L-8 EFFECTS OF SPIRAMINE ALKALOIDS ON THE PLATELET AGGREGATION

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Spiraea japonica L. F., a plant species belongs to the *Spiraea* genus of Rosaceae family, has seven varieties. There are all varieties distributed in Yunnan province of China, and *S. japonica var. acuminata* and *S. japonica var. fortunei* were used as the folk medicines to treat malaria and inflammation etc..

Since 1960s, Japanese and Russian scholars obtained C_{20}-diterpenoid alkaloids from *S. japonica* L.F.[1], also Chinese scholars got a series of Hetisine-type C_{20}-diterpenoid alkaloids from *S. japonica var. fortunei* in 1985[2]. Since then, we started the chemical and pharmaceutical studies of this complex[3]. Here we wish to report the bioactive effects of spiramine alkaloids and their derivatives on the platelet aggregation induced by AA, PAF, or ADP.

1.Isolation of Chemical Components:

22 New alkaloids and 7 new diterpenoids, together with 5 known alkaloids were isolated from *S. japonica* complex by our group(Table 1), which belong to the three types(Fig. 1):

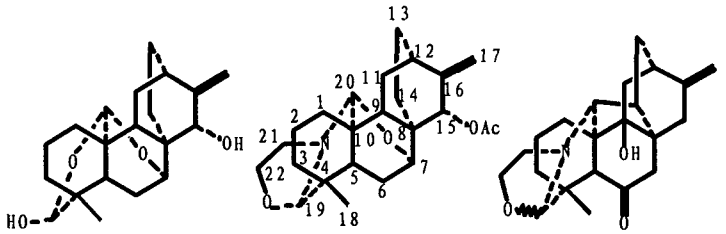


Fig.1

The major components of basic extract isolated from this complex are spiramine A, B, C and D. With spiramine F, all of above alkaloids could be reduced to same product triol. Spiramine H probably is a chemically intermediate between atisine- and hetisine-type alkaloids. Spiramine Q and T were also isolated from *S. japonica var. acuta* as the major alkaloids, which showed strong effects on platelet aggregation induced by PAF or AA. It is interested that atisane-type diterpenoids bearing aldehyde group(spiraminol) is possible biosynthetic predecessor of spiramines by our research result of biomimetic synthesis study. The chemotaxonomy study by our group implied the evolution tendency from west area to east area for *S. japonica* complex, and South-West of China especially Hengduan Mountain is the centre of modern differentiation, probably is one of the original centre of this complex[3m].

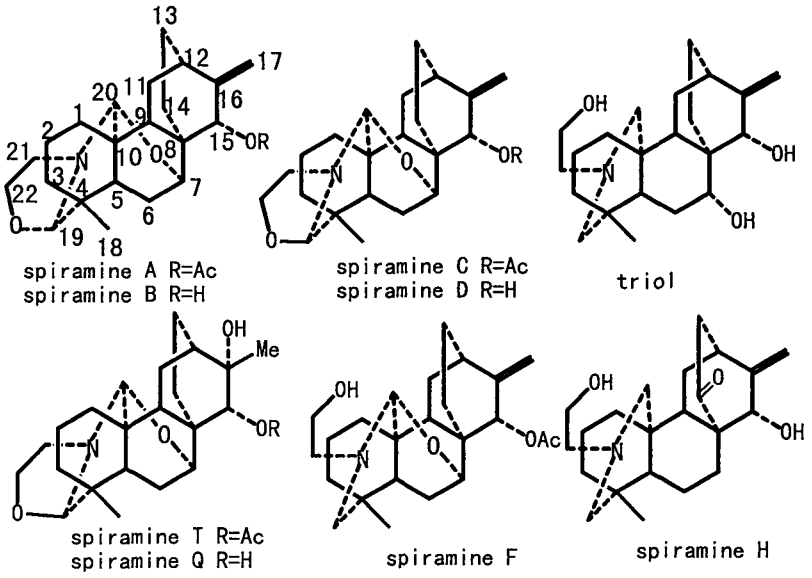


Table1. Isolated chemical constituents from *Spiraea japonica* complex

S.japonica var.-	Atisane-type	Atisine-type	Hetisine-type
acuta Yu	4	8	
incisa Yu	1	5	
ovalifolia Franch		4	
stellaris Rehd.	2	8	
acuminata Franch	1	17	
glabra (regel) Koidz	3	3	
fortunei (Planchon) Rehd.			17
S. japonica L. F.		3	5

2.Preparation of Derivatives:

Primary screening of spiramine alkaloids for anti-PAF activity revealed some information about relationship between structure-activity. In order to approach target compound for development of new drug, a series of derivatives was also prepared.

(1) Allylic rearrangement:

In the solution of 60% H_2SO_4 , spiraminol could be converted to 16-methyl-15-oxo derivatives at r. t., underwent allylic rearrangements. The products were a pair of epimers(Fig.2).

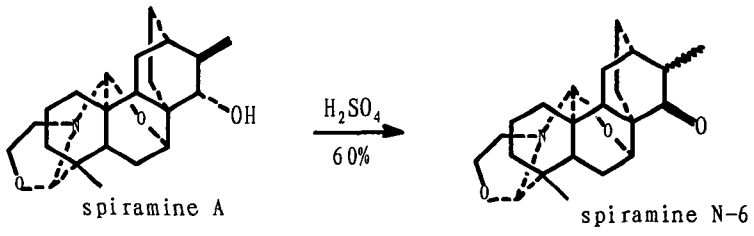


Fig.2

(2) Oxidation with $Pb(OAc)_4$:

Radical oxidation of spiramines with $Pb(OAc)_4$ afforded some interested results which have not been reported before(Fig.3).

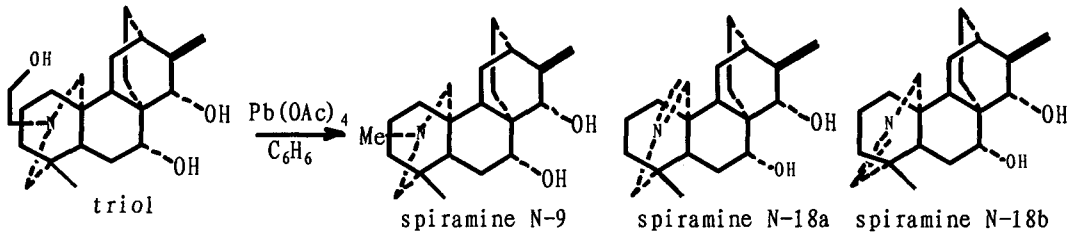


Fig.3

(3) Hydroxylation with $Hg(OAc)_4$-$NaBH_4$:

Hydroxylation at 16-C=C bond is important because the products showed higher anti-PAF activities. The stereoselectivity was about 3:1 for N-35 and N-33(Fig.4).

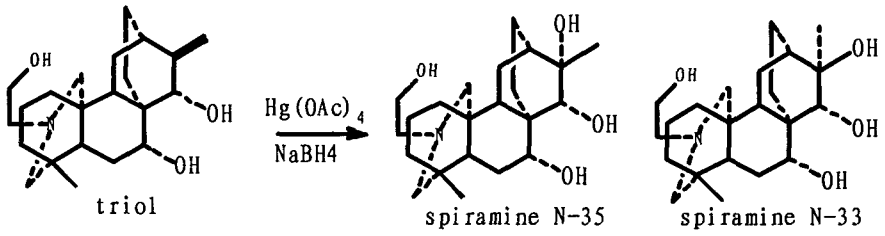


Fig.4

(4) Intramolecular Cannizarro reaction:

Treating with strong base, spiramine C or D could be converted to a mixture of spiramilactone, spiramilactone C and spiraminol N-7 through a intramolecular Cannizarro reaction . In the same condition, spiraminol and other analogies also could be converted to lactones, underwent the intramolecular Cannizarro reaction. This reaction is very important for the biosynthesis study of spiramines(Fig.5).

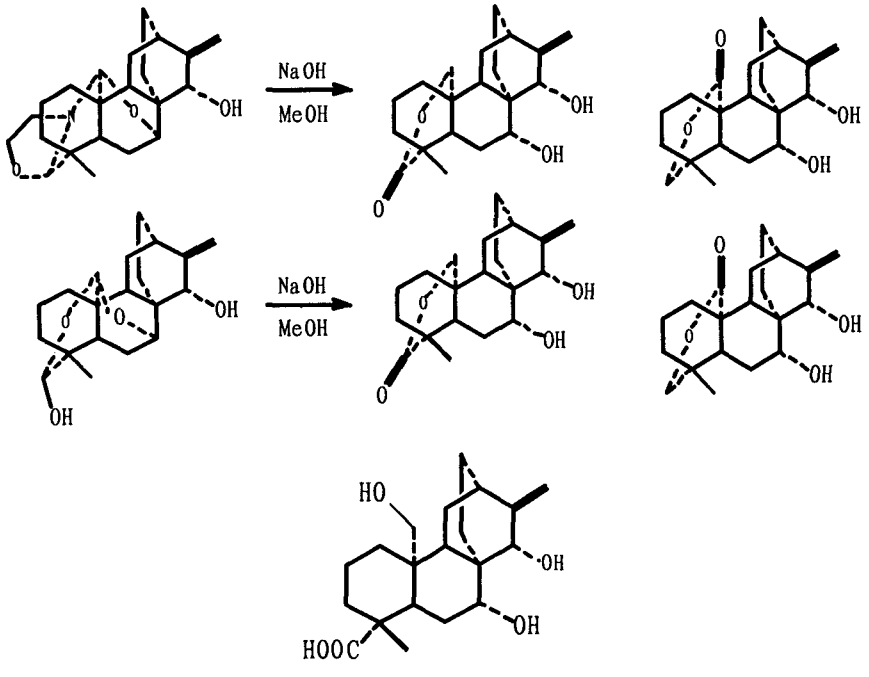
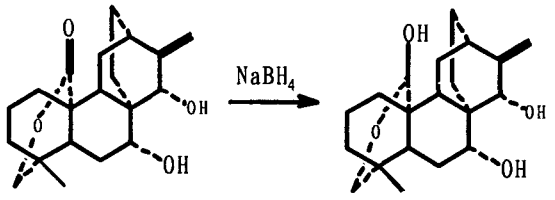


Fig.5

(5) Reduction of lactone with $NaBH_4$:

The sure fact was get that lactone could be reduced to hemialdehyde using $NaBH_4$ as the reduction reagent. This result afforded a good method for preparation of key compound of biosynthesis study from the major natural products in plants(Fig.6).



spirami lactone　　spiramine N-14

Fig.6

3.Primary Screening for Anti-PAF Activity:

Most of spiramines showed more or less ant-inflammation activities by karrageenin edema(1) and croton ear edema(2) methods[3]. This fact let us turn to investigate the effect of spiramines on anti-PAF activity because PAF could induce inflammation and platelet aggregation. Fortunately, research results confirmed our predict.

Table 2. The Effects of Spiramines and Derivatives On Rabbit Platelet aggregation induced by PAF(in vitro, sample 50mg/L, PAF 2μl)

sample + PAF	Inhibition rate% (Aggregation rate%)		
No.	1	2	3
control(H_2O, 10μl)	0 (55)	0 (51)	0 (57)
spiramine A and B	12.7 (48)	21.6 (40)	14.0 (49)
spiramine C and D	18.1 (45)	23.5 (39)	29.7 (40)
spiramine H	38.0 (34)	39.2 (31)	42.0 (33)
spiramine F	38.0 (34)	41.2 (30)	43.7(32)
spiramine N-21	54.3 (25)	60.7 (20)	59.5 (23)
spiramine N-9	63.5 (20)	49.1 (26)	49.0 (29)
spiramine N-18	81.4 (10)	74.5 (13)	80.5 (11)
spiramine Nfe-2	72.4 (15)	66.6 (17)	66.5 (19)
spiramine N-35	72.4 (15)	66.6 (17)	77.0 (13)
spiramine N-33	74.2 (14)	70.5 (15)	80.5 (11)
spiramine Q	36.2 (35)	29.4 (36)	35.0 (37)
triol	77.8 (12)	80.3 (10)	77.0 (13)
spiramine N-7	65.2 (19)	64.6 (18)	64.7 (20)

For the structure-activity relationship of spiramines and derivatives, the chemical conversion of C_{16}-exo-methylene(by Allylic rearrangement or Hydroxylation) is the key point of raising activity.

4. Effects of Spiramine N-6, Spiramine Q and Spiramine T on Platelet Aggregation Induced by PAF, AA, and ADP.

Spiramine N-6 showed good anti-PAF activity, $IC_{50}=2.6x10^{-5}$ mol/L. In vivo effects of spiramine N-6 on the platelet aggregation induced by AA, PAF or ADP

(2mg/kg, i.v.) see Table 3.

Table 3.The effects of spiramine N-6 on the platelet aggregation induced by AA, PAF or ADP (2mg/kg, i.v., n=2 rabbits, P*<0.05, P<0.01)**

	Platelet Inhibition (%)		
Time	PAF	AA	ADP
0 min	0	0	0
10 min	3.8*	75.7**	4.4*
30 min	38.3**	77.7**	22.4*
60 min	50.4**	65.3**	24.3**
90 min	40.9**	49.6**	39.9**
120 min	7.4*	19.4*	7.9*

Spiramine Q showed special anti-AA activity, $IC_{50}=1.9x10^{-5}$ mol/L. In vivo test, the effects of spiramine Q on the platelet aggregation induced by AA was very fast(after i.v. 10min, inhibition 100%, see Table 4).

Table 4.The effects of spiramine Q on the platelet aggregation induced by AA, PAF or ADP (2mg/kg, i.v., n=2 rabbits, P*<0.05)

	Aggregation (%)		
Time	PAF	AA	ADP
0 min	67.5	75.0	69.0
10 min	60.5	0.0*	62.5
30 min	67.0	0.0*	64.5
60 min	65.0	0.0*	66.4
90 min	65.0	14.0*	61.0
120 min	62.5	62.0	64.0
180 min	66.3	71.5	66.6

Spiramine T showed good anti-PAF activity, $IC_{50}=5.8x10^{-5}$ mol/L. While in vivo test, spiramine T inhibited on platelet aggregation not only induced by PAF, but also induced by AA. Even after 240min, the inhibition rate was still 77.6%(see Table 5).

Table 5.The effects of spiramine T on the platelet aggregation induced by AA, PAF or ADP (2mg/kg, i.v., n=2 rabbits, P*<0.05)

	Aggregation (%)		
Time	PAF	AA	ADP
0 min	57.2	61.6	57.2
15 min	52.8	61.4	51.0
60 min	47.8	0.6*	50.6
120 min	19.2*	0.0*	47.0
180 min	13.4*	2.4*	47.8
240 min	10.4*	13.8*	50.8

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References

[1] a. V. I. Frolova et al: Med. Prom. USSR., 18, 19(1964).
b. G. Goto et al: Tetrahedron Lett., 1369(1968).
c. K. Sasaki et al: J. Chem. Soc. B., 354(1971).
d. G. Goto et6 al: Tetrahedron Lett., 2928(1968).
e. V. D. Gorbunov et al: Khim. Prir. Soedin., 12, 124(1976).
f. V. D. Gorbunov et al: Khim. Prir. Soedin., 5, 454(1969).

[2] a. F. Sun et al: Heterocycles, 24(8), 2105(1986).
b. F. Sun et al: Heterocycles, 26(1), 19(1987).
c. F. Sun et al: Yaoxuexuebao, 20(12), 913(1985).
d. F. Sun et al: Tetrahedron Lett., 27(3), 275(1986).
e. F. Sun et al: J. Nat. Prod., 50(5), 923(1987).
f. F. Sun et al: J. Nat. Prod., 51(1), 50(1988).

[3] a. X-J Hao et al: Chem. Pharm. Bull., 35, 1670(1987).
b. M. Node et al: Heterocycles, 30, 635(1990).
c. X-J Hao et al: Acta Bota. Yun., 13(4), 452(1991).
d. X-J Hao, et al: Acta Bota. Yun., 14(3), 314(1992).
e. X-J Hao et al: Chinese Chem. Lett., 3, 427(1992).
f. X-J Hao et al: Heterocycles, 36(4), 825(1993).
g.X-S Yang et al: Acta Bota. Yun., 15(4), 412(1993).
h. X-J Hao et al: Acta Bota. Yun., 16(3), 301(1994).
i. X-J Hao et al: Phytochemistry, 38(2), 545(1995).
j. X-Y Hao et al: Guizhou Science, 13(2), 25(1995).
k. J-L Nie et al: Acta Bota. Yun., 18(2), 226(1996).
l. X. Hong et al: Chinese Chem. Lett., 7, 133(1996).
m. X-J Hao et al: Acta Bota. Yun. 19(3), 297(1997).
n. J-L Nie et al: Acta Bota. Yun. 19(3), 327(1997).
o. J-L Nie et al: Actaq Bota. Yun. 19(4), 429(1997).
p. X-J Hao et al: Phytochemistry, 48(7), 1213(1998).