The Chemistry and Biochemistry of *Spiraea japonica* Complex

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Abstract: The characteristic components of *Spiraea japonica* complex, which consists of seven varieties, are the hetisine- and atisine-type diterpene alkaloids, and the atisane-type diterpenes. From this complex, 20 hetisines, 37 atisines and 7 diterpenes were isolated during 1964–2001, including the observations of the isomerization of spiraea diterpene alkaloids having the oxazolidine ring and the interconversion of the oxazolidine rings, the configuration of the oxygen substitution at C-15 of the atisine-type alkaloids, and the interconversion relationship between the two main subtypes of the hetisine-type alkaloids. The chemotaxonomy based on structures of the reported diterpene alkaloids were suggested. The studies on the anti-inflammation, anti-platelet aggregation and neuroprotective bioactivities of the alkaloids from *S. japonica var. acuta* were reviewed as well.

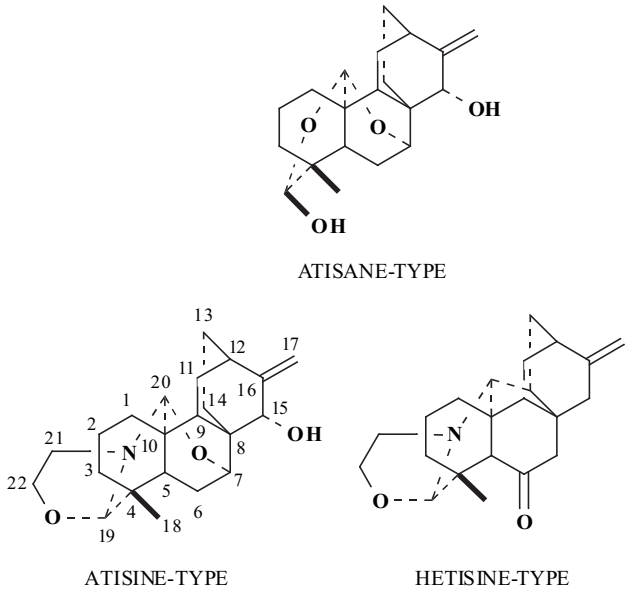
Keywords: *Spiraea japonica*, chemotaxonomy, hetisine, atisine, atisane, diterpenes, diterpene alkaloids, anti-inflammation, anti-platelet aggregation, neuroprotection.

INTRODUCTION

Spiraea japonica L. (Rosaceae) complex consisting of seven varieties including var. *acuta* Yu, var. *acuminata* Franch., var. *fortunei* Rehd., var. *glabra* Koidz., var. *ovalifolia* Franch., var. *stellaris* Rehd. and var. *incisa* Yu are widespread in Yunnan Province, China. The young leaves, fruits and roots of some of these plants have been used as diuretic, detoxicant and analgesic agents and also for the treatment of inflammation, cough, headache and toothache in traditional Chinese medicine (TCM) [1].

Studies on the chemical constituents of the complex *S. japonica* started in 1964. Frolova and coworkers reported the isolation of several alkaloids from *S. japonica* [2]. Later on to 1976, the Japanese and Russian scientists isolated eight diterpene alkaloids from this plant [3-8]. In 1985, Sun and coworkers reported the isolation of three diterpene alkaloids spirasines IV, IX and XI from *S. japonica* var. *fortunei* [9] and another twelve alkaloids spirasines I-III, V-VIII, X and XII-XV from the same plant subsequently [10-14]. All of these diterpene alkaloids were reported before 1988, and could be structurally classified into hetisine- and atisine-type (Scheme 1). Except for sipradines F and G [15], all of them are the hetisine-type alkaloids.

From 1987 [16], our group systematically studied the chemical constituents of the complex *S. japonica* including all those seven varieties mentioned above, which led to the isolation of 35 new atisine-type diterpene alkaloids [17-35] and 7 new atisane-type diterpenes [40-42]. Based on the rich chemical data, a systematic hypothesis was suggested that the complex *S. japonica* was originated in the southwest of China, and presented an evolutionary tendency from the west to the east [43]. Meanwhile, the total synthesis and bioactivities of those atisine-type alkaloids were studied as well [44-48].



Scheme. 1.

1. CHEMICAL COMPONENTS

1-1. Spiraea Diterpene Alkaloids

The studies on the alkaloid constituents of *Spiraea japonica* were initiated by Molodozhnikov and coworkers in 1964 [2].

1-1-1. Hetisine-Type Alkaloids

Up to date, 20 hetisine-type alkaloids are isolated from the complex *S. japonica* and mainly from *S. japonica* and *S.*

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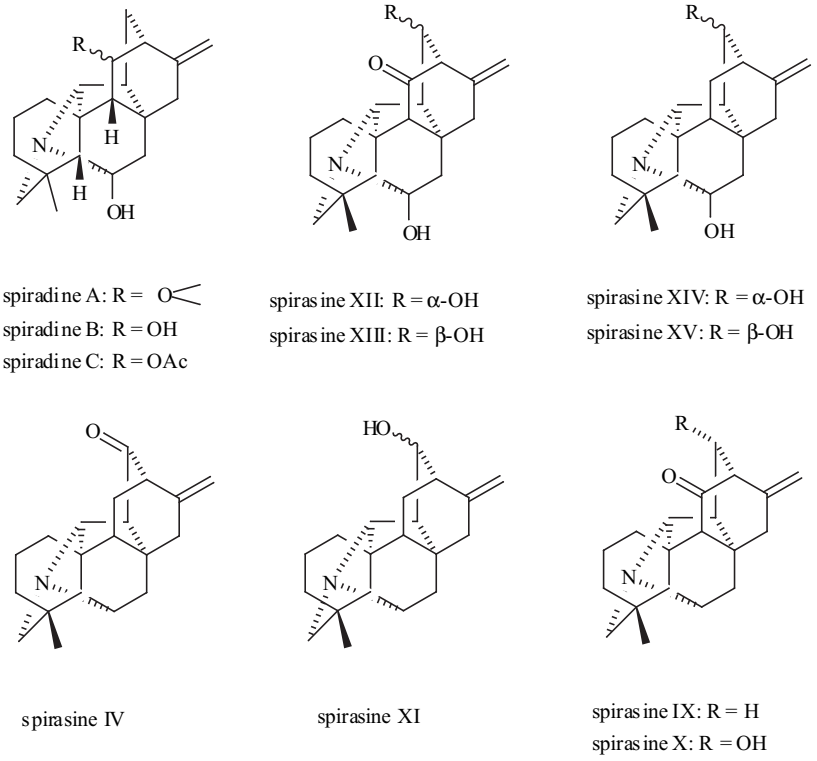


Fig. (1). The hetisine-type alkaloids bearing an N-C-6 bond.

japonica var. *fortunei*. This type of alkaloids can be structurally classified into two subtypes, one having N-C-6 linkage (Figure **1**) and the other having oxazolidine ring (Figure **2**). The degrees of oxygen-substitution varied from 1 to 4, being different from the situation in other types of diterpene alkaloids, e.g. aconitines mostly are poly-oxygenated.

Eleven hetisine-type alkaloids isolated from the complex *S. japonica* had the N-C-6 linkage. The first example is the isolation of spiradines A, B and C from *S. japonica* L. fil by Goto and coworkers in 1968 [3]. Later on, Sun and coworkers isolated another eight compounds, spirasines IV [9], IX [9], X [10], XI [9], and XII-XV [11] from *S. japonica* var. *fortunei*. Spirasines A-C and XII-XV all had

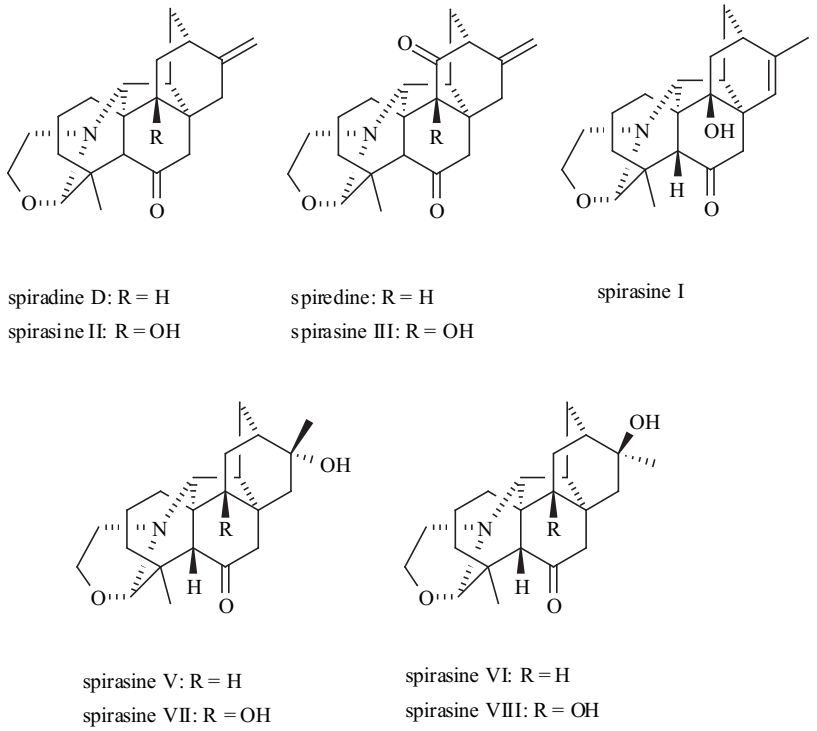


Fig. (2). The hetisine-type alkaloids bearing an oxazolidine ring.

the hydroxyl substitution at C-6. It was speculated that these compounds were transformed from their corresponding C-6 ketone derivatives under acidic conditions, however, the isolation of spirasines IV, IX, X and XI undermined this suggestion. Spirasines XII and XIII, and spirasines XIV and XV are the epimeric pairs at C-13. These structures could be reduced from their corresponding C-13 ketone derivatives that have not been isolated from the complex *S. japonica* yet. Since all of these C-9 and/or C-13 hydroxyl-substituted compounds (spirasines B and X-XV) can be transformed from their ketone derivatives chemically, their biosynthetic precursors should be isolated from *S. japonica* as well.

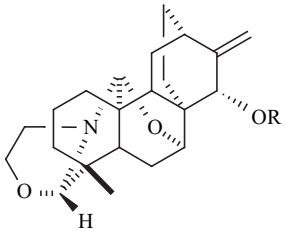
Spiradine D was the first hetisine-type spiraea alkaloid possessing the oxazolidine ring isolated from *S. japonica* L. fil by Goto and coworkers in 1968 [4]. However, until the middle of 1980's, Sun and coworkers reported seven more, including spirasines I [12], II [12], III [13], V [14], VI [14], VII [12] and VIII [12] isolated from *S. japonica* L. f. var. *fortunei*, which contribute to this subtype as a complete series with the extra oxygen substitution at C-9 (spirasines I and II), at C-11 (spiredine) [8], at both C-9 and C-11 (spirasine III) and at the C-15 exocyclic double bond hydrated products spirasines V-VIII.

1-1-2. Atisine-Type Alkaloids

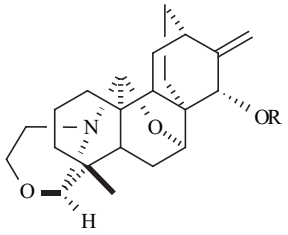
Up to date, 37 atisine-type diterpene alkaloids are isolated from the complex *S. japonica*. These compounds bear much higher structural diversity than the hetisine-type alkaloids and the half of them have the oxazolidine ring.

1-1-3. The Isolation and Structure Elucidation of Spiraea Diterpene Alkaloids

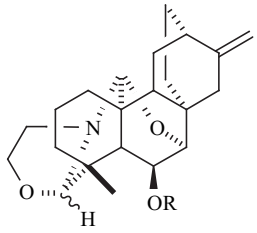
Unlike the hetisine-type alkaloids, the atisine-type alkaloids have two types of oxazolidine ring substitutions, one composed of N-C-19 via C-21, C-22 and oxygen, and the other composed of N-C-20 via C-21, C-22 and oxygen (Figure **3**). Spiradines F and G were the first two atisines isolated from *S. japonica* by Toda and coworkers in 1968 [15]. Spiramines A, B, C and D isolated from *S. japonica* var. *acuminata* by Hao and coworkers [16,17] have the same plane structure as spiradines F and G except the C-15



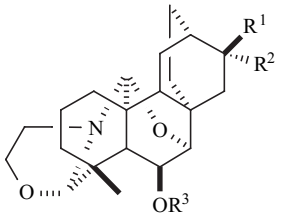
spiramine A: R = Ac
spiramine C: R = H



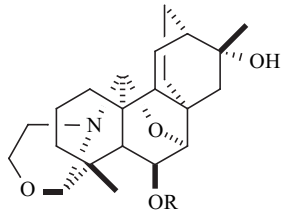
spiramine B: R = Ac
spiramine D: R = H



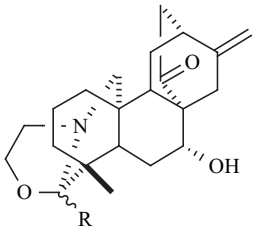
spiradine F: R = Ac
spiradine G: R = H



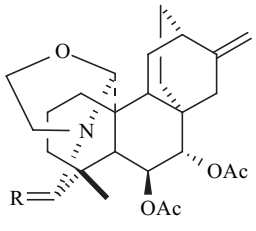
spiramine P: R^1 = OH, R_2 = Me, R^3 = H
spiramine Q: R^1 = Me, R^2 = OH, R^3 = H
spiramine U: R^1 = OH, R^2 = Me, R^3 = Ac



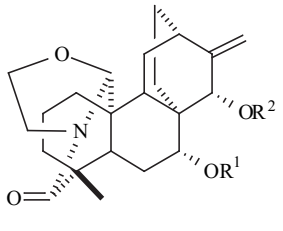
spiramine T: R = Ac
spiramine W: R = H



spiramine Z-2: R = α-H
spiramine Z-3: R = β-H



spiramine Z-1: R = 2H
spiramide: R = O<



spiramine S: R^1 = H, R^2 = Ac
spiramine V: R^1 = Ac, R^2 = H
deacetylspiramine S: R^1 = R^2 = H

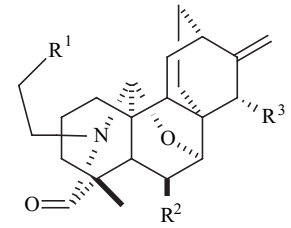
Fig. (3). The atisine-type alkaloids possessing an oxazolidine ring.

oxygen substitution instead of at C-6. However, spiramines A-D were the first epimers among the atisines isolated in pure form. Spiramines P [10,18], Q [10,18], T [19,20], U [19,20] and W [21] have the similar structures as spiramines A-G, but the C-15 exocyclic double bond was hydrated to form a chiral center with *S* or *R* configuration. Spiramines Q and W were the epimers at C-19, and all were obtained in pure form. Spiramines Z-2 and Z-3 [22,23] were the epimers at C-19 and were the only two atisines isolated from this complex both having the C-14 ketone and the oxazolidine ring. Spiramine Z-1, spiramide [24,25], spiramines S [26] and V [27], and 15-deacetyl spiramine S [22,23] have the N-C-20 oxazolidine ring. They were different from the atisines having N-C-19 oxazolidine ring without the C-7/C-20 *O*-bridge, and all did not have the hydroxyl substitution at the C-19 instead of with or without the C-19 ketone. The oxygen substitutions in the atisines bearing the oxazolidine ring were most abundant at C-7, followed by C-19, C-20, C-6, C-15, C-16 and the least at C-14.

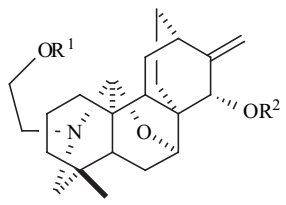
All nineteen alkaloids of this subtype have the C-15 exocyclic double bond (Figure **4**). Spiramines R [18], X [28] and Y [28] could be considered to be the oxidative open ring forms of the atisines having the oxazolidine ring, but the corresponding putative counterparts have not been isolated from the complex *S. japonica* yet. Spiramines R, X, Y, E [29,30], F [29,30] and deacetylspiramine F [23] were the only five ones having the C-7/C-20 *O*-bridge. Spiramines G [29,30], H [29], I [29] and spiratine A [25,31] were the other four alkaloids, possessing the C_2-unit substitution at the N, among the only three spiramines R, X and Y with the oxygen substitution at the C-19. Spiratine B [25,31], spiramines Z [28], J [32], K [32], L [32], M [32], N [33], O [34] and 19-deethylspiramine N [22,23] were imines. The acetone, methoxy or ethoxy substitution at the C-19 may be formed during the isolation progress, only spiratine B and 19-deethylspiramine N could be the real natural products.

1-1-3-1. The Isomerization of Spiraea Diterpene Alkaloids Having Oxazolidine Ring, the Isolation and Configure Determination of Epimers

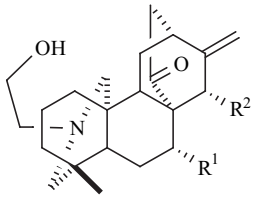
In the atisine-type diterpene alkaloids, isomerization of the oxazolidine ring in atisine via an immonium salt to form isoatisine was studied extensively [35], and the existence of the epimeric mixtures at C-20 was demonstrated by ^1H and ^{13}C NMR studies [36]. Sun and coworkers [12] reported that the oxazolidine-containing hetisine-type alkaloids such as spirasine III epimerized at C-19 in solution, display a pair of signals at ca. δ 4.4 (19*R*) and 3.7 (19*S*) in a ratio of 1:1. Hao and coworkers [16] firstly isolated two epimeric pairs of



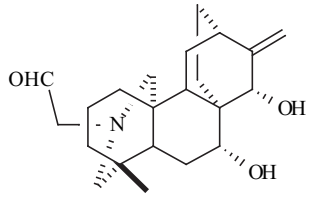
spiramine R: R^1 = OH, R^2 = H, R^3 = OAc
spiramine X: R^1 = OAc, R^2 = OAc, R^3 = H
spiramine Y: R^1 = OH, R^2 = OAc, R^3 = H



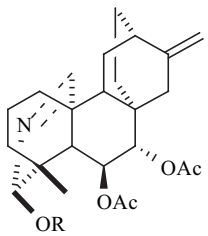
spiramine E: R^1 = R^2 = Ac
spiramine F: R^1 = H, R^2 = Ac
deacetyl spiramine F: R^1 = R^2 = H



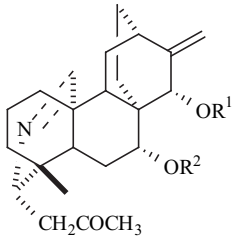
spiramine G: R^1 = OH, R^2 = H
spiramine H: R^1 = H, R^2 = OH
spiramine I: R^1 = H, R^2 = OAc



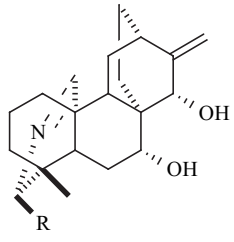
spiratine A



spiramine Z: R = Et
spiratine B: R = H

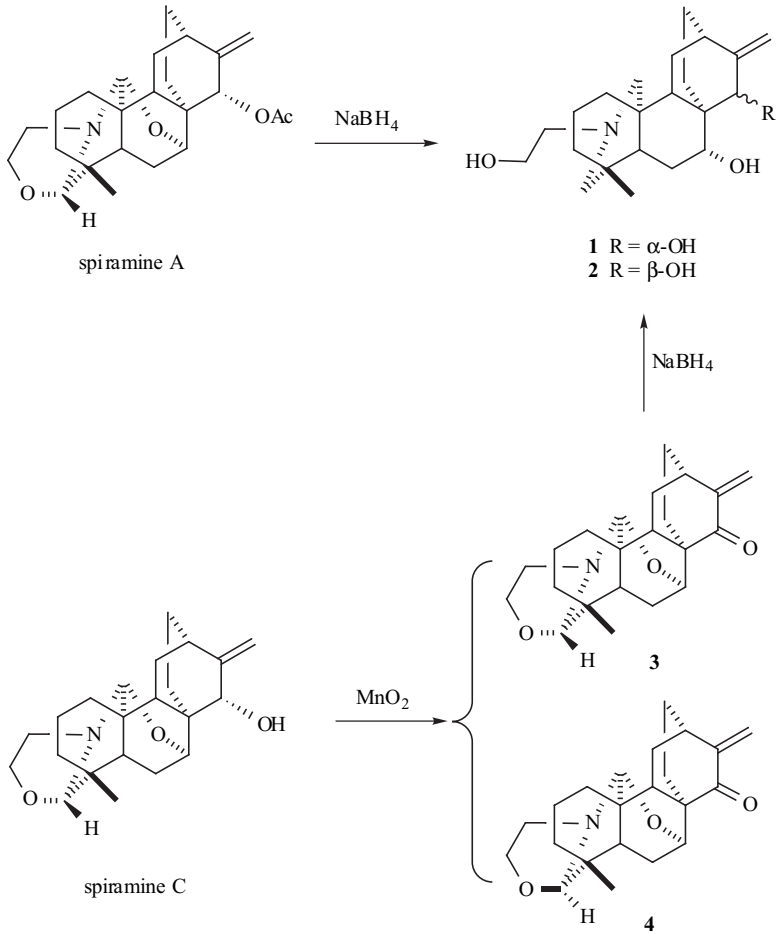


spiramine J: R^1 = R^2 = H
spiramine L: R^1 = Ac, R^2 = H
spiramine M: R^1 = H, R^2 = Ac



spiramine K: R = CH_2COCH_3
spiramine N: R = OEt
spiramine O: R = OMe
19-deethyl: R = OH

Fig. (4). The atisine-type alkaloids without an oxazolidine ring.



Scheme 2.

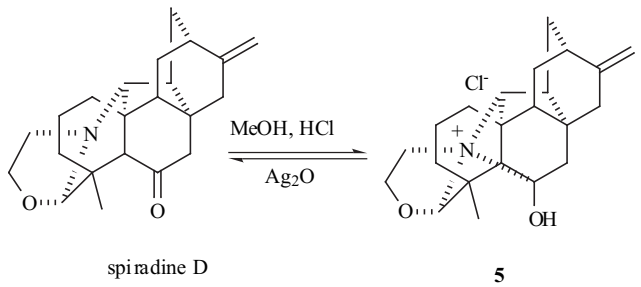
the atisine-type alkaloids spiramines A, B, C and D in pure form from *S. japonica* L. fil var. *acuminata* Franch, the former and the latter two were epimeric at C-19, respectively. The X-ray structural analysis provided the overall structure of spiramine A including the *S*-configuration at C-19 in the oxazolidine ring [17]. The epimerization rate of spiramines was slower than atisine. Spiramine A gave a 3:1 mixture of spiramines A and B after one day at room temperature in CD_3OD, while almost unchanged after two days in $CDCl_3$. Upon chromatography over silica gel or alumina oxide, quick epimerization took place. Spiramine A afforded a mixture of spiramines A and B in an approximate ratio of 5:3 on a silica gel column and 4:1 over alumina oxide after 2 h. Under basic conditions, e.g. hydrolysis of spiramines A and B with potassium hydroxide in methanol afforded an approximate 2:1 mixture of spiramines C and D, respectively [17].

1-1-3-2. Elucidation on the Configuration of Oxygen Substitution at C-15

Reduction of spiramine A with sodium borohydride afforded a triol (**1**) that was not identical with dihydroajaconine (**2**). The comparison of ^{13}C-NMR data of the triol and dihydroajaconine indicated that the acetoxy group at C-15 in spiramine A was of the α-configuration. Oxidation of spiramine C with manganese dioxide gave α,β-unsaturated ketones (**3**) and (**4**). The former was reduced with sodium borohydride to produce a triol, which was identical with dihydroajaconine determined by the spectroscopic data including optical rotation (Scheme 2) [16,17]. Up to date, only the spiramine alkaloids isolated from *Spiraea japonica* complex distributed in the southwestern China have 15-α-hydroxyl group in all of the atisine alkaloids.

1-1-3-3. Chemical Reactions

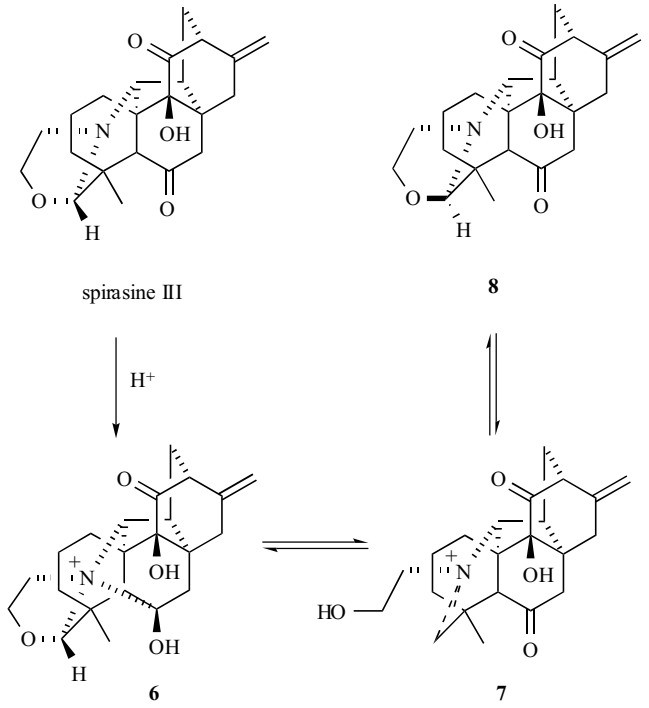
The hetisine-type alkaloids having the C-6 ketone such as spiradine D produced a quaternary salt upon being treated with MeOH-HCl at 0 °C, and this quaternary salt was recovered into the original spiradine D by shaking with silver oxide in methanol (Scheme 3) [4,37], suggesting an interconversion relationship between the two main subtypes of the hetisine-type alkaloids (Figure **1** and **2**).



Scheme 3.

As shown in Scheme 4, upon acidification, spirasine III could be transformed into the possible salt forms,

carbinolamine (**6**) and the open chain immonium (**7**), and the equilibrium between the salts was found to be changeable in different solvents as shown by the ^{13}C NMR data. In aqueous medium, the existence of (**7**) as a minor isomer was indicated by the methane signal at δ_C 185 ppm, which was observed in trifluoroacetic acid solution as well [38,39].



Scheme 4. The interconversion of the oxazolidine rings [12].

The C-16 exocyclic double bond in the hetisine-type alkaloids could be moved into C-14/C-15 with inorganic base in methanol [4].

1-2. Spiraea Diterpenes

Up to date, only seven diterpenes were isolated from the complex *S. japonica*, and they are structurally close to each other (Figure **5**). Obviously, spiraminol [33] could be readily oxidized into spiramilactone [40]. On the other hand, spiraminol (hemiacetate) could be converted into the aldehyde products **9** (Scheme 5) which would produce **10** and **11** through the intramolecular Cannizzaro reaction under the basic reaction conditions. Spiramilactone [41] and spiramilactone C [42] were the lactones of **10** and **11**, respectively.

Moreover, spiramilactone D [42] could be the C-15 exocyclic double bond hydrated product of spiramilactone C. Spiramadol is of interest because it is a dialdehyde, and its isolation strongly supported the proposed conversion of spiraminol into spiramilactone and spiramilactone C. It was speculated that if the hydroxyl group at C-7 was not acetylated, such as in the form of 7-deacetyl spiramadol, hemiacetate like spiraminol could be formed readily.

The skeleton of the *Spiraea* diterepenes was very close to the *Spiraea* diterpenoid alkaloids. It was interesting to see that both the diterpenes and the diterpenoid alkaloids were isolated from the same plants. The fact implied that there were some biogenetic pathway relationship between the diterpenes and the diterpenoid alkaloids. The biogenetic pathway of diterpenoid alkaloids has to obey the isoprene rule as the "pseudo alkaloids". The fact of the double Mannich reaction-like condensation of spiraminol with ethanolamine gave a mixture of spiramine C and D specifically (Scheme 6), implied the *Spiraea* diterpenes would be the biosynthetic precursors of the *Spiraea* alkaloids [43].

2. THE CHEMOTAXONOMY OF THE COMPLEX *S. JAPONICA*

Table **1** summarizes up to date results of chemical research on the complex *Spiraea japonica*. Based on the results of chemistry, cytology, morphology of the *Spiraea japonica* complex, it was suggested that this complex is a particular and relative impenitent group in *Spiraea*. Southwestern China especially the Hengduan Mountain is the center of modern differentiation, probably the original center of this complex. It was also suggested that some varieties of this complex would be combined [43].

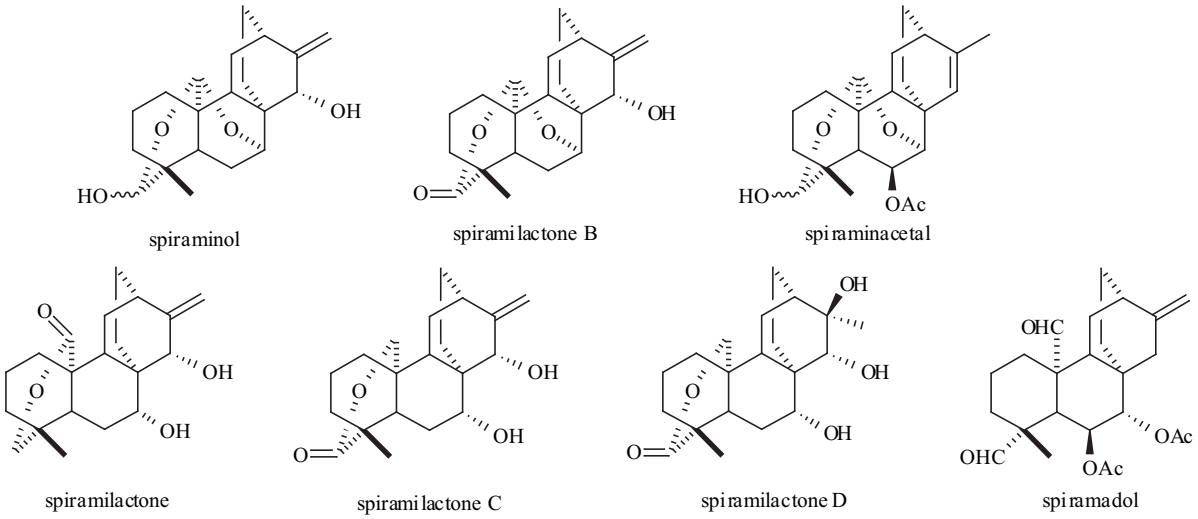
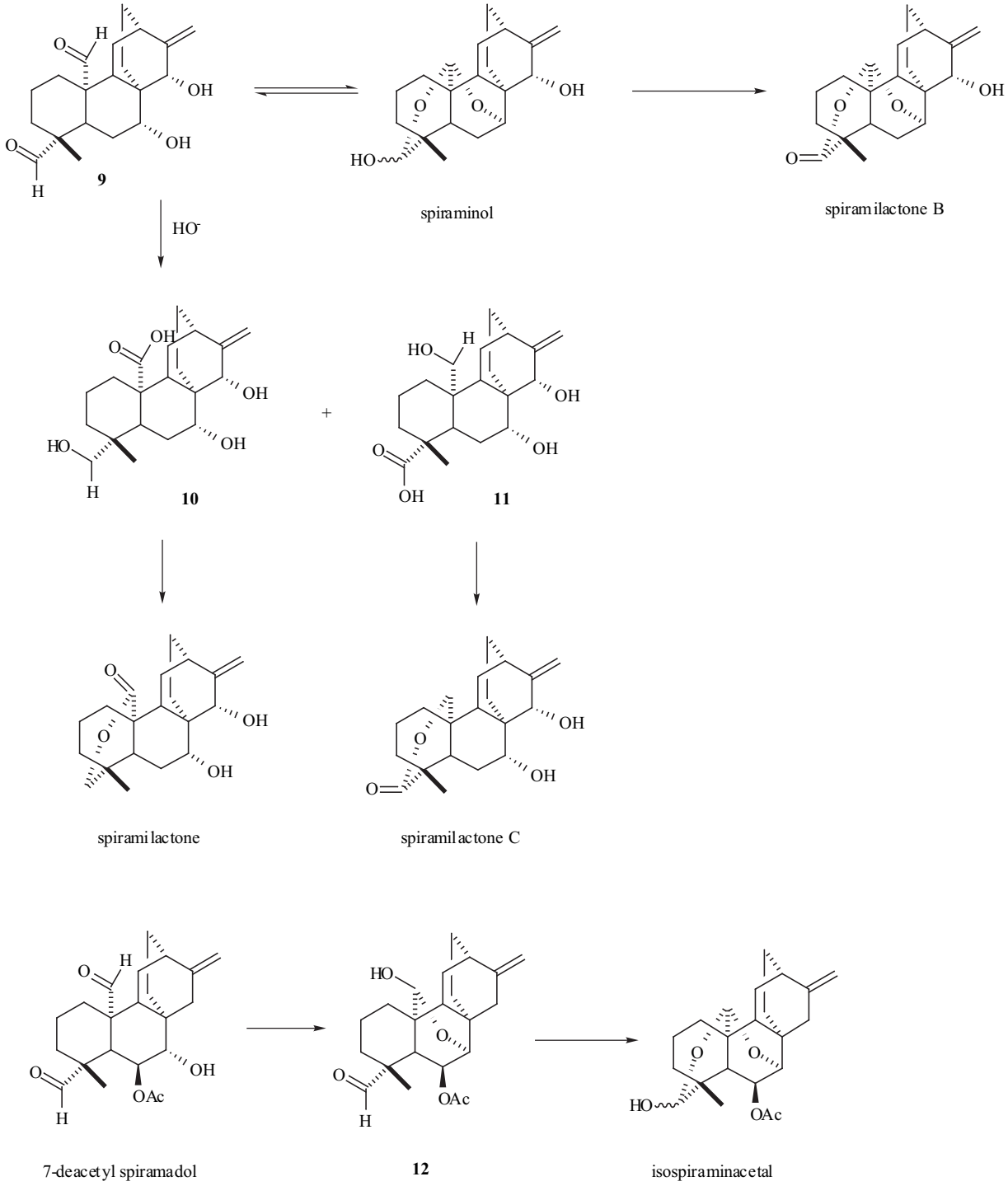
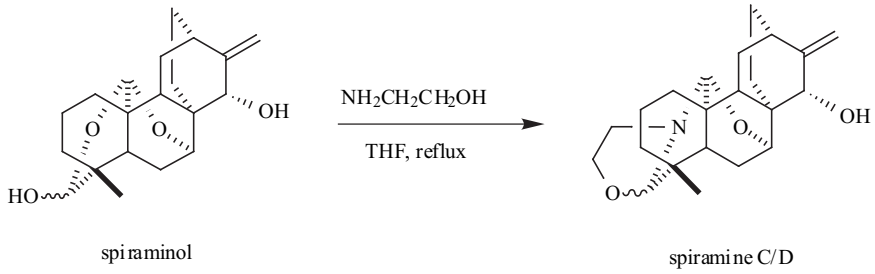


Fig. (5). The alkaloids isolated from the complex *S. japonica.*



Scheme 5.



Scheme 6.

Table 1. Distribution of Spiraea Alkaloids in the Complex *Spiraea japonica*

Species (eastern→western area)	Hetisines	Atisane and atisines
S. japonica L. fil (Japan)	spiradine A [1], spiradine B [1], spiradine C [1], spiradine D [1]	spiradine F [15], sipradine G [15]
var. *glabra* Koidz. (Kuocangshan, Hangzhou, Zhejiang)	spiredine, spirasine II, spirasine III, spirasine V, spirasine VI, spiradine C (unpublished data)	
var. *fortunei* Rehd. (Tianmushan, Hangzhou, Zhejiang)	spiredine, spirasine III (unpublished data)	
var. *fortunei* Rehd. (Guiyang, Guizhou)	spiradine A [1,9], spirasine I [11], spirasine II [11], spirasine III [12], spirasine IV [9], spirasien V [10], spirasine VI [10], spirasine VII [11], spirasine VIII [11], spirasien IX [9], spirasine X [13], spirasine XI [9], spirasine XII [14], spirasien XIII [14], spirasien XIV [14], spirasien XV [14]	
var. *acuminata* Franch. (Kunming, Yunnan)		Spiraminol [32], spiramine A [16,17], spiramine B [16,17], spiramine C [16,17], spiramine D [16,17], spiramine E [29,30], spiramine F [29,30], spiramine G [29,30], spiramine H [29,34], spiramine I [29,34], spiramine J [32], spiramine K [32], spiramine L [32], spiramine M [32], spiramine N [33], spiramine O [34], spiramine S [26], spiramine V [27]
var. *stellaris* Rehd. (Kunming, Yunnan)		Spiramilactone B [39], spiramine A [40], spiramine B [40], spiramine C [40], spiramine D [40], spiramine F [40], spiramine H [40], spiramine P [40], spiramine Q [40]
var. *ovalifolia* Franch. (Songming, Yunnan)		spiramine A [23], spiramine B [23], spiramine C [23], spiramine D [23], spiramine F [23], spiramine H [23], spiramine Z-2 [22,23], spiramine Z-3 [22,23], 19-*O*-deethylspiramine N [22,23], deethylspiramine F [22,23], deacetylspiramine S [22,23], spiramidine A [22], spiramidine B [22]
var. *incisa* Yu (Dali, Yunnan)		Spiramilactone [40], spiramine A [18], spiramine B [18], spiramine P [18], spiramine Q [18], spiramine R [18]
var. *acuta* Yu (Lijiang, Yunnan)		spiramacetal [41], spiramadol [41], spiramilactone C [41], spiramilactone D [41], spiramine A [20,21], spiramine B [20,21], spiramine C [20], spiramine D [20], spiradine F [21], spiramine P [19-21], spiramine Q [19,20], spiramine T [21], spiramine U [19-21], spiramine W [21], spiramine X [28], spiramine Y [28], spiramine Z [28], spiramide [24,25], spiratine A [25,31], spiratine B [25,31]
var. *fortunei* Rehd. (Tengchong, Yunnan)	spiredine, spiradine A, spirasine V, spirasine VI (unpublished data)	

3. THE BIOACTIVITIES AND ACTIVE COMPONENTS OF *SPIRAEA JAPONICA*

3-1. The Outline Studies on the Bioactivities of *S. japonica*

Based on the background of applications as a folk medicine in China, studies on the anti-inflammation, anti-platelet aggregation, neuroprotective bioactivities of the alkaloids from *S. japonica var. acuta* were carried out step by step (Figure **6**). In the bioactivity screening cycle, PAF (platelet-activating factor) was the key link point because it was both the intermediate of inflammation and the inducer of platelet aggeregation.

3-2. The Anti-Inflammatory Activity of Spiraea Diterpene Alkaloids

Orally administration of the EtOH extract (200 $mg \cdot kg^{-1}$) and the crude alkaloid (3.4 $mg \cdot kg^{-1}$) of *S. japonica* var. *acuta* significantly inhibited mouse ear swelling induced by xylene, with an inhibitory rate of 27.7 and 33.5 %, whereas the neutral fraction at 20 $mg \cdot kg^{-1}$ was inactive with an inhibitory rate of 7.5 %. This finding suggests that the extracts of *S. japonica* var. *acuta* have anti-inflammatory activity, and the active fraction was the crude alkaloid not the neutral fraction. Moreover, the anti-inflammatory activity of the crude alkaloid at 3.5 $mg \cdot kg^{-1}$ was equivalent to that of aspirin at 200 $mg \cdot kg^{-1}$, indicating that the crude alkaloid has potent anti-inflammatory effect (unpublished data).

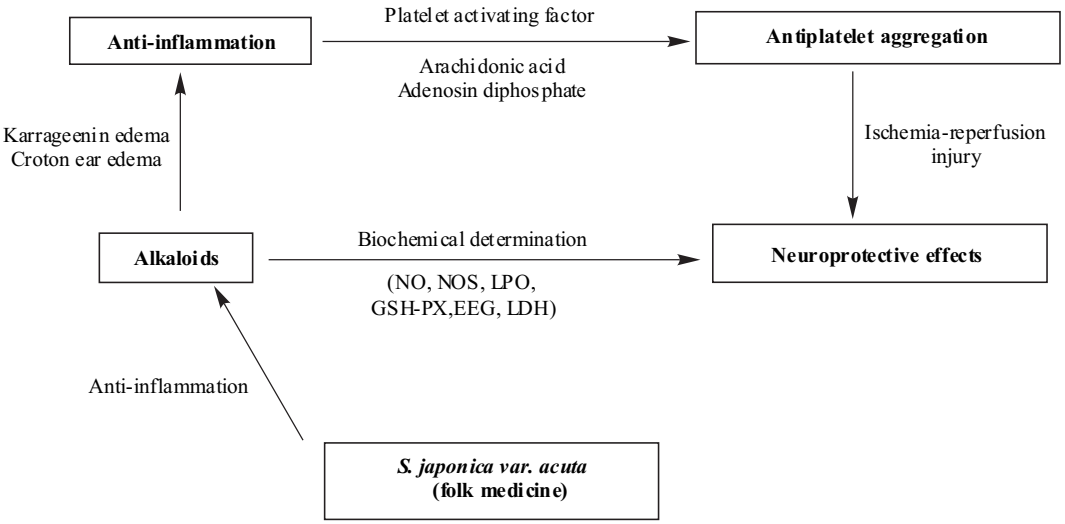


Fig. (6). Bioactivities of the alkaloids from *S. japonica* var. *acuta*.

3-3. The Antiplatelet Aggregation Activities of Spiraea Diterpene Alkaloids

The ethanol extract of *S. japonica* var. *acute* and the lipophilic portion of the crude alkaloid significantly inhibited aggregation of rabbit platelet induced by PAF *in vitro* in a concentration-dependent manner with IC_{50} at 735.1 ± 73.1 mg.L^{-1} and 101.8 ± 6.8 mg.L^{-1}, respectively (Figure **7**), while they had no inhibition on the ADP- or AA-induced aggregation and exhibited selective inhibitions on the platelet aggregation induced by PAF. The neutral part and water-soluble portion of the crude alkaloid had no evident inhibition against the aggregation of rabbit platelet induced by PAF, indicating that the alkaloids were the antiplatelet aggregation components.

A series of the atisine-type diterpene alkaloids including spiramines A and C, spiradine F, spiramine Z-2, deacetylspiramine F and 15-deacetylspiramine S as well as the synthetic derivatives spiramines C1-4 and F1-4 of spiramine C and spiradine F (Figure **8**) [47], respectively, were evaluated for the ability of inhibiting aggregation of the rabbit platelet induced by AA (arachidonic acid), ADP (adenosine-5'-diphosphate) and PAF (platelet-activating factor) *in vitro*. All of the atisine-type alkaloids investigated in this study significantly inhibited the PAF-induced platelet aggregation in a concentration-dependent manner, while they had no inhibition on the ADP- or AA-induced aggregation, exhibited selective inhibitions on the platelet aggregation induced by PAF except for spiramine C1. Spiramine C1 concentration-dependently inhibited the platelet aggregation induced by PAF, ADP and AA with IC_{50} at 30.5±2.7, 56.8±8.4 and 29.9±9.9 μM, respectively, suggesting a non-selective antiplatelet aggregation action. The inhibitory effect of spiramine C1 on AA was as potent as aspirin.

Preliminary studies on the structure-activity relationships about inhibiting the PAF-induced aggregation showed that the oxygen substitution at C-15 position and the presence of an oxazolidine ring in the spiramine alkaloids were essential to their antiplatelet aggregation effects. These results suggest that the atisine-type alkaloids isolated from *S. japonica* are a class of novel antiplatelet aggregation agents [47].

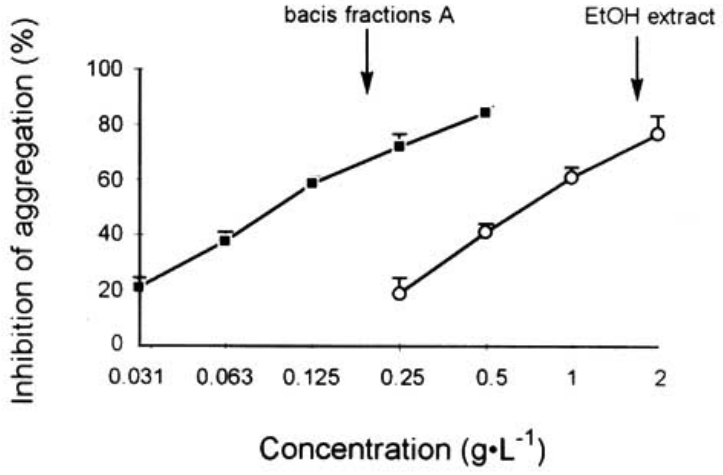


Fig. (7). Effects of the EtOH extract and basic fraction A of *S. japonica* var. *acuta* on PAF (4.5 nmol·L^{-1})-induced platelet aggression *in vitro* ($x \pm s$, $n = 4$).

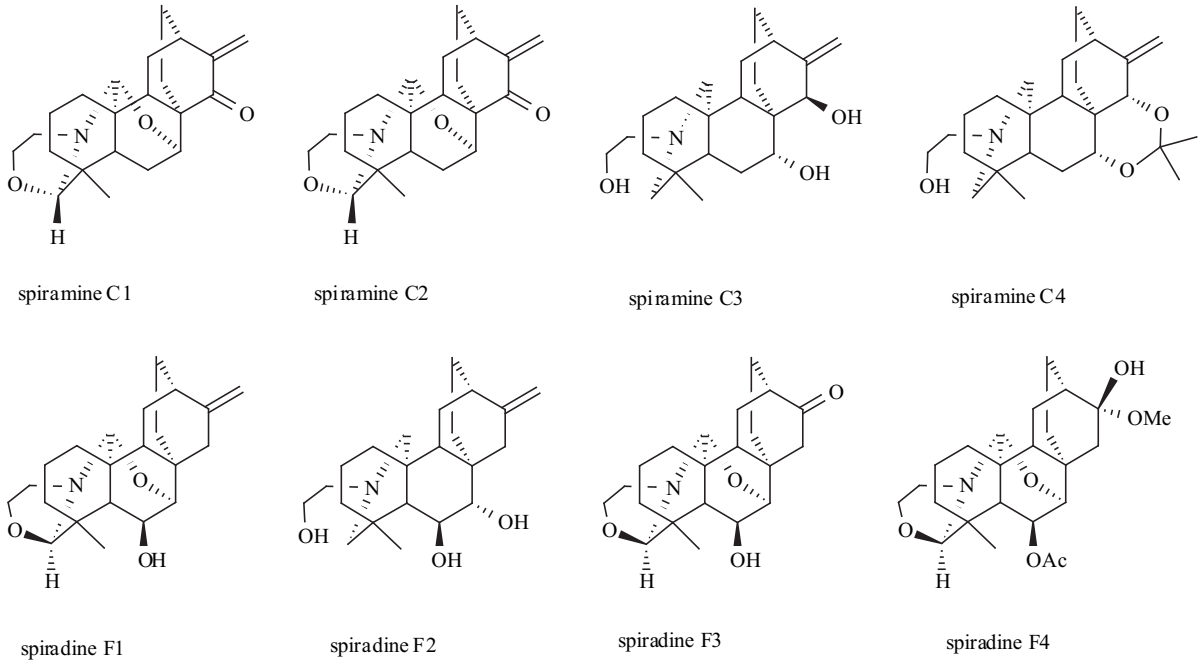


Fig. (8). Synthetic derivatives of spiramine C and spiradine F.

3-4. The Neuroprotective Effects of *S. japonica* [46,48]

The neuroprotective effects of the EtOH extract of *S. japonica* var. *acuta* on the cerebral ischemia-reperfusion injury produced by 10-min bilateral occlusion of the common carotid arteries followed by a 5-day reperfusion in gerbils were investigated. The results showed that intragastric the EtOH extract (20 $mg.kg^{-1}$) markedly enhanced the recovery of EEG amplitude during reperfusion and decreased the water content in cortex, which were much stronger than that of GBE (Figure **9**). It was suggested that the EtOH extract of *Spiraea japonica* var. *acuta* exhibited the protective effects on the cerebral ischemia-reperfusion injury in gerbils.

The neuroprotective effects of spiramine T isolated from *S. japonica* var. *acuta* on the cerebral ischemia-reperfusion injury produced by 10-minute's bilateral occlusion of the common carotid arteries followed by a 5-day's reperfusion in gerbils were investigated. Intravenous spiramine T (0.38, 0.75 and 1.5 $mg.kg^{-1}$) markedly reduced the stroke index, and enhanced the recovery of EEG amplitude during reperfusion and decreased the concentrations of cortex calcium and LPO in a dose-dependent manner. However, no significant effects on water and sodium contents were observed [46]. Spiramine T (1.0 and 2.0 $mg.kg^{-1}$) markedly increased the GSH-PX activity, and inhibited the increase of NOs activity and nitric oxide production of cortex during the ischemia -reperfusion in gerbils [48]. These results suggested that spiramine T exhibited protective effects on the cerebral ischemia-reperfusion injury in gerbils, and its mechanism might be related to the inhibition of calcium overloading, anti-peroxidation, modulation of endogenous antioxidant

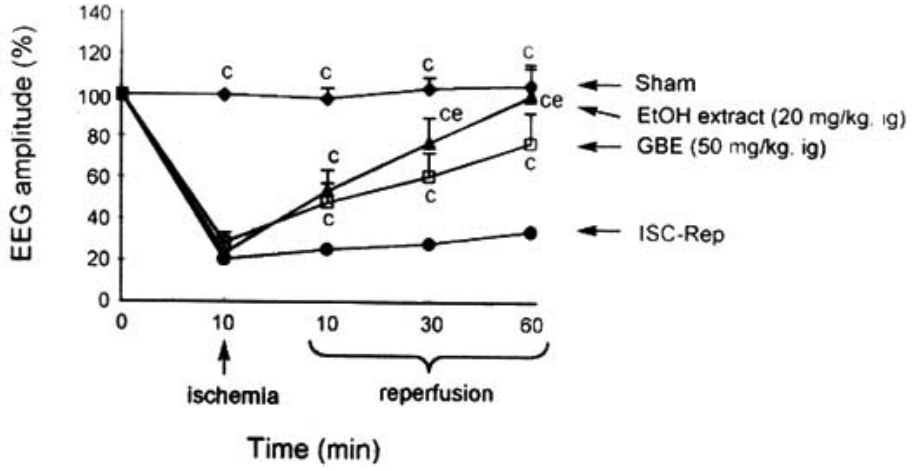


Fig. (9). Effect of the intragastric EtOH extract of *Spiraea japonica var. acuta* on EEG during cerebral ischemia/reperfusion in gerbils ($x \pm s$, $n = 8$) $^{c}P < 0.01$ for comparison with Isc-Rep group; $^{e}P < 0.05$ for comparison with GBE group (Student's *t*-test).

enzymatic activities, and reduction of the formation of nitric oxide.

SUMMARY

Spiraea japonica L. f. is a typical species complex widespread in East Asia. Except for *S. japonica var. japonica*, all of the complex can be found in Shouthwest China. There are more than 60 diterpene alkaloids and 7 diterpenoids isolated from *Spiraea japonica*, some of them were showed bioactivities of anti-inflammation, anti-platelet aggregation and neuroprotective effects. It was noteworthy that some alkaloids such as spiramine T and the extracts of the varieties such as *S. japonica var. acuta* showed markedly neuroprotective effects under the cerebral ischemia-reperfusion injury, and the different chemicals attributed to the neuroprotective effects under the ischemia-reperfusion by the different manners [49].

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