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Studies on the Sedative Alkaloids from *Zizyphus spinosus* Semen

Byung Hoon Han, Myung Hwan Park and Jeong Hill Park
Natural Products Research Institute, Seoul National University, Seoul 110, Korea

Abstract—A number of sedative alkaloids were isolated from Sanjoin(酸棗仁), the seeds of *Zizyphus vulgaris* Lamark var. *spinosus* Bunge (Rhamnaceae) which is an important Chinese medicinal material used to treat insomnia and sometimes to treat sleepiness. Those compounds were designated as Sanjoinine-A, B, C, D, etc. depending on the order of increasing polarity. Sanjoinine-A, $C_{31}H_{42}N_4O_4$, mp 249°, $[\alpha]_D^{27}$−316, Sanjoinine-B, $C_{30}H_{40}N_4O_4$, mp 212~4°, Sanjoinene, $C_{29}H_{35}N_3O_4$, mp 281~2°, $[\alpha]_D^{22}$−272, Sanjoinine-D, $C_{32}H_{46}N_4O_5$, mp 256~8°, $[\alpha]_D^{22}$−53.6, Sanjoinine-F, $C_{31}H_{42}N_4O_5$, mp 228~9°, $[\alpha]_D^{22}$−215, and Sanjoinine-G_1, $C_{31}H_{44}N_4O_5$, mp 236~8°, $[\alpha]_D^{22}$−68.6, were found as 14-membered cyclic peptide alkaloids, Sanjoinine-G_2, $C_{30}H_{42}N_4O_4$, mp 182°, $[\alpha]_D^{22}$−79.2 as being open chain peptide alkaloid, and Sanjoinine-E, $C_{19}H_{21}NO_2$, mp 166°, $[\alpha]_D^{20}$−146.2, N-Methylasimilobine, $C_{18}H_{19}NO_2$, mp 193~5°, $[\alpha]_D^{20}$−204, Sanjoinine-Ia, $C_{18}H_{19}NO_2$, mp 155~7°, $[\alpha]_D^{20}$−140, Sanjoinine-Ib, $C_{19}H_{21}NO_4$, mp 184°, Sanjoinine-K, $C_{16}H_{19}NO_3$, mp 159~61°, $[\alpha]_D^{20}$+35, Caaverine, $C_{17}H_{17}NO_2$, mp 204°, $[\alpha]_D^{20}$−80, and Zizyphusine, $C_{20}H_{24}NO_4$ mp 214~6°, $[\alpha]_D^{20}$+317 as being aporphine alkaloids. The heat treatment of the cyclic peptide alkaloids produced their isomeric products which showed enhanced sedative activity. The chemical structure of the isomeric products will be discussed.

Keywords—*Zizyphus vulgaris* · Rhamnaceae · cyclic peptide alkaloid · aporphine alkaloid · sedative activity · sanjoinine-A · frangufoline

Sanjoin(酸棗仁) the seeds of *Zizyphus vulgaris* Lamark var. *spinosus* Bunge (Rhamnaceae) is a traditional medicinal material which is used in the oriental medicine as an important hypnotic agent to treat insomnia and as a sedative agent.[1,2,3] As the usual cases of Chinese medicine, Sanjoin is used as hypnotic agent when it is roasted. Some Chinese medicinal books also describe that raw Sanjoin is used to treat excessive sleepiness caused by physical emaciation.[1] Some other books also decribe that roasting the Sanjoin potentiates the hypnotic activity, but that excessive roasting in terms of too high temperature or too long heat treatment will reduce the activity.[4] Those ethnopharmacological aspects tempted many scientists to undertake the chemical and pharmacological studies on Sanjoin for the isolation and identification of its effective components. Followings are the summary of the scientific papers of

Sanjoin published yet.

1) Oriental medicine and folkmedicine[1-4]
   Hypnotics
   Sedative
   Nervine tonics for insomnia
   Anti-arrhythmia
2) Pharmacology
   Hypnotic.....Kawaguchi, R.[5]
   Major tranquilizer...Kim, E.C.[6]
   Sedative, Papaverine-like analgesic, Anti-inflammatory...Watanaba, I[7]
   Sedative.....Woo, W.S., Shin, K.H., Ahn, Y.S.[8-10]
   Anti-arrythmic.....Cho, T.S.[11]
3) Chemistry
   *Flavonoid[12-14]
   Swertisin.....Woo, W.S.
   Spinosin and its derivatives...Woo, W.S.
   *Terpenoid and Saponin
   Betulinic acid.....Kawaguchi, R.[5]
   Betulin .....Shibata, S.[15]
   Jujubogenin .....Kawai, K.[16]
   Ebelinlactone .....Shibata, S.[15]
   Jujuboside A, B.....Otsuka, H.[17,18]

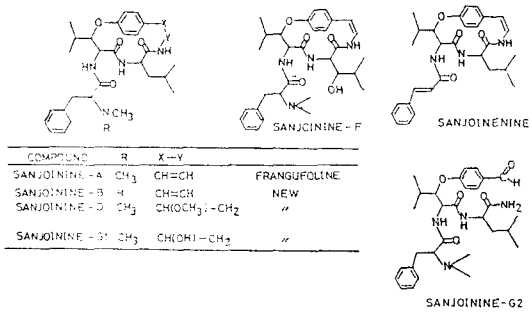
Saponin and flavonoids were reported as being the effective components for the sedative activity of Sanjoin, but the effective doses ED_{50} for the substances were shown somewhat higher.[9,10] On the other hand some scientist in Korea reported the hypnotic activity something like of major tranquilizer activity in the alkaloid fraction of the Sanjoin although he was not successful in the isolation and identification of the alkaloids.[6] Based on this background, the authors attempted to isolate the alkaloids from the extract of Sanjoin and finally we could isolate fourteen kinds of alkaloids in a crystalline state. Table I shows the summary of physico-chemical properties and the isolation yields of the alkaloid components isolated in our laboratory. Those compounds were designated tentatively as Sanjoinine A, B, C, D, etc. in the decreasing order of their polarity.

By the application of spectral analysis of CMR, PMR, Mass, IR and UV in combination with some chemical studies, the chemical struc-

Table I. Physicochemical properties and isolated yields of sanjoin alkaloids

Compound	Molecular formular	Molecular weight	mp	$[\alpha]_D$	Yield
Sanjoinine-A	$C_{31}H_{42}N_4O_4$	534	249°C	−316°	6×10^{-3}%
Sanjoinine-B	$C_{30}H_{40}N_4O_4$	520	212~4°C	—	5.5×10^{-4}%
Sanjoinine-D	$C_{32}H_{46}N_4O_5$	566	256~8°C	−53.6°	4×10^{-5}%
Sanjoinine-F	$C_{31}H_{42}N_4O_5$	550	228°C	−215°	1.3×10^{-4}%
Sanjoinine-G_1	$C_{31}H_{44}N_4O_5$	552	236~8°C	−68.6°	3.5×10^{-5}%
Sanjoinine-G_2	$C_{30}H_{42}N_4O_5$	538	182°C	−79.2°	1.6×10^{-4}%
Sanjoinenine	$C_{29}H_{35}N_3O_4$	489	281~2°C	−272.5°	2.2×10^{-4}%

Compound	Name	mp	$[\alpha]_D$	Yield
Sanjoinine-E	Nuciferine	166°C	−146.2°	2.7×10^{-5}%
Sanjoinine-Ia	Nornuciferine	155~7°C	−140°	1.2×10^{-4}%
Sanjoinine-Ib	Norisocorydine	184°C	—	8.7×10^{-5}%
Sanjoinine-K	(+)-Coclaurine	159~161°C	+35°	1.4×10^{-3}%
N-Methylasimilobine		193~5°C	−204°	5×10^{-6}%
Caaverine		204°C	−80°	6.8×10^{-5}%
Zizyphusine(NEW)		214~216°C	+317°	6.2×10^{-3}%



COMPOUND	R	X—Y	
SANJOININE-A	CH_3	CH=CH	FRANGUFOLINE
SANJOININE-B	H	CH=CH	NEW
SANJOININE-D	CH_3	$CH(OCH_3)-CH_2$	〃
SANJOININE-F	CH_3	$CH(OH)-CH_2$	〃

Chart 1. Peptide alkaloids from the seed of *Zizyphus vulgaris* var. *spinosus.*

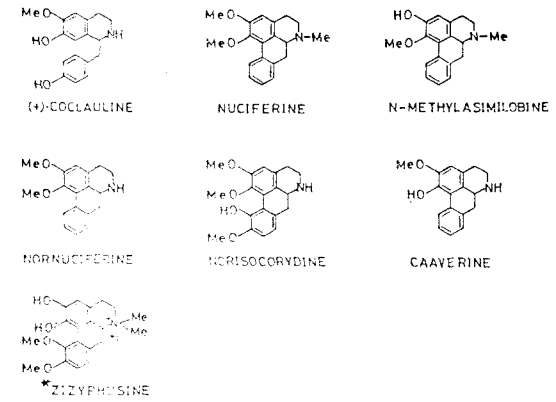


Chart 2. Aporphine and tetrahydroisoquinoline alkaloids from the seed of *Zizyphus vulgaris* var. *spinosus.*

tures of all alkaloids were elucidated and they are summarized as above. As shown in Charts, seven alkaloids have peptide nature and the others are aporphine series. Six peptide alkaloids have cyclic structure with one N, N-dimethylpheylalanine as a branched chain. Sanjoinine G_2 is one exceptional compound which is not cyclic. Sanjoinine-A one of the rich components in the alkaloids fraction was found identical with already known substance frangufoline which was isolated from other plant such as *Rhamnus frangula,*[19] *Zizyphus mauritiana,*[20] *Melochia corchorifolia,*[21] *Euonymus europeaus,*[22] *Discaria longispina*[23] and *Scutia buxifolia.*[23] Other peptide alkaloids are considered as being new substances as yet undescribed. As shown in Chart 2, all aporphine alkaloids and benzylisoquinoline alkaloids except Zizyphusine are known components isolated from other plants. Zizyphusine a quarternary ammonium alkaloid seems to be also a new compound. The sedative activities were evaluated by hexobarbital induced sleeping time prolongation test on some alkaloids, each one representing the cyclic peptide, aporphine-series, benzylisoquinoline and quarternary ammonium alkaloids. The results are summarized in Table II.

As shown in Table II, Sanjoinine-A one of the cyclic peptide alkaloids and nuciferine one of the aporphine alkaloids showed strong prolongation activity on hexobarbital induced sleeping time of mice. The sedative activities of aporphine alkaloids have been known already as having major tranquilizer nature but that of cyclic peptide is the target of our studies as yet undescribed. The

Table II. Sedative activity of sanjoin-alkaloids;
Samples were orally administered 1hr before hexobarbital-Na (50mg/kg) ip injection to mice (n=6~7, sleeping time in min. mean±S.E.)

	Cyclopeptide	Aporphine		Tetrahydrobenzyl-isoquinoline
	Sanjoinine-A	Nuciferine	Zizyphusine	Coclaurine
Control	16.3± 9.8	27.8±10.4	20.6± 2.1	20.6± 2.1
3mg/kg	26.1±13.1 (+60%)	33.3±13.8 (+19.7%)		
10mg/kg	30.6±19 (+87%)	52.4±17.5 (+88.4%)	20.0± 5.9	16.8± 4.3
33mg/kg			22.2± 6.9	16.1± 8.0

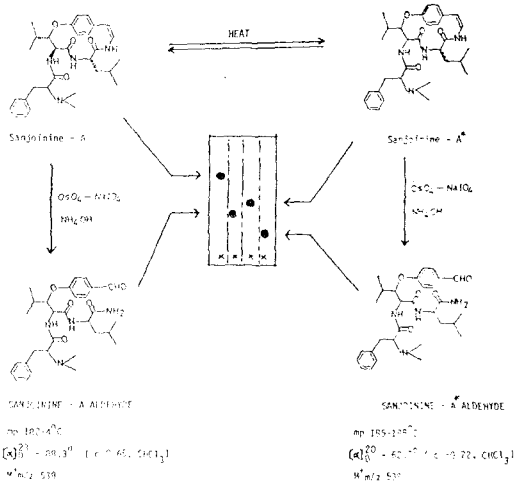
Table III. Sedative activity of sanjoinine-A and sanjoinine-A*; Samples were administered intraperitonearlly 30min. before hexobarbital-Na (50mg/kg) ip injection to mice (n=6~7), sleeping time in min., mean±S.E.

	Control	Sanjoinine-A			Sanjoinine-A*		
		1mg	3mg	10mg/kg	1mg	3mg	10mg/kg
Exp. 1	18.3±8.1	26.1± 7.1 (+42.6%)	30.2± 7.2 (+65%)	—	32.8±12.4 (+79.2%)	33.2±12.2 (+81.4%)	—
Exp. 2	11.5±4.1	—	25.8±18.5 (+124%)	45.1±11.9 (+292%)	—	39.3±16.1 (+241%)	46.0±23.9 (+300%)

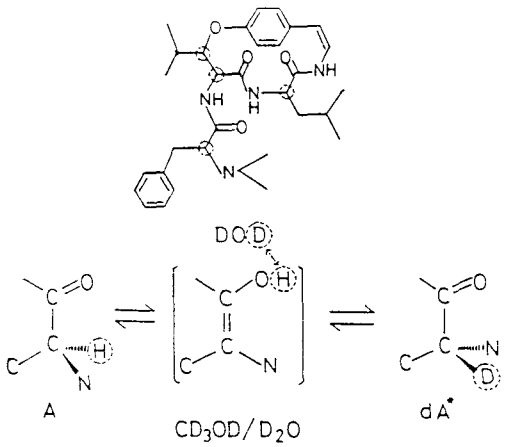
sedative activity of Sanjoinine-A at 3mg/kg-dose was potent enough to see the cyclic peptide alkaloids as being the effective components of Sanjoin. In order to see whether the potentiation of the sedative activity of Sanjoin by roasting treatment is reflected in the molecular level of sedative alkaloid components, the Sanjoinine A was treated to high temperature by exposing to 210° bath in sealed stainless steel tube for 10 minutes. On the heat treatment of Sanjoinine A, it showed an artefact spot on thin layer chromatogram which could be clearly isolated by column chromatograpy. This artefact showed clearly different physico-chemical properties, but surprisingly a part of it reverted to the original substance when it was exposed to same high temperature, suggesting reversible isomerization between Sanjoinine A and its artefact at high temperature. The artefact isomer of Sanjoinine-A was designated tentatively as Sanjoinine-A*. The comparative evaluation on the sedative activity of Sanjoinine-A and Sanjoinine-A* was conducted. As described in Table III Sanjoinine-A* showed highly enhanced sedative activity than that of Sanjoinine-A.

This finding drew our deep interest since it is reflecting the ethnopharmacological aspects of Sanjoin in our molecular pharmacological studies on Sanjoinine-A and A*. In order to elucidate the mechanism of reversible isomerization of Sanjoinine-A and Sanjoinine-A*, we elucidated the chemical structure of the Sanjoinine-A*. The reversible isomerization of those substances means no covalent bond cleavage during the heat treatment. Without covalent bond cleavage, there would be two possibilities for the mechanism of reversible isomerization. One would be conformational isomerization arising from the rigid ring structure of fourteen membered cycle. The other possibility will be the inversion of some chiral center in three amino acid residues at high temperature. If former possibility is true, the ring opening of the both isomers will produce same cleavage products. To see the former possibility, the fourteen membered ring of the both isomers were cleaved by the successive oxidation with osmium tetroxide and periodate at the olefinic bond in the ring as shown in Scheme 1.

The cleavage products from Sanjoinine-A and



Scheme 1. Ring opening of sanjoinine-A and sanjoinine-A*.



Scheme 2. Isomerization mechanism of sanjoinine-A at high temperature.

Sanjoinine-A* showed distinctly different Rf values on TLC. This result negates the former possibility and rather suggests later possibility. It is an already known fact that amino acids produce racemate at high temperature through a mechanism of a α-hydrogen mobilization by keto-enol tautomerism. Thus the α-hydrogen in α-amino acid will be exchanged with the protons in the protic solvent at high temperature. In order to see the later possibility, we prepared the Sanjoinine-A* by heating Sanjoinine-A at high temperature in D_2O-solvent. The deuterated Sanjoinine-A* was isolated by chromatographic purification and it showed same physico-chemical properties of ordinary Sanjoinine-A* except that it showed some different PMR, CMR and Mass spectral data.

In the PMR data of the deuterated Sanjoinine-A* (Fig. 1) we could clearly recognize that α-proton of N, N-dimethylphenylalanine was disappeared and that the β-protons in the N, N-dimethylphenylalanine appeared as AB-quartet instead of multiplet due to further coupling to α-proton. This fact suggests that the isomer of Sanjoinine-A must be produced by high temperature treatment through the inversion of one

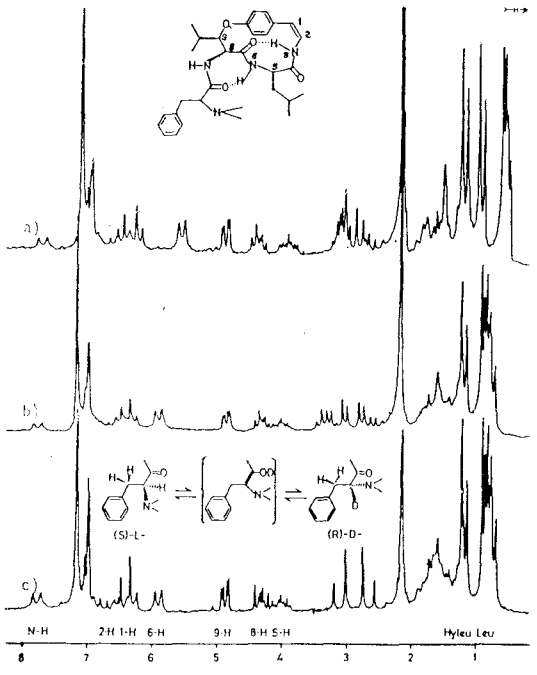


Fig. 1. ^{1}H-NMR spectrum of sanjoinine-A and its sanjoinine-A*, dA*.
a) Sanjoinine-A b) Sanjoinine-A*
c) Sanjoinine-dA*(deuterium labelled)

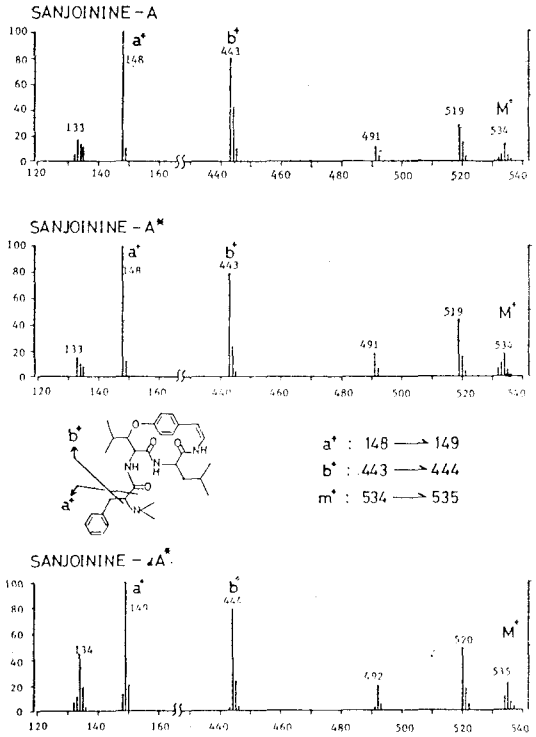


Fig. 2. Mass spectra of sanjoinine-A, sanjoinine-A* and sanjoinine-dA* (deuterium labelled).

chiral center in N, N-dimethylphenylalanine out of four chiral centers in the Sanjoinine-A. The CMR data supported also our finding that the carbon peak at 70.6ppm arising from α-carbon of N, N-dimethylphenylalanine was disappeared in the CMR spectrum of deuterated Sanjoinine-A′ due to its increased relaxation time.

Mass spectra of deuterated Sanjoinine-A* supported also the inversion of α-carbon of N, N-dimethylphenylalanyl side chain. As shown in Fig. 2, mass-spectra of the deuterated Sanjoinine-A* showed one mass-unit higher shift in the following diagnostic fragment ions than those of corresponding non-deuterated compound; which imply the inversion of only one amino acid N, N-dimethylpheylalanine residue.

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