all GR grade. Sanjoin was purchased from the local herbal drugs market in Seoul.

Chromatographic conditions: The mobile phase consisted of A: 0.05 M potassium phosphate (pH=3.5) /Acetonitrile=90/10, B: 0.05 M potassium phosphate (pH=3.5)/Acetonitrile=60/40, linear gradient from 0% to 75% of B between time 5 to 50 min. The flow rate was 0.7 ml/min. Detection of the peaks at 254 nm and the sensitivity was set at 0.05 AUFS.

Preparation of the standard solutions: Standard alkaloids were dissolved in chloroform/methanol=5/1 with following concentrations; sanjoinine-A 0.352 mg/ml, sanjoinine-F 0.036 mg/ml, sanjoinine-G_2 0.024 mg/ml, lysicamine 0.012 mg/ml, sanjoinine-K 0.488 mg/ml, juzirine 0.0173 mg/ml, caaverine 0.28 mg/ml.

Preparation of the sample solution: Crashed sanjoin (200 g) was extracted three times with *n*-hexane and the residue was extracted three times with methanol. Hexane and methanol extracts were subjected to further solvent fractionation to yield alkaloidal fraction according to Han's method[6,7]. The alkaloidal fraction was dissolved in 10 ml of chloroform/methanol=5/1. The solutions were filtered through 0.45 μm membrane filter and applied to HPLC.

Assay: Equal volumes (10 μl) of standard and sample solutions were injected into the HPLC and chromatographed under the conditions described above. The chromatographic peaks in sample solution were identified by the conjection with the standards, and were determined by the comparison of peak areas.

Procedure of the extraction and isolation of alkaloids

Crushed seeds of *Zizyphus jujuba* (100 kg) were extracted with boiling *n*-hexane (90 *l*×2) and MeOH (90 *l*×2) successively. MeOH extract (4.8 kg) was suspended in water (10 *l*) and extracted with Et_2O (15 *l*×4). Hexane fraction (17 kg) and Et_2O fraction were extracted with 5% HCl and the aqueous phase was basified with c-NH_4OH to pH=10 and then extracted with $CHCl_3$ to yield alkaloidal fraction (total 16.5 g).

Isolation of lysicamine

Alkaloidal fraction (16.5 g) was chromatographed over silica gel (Merck Art. 9385, 4 cmφ×52 cm) and eluted with $CHCl_3$ to yield Fr. I (126 mg), Fr. II (150 mg), Fr. III (140 mg), Fr. IV (14.5 g). Fr. II (150 mg) was further chromatographed over silica gel 9385 with $CHCl_3$-EtOAc (5:1) to yield lysicamine (5 mg).

mp. 212℃; UV: λ_{max} (MeOH) (log ε): 235 (4.48), 271 (4.40), 308 (3.77), 400 (3.96); IR (cm^{-1}, KBr): 1675 (C=O); ^{1}H-NMR ($CDCl_3$, TMS) δ ppm: 4.02 (3H, s, 2-OCH_3), 4.10 (3H, s, 1-OCH_3), 7.22 (1H, s, 3-H), 7.78-7.55 (2H, m, 9-H, 10-H), 7.79 (1H, d, *J*=4.8 Hz, 4-H), 8.58 (1H, dd, *J*=7.7, 1.9 Hz, 8H), 8.91 (1H, d, *J*=4.8 Hz), 9.17 (1H, dd, *J*=8, 1.6 Hz); MS [EI^+, m/z] (Rel. Int%): 291 (M^+, 37.3), 249 (18.4), 248 (100), 233 (19.6), 220 (5.4), 205 (10.1).

Isolation of juzirine

Fr. IV was chromatographed over silica gel (Merck Art. 9385) with $CHCl_3$-MeOH (80:1) to give Fr. IV-1 (300 mg), Fr. IV-2 (600 mg), Fr. IV-3 (300 mg), Fr. IV-4 (1 g), Fr. IV-5,6 (1.8 g) and Fr. IV-7 (9 g). Fr. IV-7 was further chromatographed over silica gel with $CHCl_3$-MeOH (5:1) to give from Fr. IV-7-1 to Fr. IV-7-7. Juzirine (40 mg) was crystallized from Fr. IV-7-5.

mp. 203-205℃; IR (cm^{-1}, KBr): 3380 −3200 cm^{-1} (-OH); UV (MeOH): λ_{max} (log ε): 239 (4.48), 273 (3.60), 280 (3.60), 320 (3.30), ^{1}H-NMR (80 MHz, DMSO) δ ppm: 9.72 (1H, s), 9.07 (1H, s), 8.16 (1H, d, *J*=5.5 Hz), 7.46 (1H, d, *J*=5.5 Hz), 7.44 (1H, s), 7.26 (1H, s), 6.97 (2H, d, *J*=7.0 Hz), 6.62 (2H, d, *J*=7.0 Hz), 4.29 (2H, s), 3.90 (3H, s); MS [EI^+, m/z] (Rel. Int%): 281 (3.6), 280 (8.7), 265 (1.4),248 (2.3),

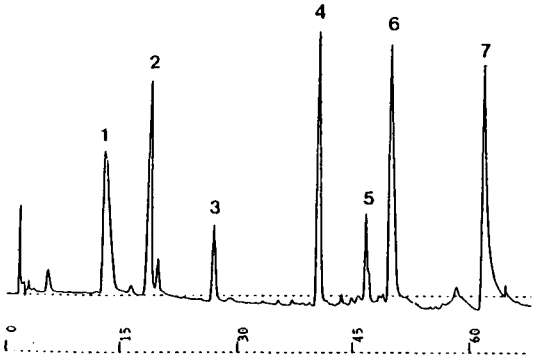


Fig. 1. Chromatogram of standard alkaloids.
Column: RP-Select B (150 mm×4.0 mm I.D., 5 μm), Flow-rate: 0.7 ml/min. Detector: UV 254 nm, Conditions: linear gradient of eluent B from 0% to 75% between 5 to 50 min, Peaks: 1=sanjoinine-K; 2=juzirine; 3=caaverine; 4=sanjoinine-F; 5=sanjoinine-G_2; 6=sanjoinine-A; 7=lysicamine.

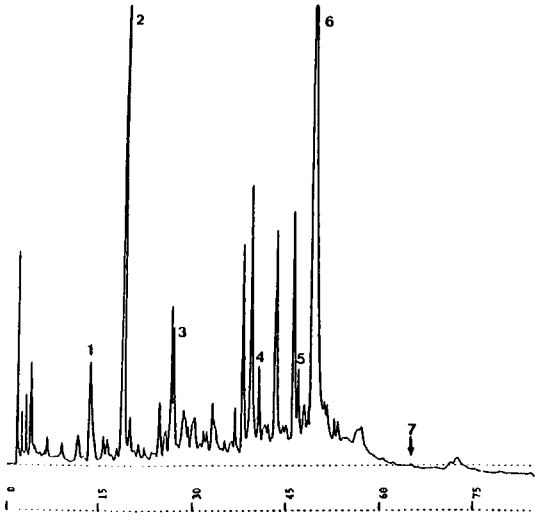


Fig. 2. Chromatogram of alkaloid fraction of the seeds of *Zizyphus jujuba*.
Conditions as in Fig. 1, Peaks: 1=sanjoinine-K; 2=juzirine; 3=caaverine; 4=sanjoinine-F; 5=sanjoinine-G_2; 6=sanjoinine-A; 7=lysicamine.

Table I. Contents of the alkaloids in the seeds of *Zizyphus jujuba*

Alkaloids	*Content in sanjoin (%)
Sanjoinine-A (Frangufoline)	(9.73± 0.91) $\times 10^{-3}$%
Sanjoinine-F	(11.09± 1.90) $\times 10^{-4}$%
Sanjoinine-G_2	(2.57± 0.65) $\times 10^{-4}$%
Sanjoinine-K	(2.38± 0.54) $\times 10^{-3}$%
Caaverine	(4.30± 0.54) $\times 10^{-3}$%
Juzirine	(0.94± 0.27) $\times 10^{-4}$%
Lysicamine	(1.23± 0.62) $\times 10^{-4}$%

*Mean± S.D. (n=4)

237 (3.2), 220 (36.4), 205 (100), [Cl^+, reactant gas: CH_4, m/z] (Rel. Int%): 310 (25.9), 283 (14.7), 282 (100), 281 (86.9), 264 (2.4).

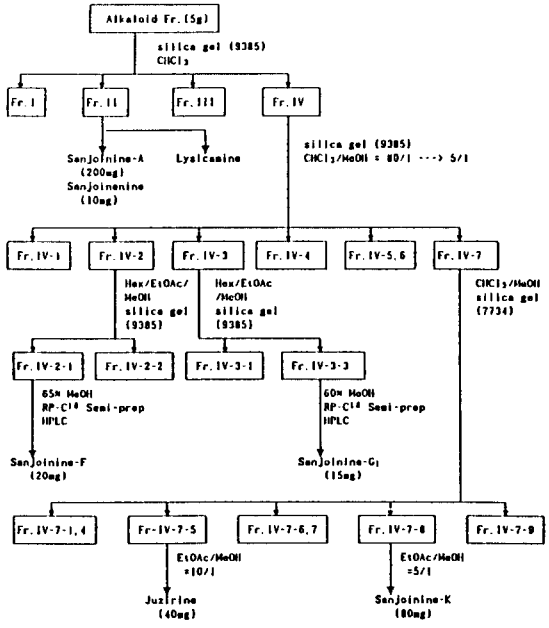
RESULTS AND DISCUSSION

Fig. 1 shows the chromatogram of standard alkaloid mixture, which could be separated completely by gradient elution. Fig. 2 shows the chromatogram of the plant extract. Many solvent systems were attempted to resolve the alkaloids, potassium phosphate buffer was better than sodium phosphate buffer or potassium acetate buffer and optimal pH was found at 3.5. The RP-Select B column, designed for the better resolution of the basic compounds, was better than RP-C_{18} column in resolution of the alkaloids. Table I shows the analytical results, the contents of alkaloids in "sanjoin".

Two known alkaloids, but have not been isolated from "sanjoin", juzirine and lysicamine were isolated from the ether soluble fraction by silica gel column chromatography using $CHCl_3$/EtOAc and EtOAc/MeOH solvent system following Scheme 1.

Lysicamine ($C_{18}H_{13}NO_3$)[8], mp 212℃, gave a yellowish-red color with Dragendorff's, reagent. The ^{1}H-NMR spectrum of lysicamine exhibited signals at δ 4.02 (3H, s, 2-OCH_3), 4.10 (3H, s, 1-OCH_3), and aromatic protons between δ 7.2-9.2. The mass spectrum showed m/z 291 (M^+), 276 (M^+ 15), 261 (M^+ 30), 248 (M^+ -15-28, 100%). These data coincided with the reported data for lysicamine[10].

Juzirine ($C_{17}H_{15}NO_3$)[9], mp 203-205℃, gave a positive Dragendorff's test (redish brown), and showed mass fragmentation of typical benzylisoquinoline alkaloid pattern (m/z 281 (M^+), 280 (100%), 265, 248,



Scheme. 1. Isolation procedure of alkaloid fraction of "sanjoin", the seeds of *Zizyphus jujuba*.

237, 220)). The ^{1}H-NMR spectrum of the juzirine showed signals of the protons of methoxy group at δ 3.9 (3H, s), one methylene group at δ 4.2 (2H, s), two hydroxy group at δ 9.0 and 9.7 and eight aromatic protons between δ 6.5-8.0. These data coincided with the reported data for the juzirine[11].

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