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Alkaloids are the Sedative Principles of the Seeds of *Zizyphus vulgaris* var. *spinosus*

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Abstract □ Sedative principles of the seeds of *Zizyphus vulgaris* var. *spinosus* have been characterized as sanjoinine-A (frangufoline), nuciferine and their congeners. Also, heat-treatment of sanjoinine-A produced a more active artifact, sanjoinine-Ahl, which provides a scientific basis for heat-processing (roasting) of this Oriental medicine.

Keywords □ *Zizyphus vulgaris* var. *spinosus*, rhamnaceae, sedative activity, cyclopeptide alkaloid, aporphinoid, sanjoinine-A (frangufoline), heat-processing, sanjoinine-Ahl.

Sanjoin (酸棗仁), the seeds of *Zizyphus vulgaris* var. *spinosus* Bunge (Rhamnaceae) has been used as the most important hypnotic agent in Chinese medicine to treat insomnia as sedatives and nervine tonics.[1-3] In earlier studies on the pharmacological aspect, hypnotic[4], tranquilizer[5], sedative[6-9], analgesic[6], antiinflammatory[6], antiarrhythmic[10], and hypotensive[11] activities have been described. Chemical studies in connection with active principles, saponin and flavonoid whose effective doses were shown to be somewhat higher as a pure substance[8,9], have been already reported. Kim[5] has reported a major tranquilizer activity in the alkaloid fraction, however, he didn't isolate this active alkaloid in a pure state. On the other hand, in a Chinese Materia Medica book[12] it was described that roasting the seeds potentiates a hypnotic activity, but this heat-processing at too high temperature or for too long time reduces the activity. Present paper describes a sedative activity of the isolated alkaloid components and also a new finding that the ethnopharmacological experiences concerned with heat treatment was demonstrated as being true by our molecular pharmacological studies using the isolated alkaloids.

EXPERIMENTAL METHODS

Animals

Male albino mice weighing 22-24g were used and fed laboratory chows and tap water and maintained in a constant temperature environment throughout the experiments.

Hexobarbital-induced sleeping time prolongation test

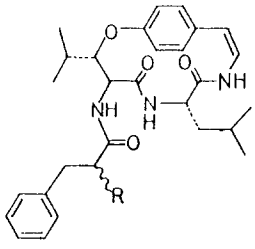
Six or seven animals in each group were injected with 50 mg/kg of hexobarbital sodium in a saline at 60 min. after oral administration, or 30 min. after intraperitoneal injection of the samples. The sleeping time was measured from a loss of righting reflux to recovering.

Extraction and fractionation

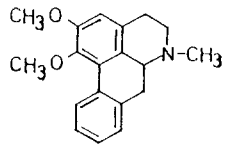
Zizyphus seeds (5.5 kg) were crushed and extracted with 18 *l* of benzene under reflux for ten hours two times. Benzene extract (1865 g) was suspended in 2.5 *l* of 8% HCl and extracted with 2.5 *l* of Et_2O four times. The aqueous phase was basified with c-NH_4OH to pH 9 and extracted with Et_2O 2.5 *l* three times to give alkaloid fraction (0.65 g). The residue after benzene extraction was extracted with boiling MeOH 18 *l* two times to give MeOH extract (440 g). MeOH extract was suspended in water (1 *l*) and extracted with Et_2O (1 *l* × five times) and then n-BuOH (1 *l* × 3 times) extraction followed to give ether fraction (180 g), butanol fraction (46 g) and water fraction (213 g). Alkaloid fraction of ether extract was prepared as above (0.52 g). Samples for mice were prepared in a saline (0.2 ml/20 g body weight).

Preparation of sanjoinine-Ahl

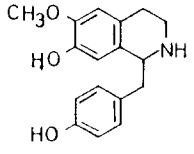
Sanjoinine-A (300 mg) was dissolved in 50 ml of MeOH and mixed with alkaloid-free purified oil of the Zizyphus seeds and then MeOH was removed under vacuum. The solution was heated to 260 °C



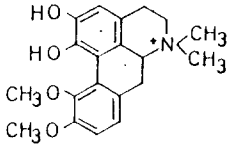
Sanjoinine-A(frangufoline)
R = —N(CH$_3$)$_2$ (*S*)
Sanjoinine-Ah$_1$
R = ···N(CH$_3$)$_2$ (*R*)



Nuciferine



Coclaurine



Zizyphusine

Fig. 1. Structures of alkaloids tested for sedative activity from Zizyphus seeds.

within three minutes and poured into 250 m*l* of Et_2O and extracted with 5% HCl (120 m*l* × 2). The 5% HCl layer was washed exhaustively with Et_2O and basified with c-NH_4OH to pH 10 and extracted with $CHCl_3$ (200 m*l* × 3). The $CHCl_3$ extracts were dried over Na_2SO_4 and evaporated to dryness (210 mg). The residue was subjected to silica gel flash column chromatography (2 × 22 cm) and eluted with $CHCl_3$: MeOH (50:1 ⟶ 30:1 ⟶ 20:1) to yield unchanged sanjoinine-A (90 mg) and sanjoinine-Ahl rich fraction. This sanjoinine-Ahl fraction was purified by preparative thin layer chromatography. The solid obtained from the band Rf 0.36 ($CHCl_3$:MeOH = 20:1) was crystalized from $CHCl_3$-MeOH to yield needles (30 mg).

RESULTS AND DISCUSSION

In order to re-evaluate the sedative activity and to isolate its active principles from Zizyphus seeds, organic solvent extraction and fractionation were carried out with monitoring the biological activity in an each fraction by prolongation of the hexobarbital sodium induced sleeping time in mice. As shown in Table I, methanol extract prolonged the hexobarbital induced sleeping time more than 67% as compared to that of control. Benzene extract was not much active[8] (data were not shown) because active principles were largely diluted with a lot of oil in the seeds. The alkaloid contents in the benzene soluble, ether soluble or butanol soluble fraction could be increased by partial purification to give sedative activity, however final water soluble fraction gave no sedative activity. Total activity in benzene and ether soluble alkaloid fraction was almost same with that of BuOH soluble fraction, however, specific activity of alkaloid fractions (sleeping time

Table I. Fractionation scheme and sedative activity of fractions of Zizyphus seeds

Zizyphus Seeds (5.5 kg)
↓
Benzene extract (1865 g) ⟶ Alkaloid fraction (0.65 g)
↓
Methanol extract (440 g) ⟶ Ether fraction (180 g)
↓ ↓
Butanol fraction (46 g) Alkaloid fraction (0.52 g)
↓
Water fraction (213 g)

Fraction	Sleeping Time	
	Control	Sample
Methanol extraction (1.0 g/kg)	27.7 ± 7.6	46.5 ± 17.3
C_6H_6 alkaloid fraction (50 mg/kg)	23.6 ± 11.8	29.2 ± 11.7
Ether fraction (0.5 g/kg)	27.7 ± 7.6	30.3 ± 11.0
Butanol fraction (0.5 g/kg)	28.8 ± 16.3	41.2 ± 15.4
Water fraction (0.5 g/kg)	28.8 ± 16.3	27.5 ± 14.3

Samples were orally administered 60 min. before hexobarbital-Na 50 mg/kg *i.p.* injection. Time in min., mean ± S.E., n = 6-7.

prolongation % per gram of sample) was roughly six times greater than that of BuOH fraction. Since the thin layer chromatograms of two alkaloid fractions were similar each other, two fractions were combined and subjected to further isolation procedures.

Repeated silica gel column chromatography and preparative thin layer chromatography allowed us to isolate fifteen alkaloids as a crystalline state in a low isolated yield 10^{-5} - 10^{-3} % of the seeds.[13] Their structure were determined by chemical and spectral analysis.[13] Eight alkaloids revealed peptide nature, six alkaloids aporphinoids and one tetrahydrobenzylisoquinoline. Sanjoinine-A (frangufoline), nuciferine, coclaurine and zizyphusine were isolated in sufficient amount and therefore were selected as the representative compounds of each alkaloid series namely cyclic peptide, aporphinoid, benzylisoquinoline and quaternary ammonium alkaloid, respectively for the sedative activity test in animals.

As shown in Table II, sanjoinine-A (frangufoline) and nuciferine showed strong prolongation activity, while coclaurine and zizyphusine were not. The sedative activity of nuciferine has been already reported as having major tranquilizing nature.[14] The sedative activity of sanjoinine-A at dose of 3 mg/kg was potent enough to see the cyclopeptide alkaloid as one of the effective components and this was the first finding of sedative activity in cyclopeptide alkaloids. Actually these alkaloids exist as a mixture of certain ratio in Zizyphus seeds, hence some drug interactions such as synergic or counteracting may be expected. In order to clarify this possibility sanjoinine-A was co-administered with nuciferine and coclaurine respectively. From the results summarized in Tables III and IV, only additive effect between sanjoinine-A and nuciferine was observed. Coclaurine was considered to have no contribution to sedative effect of Zizyphus seeds. On the sedative effect of butanol fraction, pure isolated quaternary ammonium type alkaloid, zizyphusine (isolated yield 6.2×10^{-3}% of the seeds) showed no activity (Table II), but butanol fraction itself showed some sedative activity. This could be explained as following; the activity of butanol fraction may come from small amount of aporphine alkaloids such as caaverine, N-methylas-

Table IV. Effect of co-administration of sanjoinine-A and coclaurine on the sleeping time

Control	Sanjoinine-A 1 mg/kg	Sanjoinine-A 1 mg/kg + Coclaurine 10 mg/kg	Coclaurine 10 mg/kg
34.1 ± 11.5	47.8 ± 17.5	50.7 ± 11.4	38.6 ± 13.8

Samples were administered *i.p.* 30 min. before hexobarbital sodium 50 mg/kg *i.p.* injection. Time in min., n = 7, mean ± S.E.

Table II. Sedative activity of alkaloids from Zizyphus seeds

	Cyclopeptide	Aporphine		Tetrahydrobenzylisoquinoline
	Sanjoinine-A	Nuciferine	Zizyphusine	Coclaurine
Control	16.3 ± 9.8	27.8 ± 10.4	20.6 ± 2.1	20.6 ± 2.1
3 mg/kg	26.1 ± 13.1	33.3 ± 13.8		
10 mg/kg	30.6 ± 19	52.4 ± 17.5	20.0 ± 5.9	16.8 ± 4.3
33 mg/kg			22.2 ± 6.9	16.1 ± 8.0

Samples were orally administered 1 hr before hexobarbital-Na (50 mg/kg) *i.p.* injection to mice. n = 6-7, sleeping time in min., mean ± S.E.

Table III. Effect of co-administration of sanjoinine-A and nuciferine on the sleeping time

Control	Sanjoinine-A 1 mg/kg	Sanjoinine-A 1 mg/kg + Nuciferine 2.5 mg/kg	Sanjoinine-A 1 mg/kg + Nuciferine 5 mg/kg	Nuciferine 5 mg/kg
32.1 ± 23.8	50.8 ± 11	46.1 ± 11.3	97.5 ± 31	83.3 ± 19.5

Samples were administered *i.p.* 30 min. before hexobarbital-Na 50mg/kg *i.p.* injection. Time in min., n = 7, mean ± S.E. S.E.

Table V. Effect of sanjoinine-A and sanjoinine-Ahl on hexobarbital induced sleeping time.

	Control	Sanjoinine-A			Sanjoinine-Ahl		
		1 mg	3 mg	10 mg/kg	1 mg	3 mg	10 mg/kg
Exp. 1	18.3 ± 8.	26.1 ± 7.1	30.2 ± 7.2*		32.8 ± 12.4*	33.2 ± 12.2*	
Exp. 1	11.5 ± 4.1		25.8 ± 18.5**	45.1 ± 11.9***		39.3 ± 16.1***	46.0 ± 23.9***

Samples were administered intraperitoneally 30 min. before hexobarbital -Na(50mg/kg) *ip* injection to mice. n-6-7, sleeping time in min., mean ± S.E.
*P<0.05, **P< 0.01, ***p<0.001

similobine, norisocorydine *etc.* (these were identified on TLC) which possibly make salts with flavonoids[15,16] in this fraction, consequently these are hard to be removed completely by solvent partition.

A comparative evaluation on the sedative activity of sanjoinine-A and sanjoinine-Ahl revealed that heat-induced sanjoinine-Ahl[17] showed a highly enhanced sedative activity than that of sanjoinine -A. The other cyclopeptide alkaloids also produced their isomers with the same tendency by heat treatment (data were not shown) and also nuciferine was converted to somewhat less effective lysicamine whose activity is available in the seeds of *Z. vulgaris* var. *spinosus*.[18] Therefore roasting the Zizyphus seeds converts cyclopeptide alkaloids to more potent isomers, which provides a scientific explanation for ethnopharmacological experiences on the Zizyphus seeds.

ACKNOWLEDGEMENT

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LITERATURE CITED

1. Huh, J.: *Dong Eui Bo Gam,* Namsandang, Seoul, Korea, p216 (1981).
2. Namba, T.: *Coloured Illustrations of Wakan-Yaku,* Boyuksa, Japan, p321 (1980).
3. Lee, H.S.: *Folk medicine,* Gye-chuk munhwasa, Seoul, Korea p102 (1975).
4. Kawaguchi, R., and Kim, K.W.: Constituents of the seeds of *Zizyphus vulgaris* Lamark var. *spinosus* Bunge. *J. Pharm. Soc. Jpn.,* **60,** 343 (1940), *idem, ibid.,* **60,** 595 (1940).
5. Kim, E.C.: Studies of sedative and psychotropic actions of Zizyphi spinosi Semen. *J. Pharm. Soc. Korea,* **15,** 53 (1971).
6. Watanabe, I., Saito, H., and Takagi, K.: Pharmacological studies of Zizyphus seeds. *Japan J. Pharmacol.,* **23,** 563 (1973).
7. Shibata, M., and Fukushima, M.: Acute toxicity and sedative action of Zizyphus seeds. *Yakugaku Zasshi,* **95**(4), 465 (1975).
8. Woo, W.S., K.H., and Kang, S.S.: Chemistry and pharmacology of flavone-C-glycoside from Zizyphus seeds. *Kor. J. Pharmacog.,* **11,** 141 (1980).
9. Shin, K.H., Woo, W.S., and Lee, C.K.: Sedative action of flavonoids and saponin from the seeds of *Zizyphus vulgaris* var. *spinosus* Bunge. *Kor. J. Pharmacog.,* **12**(4), 203 (1981).
10. Cho, T.S., Ro, J.Y., and Hong, S.S.: Pharmacological action of Zizyphi Semen extract on heart. *Korean J. Pharmacol.,* **12**(2), 13 (1976).
11. Ahn, Y.S., Kim, K.H., Cho, T.S., Kim, W.J., and Hong, S.S.: Pharmacological Studies of Zizyphus seed extract on central nervous system and blood pressure. *Korean J. Pharmacol.,* **18**(1), 17 (1982).
12. 陳存仁, 図説漢方医薬大事典, Vol. 4, 講談社, 日本, p. 36 (1982).
13. Han, B.H., and Park, M.H.: Some parts of this work were presented at the 5th Asian Symposium on Medicinal Plants and Spices, Seoul, Korea, August 1984 and reviewed in "Folk Medicine - The Art and the Science, American Chemical Society, Washington, D.C., p205-215 (1986). Details are submitted for publication
14. Bhttacharya, S.K., Bose, R., Ghosh, P., Tripathi, V.J., Ray, A.B., and Dasgupta, B.: Psychopharmacological studies on (-)-nuciferine and its Hofmann degradation product atherosperminine. *Psychopharmacology* **59,** 29(1978).
15. Woo, W.S., Kang, S.S., Shim, S.H., Wagner, H., Chari, V.M., Seligmann, O., and Obermeier, G.: The structure of spinosin from *Zizyphus vulgaris* var. *spinosus.*

Phytochemistry **18**, 353 (1979).

16. Woo, W.S., Kang, S.S., Wagner, H., Seligmann O., and Chari, V.M.: Alkylated flavone-C-glycoside from the seeds of *Zizyphus jujuba*. *Phytochemistry* **19**, 1791 (1980).
17. Han, B.H., Park., J.H., Park, M.H., Han, Y.N., and Park, M.K.: Absolute configuration of sanjoinine-A (frangufoline) and its heat induced artifact: Sanjoinine-Ahl. *Arch. Pharm. Res.* **10**(3), 200(1987).
18. Han, B.H., and Park, M.H. Sedative activity and its active components of Zizyphi fructus. *Arch. Pharm. Res.* **10**(4), 208-211(1987).