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ANTIPLATELET ACTIONS OF APORPHINOIDS FROM FORMOSAN PLANTS

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Abstract: Seventeen aporphines were tested for antiplatelet activity. L-(+)-hernovine HCl and 7-hydroxydehydrothalicsimidine strongly inhibited platelet aggregation induced by adenosine 5'-diphosphate (ADP), arachidonic acid (AA), collagen, and platelet-activating factor (PAF). The latter showed the strongest antiplatelet activity with an IC_{50} of 70.4 μM against AA-induced platelet aggregation.

Keywords: Alkaloids, Anticoagulants, Natural products, Plants

Platelets play an important role in the hemostatic process, and their aggregation can cause arterial thrombosis. Accordingly, compounds with antiplatelet activity can be useful therapeutic agents. The isoquinoline alkaloids display numerous biological activities[1,2], including antiplatelet activity. The Formosan (Taiwanese) Annonaceae and Lauraceae plants have been as the subject of phytochemical studies in our laboratory for many years, and these families have considerable potential as a source of the isoquinoline alkaloids. Recently, we demonstrated that (+)-laurotetanine, (-)-discretamine, atherosperminine, liriodenine and octeine possess marked antiplatelet and vasorelaxing effects.[3,4] Previously[5], we described the antiplatelet activity of twenty aporphines. As a continuing investigation on the structure-activity relationships of these compounds for their antiplatelet effects, members of a series of aporphinoids were tested. In this paper, we will report the antiplatelet activity of twelve aporphines, which were isolated from Formosan plants, and five semi-synthetic derivatives.

We evaluated the antiplatelet effects of L-(+)-hernovine HCl (**1**), (+)-*N*-methylhernovine picrate (**2**), (+)-*N*-methyllaurotetanine MeI (**3**), (+)-laurolitsine picrolonate (**4**), (+)-*N*-methyl-10-*O*-methylhernovine (**5**), (+)-nornuciferine (**6**), L-(+)-nandigerine HBr (**7**), (+)-*N*-methylovigerine picrate (**8**), (-)-roemerine HCl (**9**), (+)-*O,O,N*-trimethylhernovine MeI (**10**), (+)-*N*-methylhernovine MeI (**11**), (+)-magnoflorine picrate (**12**), (+)-*O,N*-dimethylnandigerine MeI (**13**), (+)-*O,N*-diacetylactinodaphnine (**14**), 7-hydroxydehydrothalicsimidine (**15**), 7-hydroxydehydroglaucine (**16**), and 7-dehydrolirinidine (**17**) on the aggregation of washed rabbit platelets

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induced by adenosine 5'-diphosphate (ADP, 20 μM), arachidonic acid (AA, 100 μM), collagen (10 μg/ml), platelet-activating factor (PAF, 2 ng/ml). Aspirin was used as a reference control. The results are shown in Tables 1 and 2.

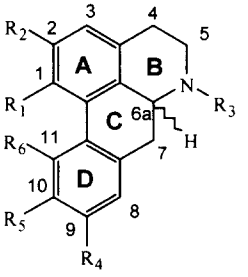

Fig.1. Structures of aporphines

Compound	Config of 6a	R_1	R_2	R_3	R_4	R_5	R_6
L-(+)-hernovine HCl (**1**)	S	OCH_3	OH	H	H	OH	OCH_3
(+)-*N*-methylhernovine picrate (**2**)	S	OCH_3	OH	CH_3	H	OH	OCH_3
(+)-*N*-methyllaurotetanine MeI (**3**)	S	OCH_3	OCH_3	$(CH_3)_2I^-$	OH	OCH_3	H
(+)-laurolitsine picrolonate (**4**)	S	OCH_3	OH	H	OH	OCH_3	H
(+)-*N*-methyl-10-*O*-methylhernovine (**5**)	S	OCH_3	OH	CH_3	H	OCH_3	OCH_3
(+)-nornuciferine (**6**)	S	OCH_3	OCH_3	H	H	H	H
L-(+)-nandigerine HBr (**7**)	S	OCH_2O		H	H	OH	OCH_3
(+)-*N*-methylovigerine picrate (**8**)	S	OCH_2O		CH_3	H	OCH_2O	
(-)-roemerine HCl (**9**)	R	OCH_2O		CH_3	H	H	H
(+)-*O,O,N*-trimethylhernovine MeI (**10**)	S	OCH_3	OCH_3	$(CH_3)_2I^-$	H	OCH_3	OCH_3
(+)-*N*-methylhernovine MeI (**11**)	S	OCH_3	OH	$(CH_3)_2I^-$	H	OH	OCH_3
(+)-magnoflorine picrate (**12**)	S	OH	OCH_3	$(CH_3)_2X^-$	H	OCH_3	OH
(+)-*O,N*-dimethylnandigerine MeI (**13**)	S	OCH_2O		$(CH_3)_2I^-$	H	OCH_3	OCH_3
(+)-*O,N*-dimethylactinodaphnine (**14**)	S	OCH_2O		OCH_3	OCH_3	OCH_3	H

As indicated in Table 1, at a concentration of 100 μg/ml, **1** and **15** strongly inhibited platelet aggregation induced by all four agents (ADP, AA, collagen, and PAF). Among all tested compounds, only **1** and **15** significantly inhibited ADP-induced platelet aggregation. Compounds **2, 6, 9** and **16** were strong inhibitors of platelet aggregation induced by AA, collagen, and PAF, and **9** also showed slight but significant inhibition of the aggregation induced by ADP. Compounds **4** and **17** showed strong inhibitory effects on aggregation induced by AA and collagen, and these two compounds also showed slight but significant inhibition of the aggregation induced by PAF. In this study, **7** and **8** were strong inhibitors of collagen-induced platelet aggregation, and this activity was fairly selective. The quaternary aporphine alkaloids **3, 10, 11, 12** and **13** did not inhibit platelet aggregation induced by ADP, AA, collagen, or PAF.

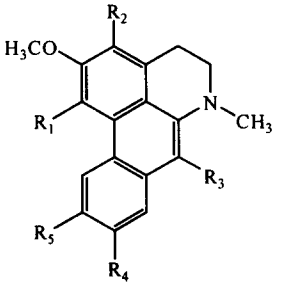

Fig.2. Structures of dehydroaporphines

Compound	R_1	R_2	R_3	R_4	R_5
7-hydroxydehydrothalicsimidine (**15**)	OCH_3	OCH_3	OH	OCH_3	OCH_3
7-hydroxydehydroglaucine (**16**)	OCH_3	H	OH	OCH_3	OCH_3
7-dehydrolirinidine (**17**)	OH	H	H	H	H

These results indicated that, in general, dehydroaporphine alkaloids are significantly active against AA- and collagen-induced aggregation, and inhibition of PAF-induced aggregation was enhanced if a hydroxy group was attached at the C-7 position.

Among the aporphine salts, hernovine hydrochloride (**1**) was extremely inhibitory and was more potent than the parent free base against ADP-, AA-, and collagen-induced aggregation (see data in reference 6). Two additional hernovine salts were tested: methyl picrate **2** and methiodide **11**. Compound **2** showed significant inhibition of AA-, collagen-, and PAF-induced aggregation, but **11** did not. Likewise, the methiodide salt **3** of *N*-methyllaurotetanine was inactive, while the parent aporphine showed strong inhibition

of AA-, collagen-, and PAF- induced aggregation as well as significant inhibition of ADP-induced platelet aggregation (see data in reference 5). Thus, the parent aporphines and their salts have varying effects on the inhibition of aggregation induced by ADP, AA, collagen and PAF.

Table 1. Effects of aporphine alkaloids on platelet aggregation induced by ADP, AA, collagen and PAF in washed rabbit platelets.[a]

Compound[b]	Aggregation (%)			
	ADP	AA	Collagen	PAF
1	10.5±8.6***	0.0±0.0***	0.0±0.0***	4.0±3.2***
2	62.8±14.9	34.9±17.7**	10.0±6.4***	18.3±15.8***
3	87.6±1.1	88.1±0.6	81.3±4.7	82.7±2.1*
4	92.1±0.3	0.0±0.0***	4.6±1.4***	79.3±2.5*
5	89.4±1.2	86.5±1.7	83.5±3.9	78.6±1.8**
6	77.1±2.5	0.0±0.0***	0.0±0.0***	30.6±2.2***
7	91.8±1.2	60.0±4.5**	0.0±0.0***	91.6±1.4
8	90.2±1.2	77.3±3.5*	0.0±0.0***	74.6±4.7
9	67.2±6.5**	0.0±0.0***	0.0±0.0***	27.8±3.9***
10	90.2±1.3	71.0±10.7	87.9±0.5	85.6±1.4*
11	90.7±0.4	83.7±1.2	89.8±0.6	85.5±2.1
12	86.1±1.1	84.4±1.9	85.0±0.5*	89.9±1.1
13	88.6±0.3*	89.0±2.4	77.6±7.6	83.9±2.0
14	86.8±2.5*	80.9±0.8*	87.3±2.4	92.2±1.3
15	34.5±18.1**	0.0±0.0***	0.0±0.0***	0.0±0.0***
16	78.3±3.4	21.8±9.7***	12.7±6.3***	0.0±0.0***
17	86.3±0.6	0.0±0.0***	0.0±0.0***	51.7±8.3*
Aspirin	77.9±1.9	0.0±0.0***	87.8±1.5	90.4±1.1
Control	82.2±1.6	86.7±0.4	89.0±0.6	90.5±1.1

[a] Platelets were preincubated with test compound, aspirin, or DMSO (0.5%, control) at 37 ℃ for 3 min, then ADP (20 μM), AA (100 μM), collagen (10 μg/ml) or PAF (2 ng/ml) was added. Percentages of aggregation are presented as means±S.E. (n=3-5); *p <0.05, **p <0.01, and ***p <0.001 as compared with the respective control.

[b] The concentration of each test compound was 100 μg/ml; aspirin was administered at 25 μg/ml.

As indicated in Table 2, **15** was the most effective antiplatelet compound; it inhibited both AA- and PAF-induced platelet aggregation with IC_{50} values of 70.4 and 11.8 μM, respectively.

In general, the antiplatelet effects of the aporphine alkaloids evaluated were different from that of aspirin, which is a cyclooxygenase inhibitor[7]. Aspirin completely inhibited AA-induced platelet aggregation, but not that of other inducers. In contrast, many aporphines including **15** showed significant inhibition in multiple assays. Thus, the mechanism(s) of action for **15** and other aporphines may be different from that of aspirin. It is possible compound **15** may inhibit TXA_2 formation in washed rabbit platelets[8]; however, the mechanism of action requires further investigation.

The structure-activity relationships and antiplatelet actions of aporphinoids present in this communication will provide a rational basis for design new alkaloids drugs in future. Furthermore, our experimental and biological evaluation[3-5] of the local alkaloid products from Formosan plants also demonstrate the local herbal medicines can be used as powerful drugs for the treatment of cardiovascular diseases.

Table 2. IC_{50} (μM) of test compounds on the platelet aggregation induced by AA, collagen and PAF in washed rabbit platelets.

Compound	AA	Collagen	PAF
1	167.7±32.6	—	—
4	149.2±35.1	—	—
6	210.6±12.5	—	—
8	—	193.5±7.1	—
9	195.0±54.8	88.2±19.7	—
15	70.4±3.5	—	11.8±2.5
16	—	—	52.6±9.5
17	171.3±47.6	—	—
Aspirin	164.2±5.8	>1000	>1000

Platelets were preincubated with DMSO (0.5%, control), aspirin or test compounds at 37 °C for 3 min, then arachidonic acid (AA, 100 μM), collagen (10 μg/ml) or PAF (2 ng/ml) was added. IC_{50} (μM) is the concentration at which 50% inhibition of platelet aggregation was reached as calculated from the dose-response curve. IC_{50} are presented as means ±S.E. (n = 3 - 5).

Experimental

MATERIALS- Aporphines (Figure 1) were either isolated from plant sources (**1**, **7** and **9** from *Neolitsea variabillima*[9], **4** from *Litea hayatae*[10], **5** and **8** from *Lindera oldhamii*[11], **6** from *Annona squamosa*[12], **9** from *Neolitsea aurata*[13], **12** from *Magnolia kachirachirai*[14], **15**, **16** and **17** from *Annona purpurea*[15,16], respectively) or prepared by semisynthesis (**2**[17], **3**[17], **10**[9], **11**[18], **13**[18], and **14**[19]).

PLATELET AGGREGATION ASSAY- The platelet aggregation assays were carried out according to reference[5].

DATA ANALYSIS- The experimental results are expressed as means±S.E. and accompanied by the number of observations. A one-way analysis of variance (ANOVA) was used for multiple comparison, and if significant variation occurred between treatment groups, the mean values for inhibitors were compared with those for controls by the Student's *t* test, and *p* values of less than 0.05 were considered to be statistically significant.

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