PHARMACOLOGICAL STUDIES ON "RHODOTOXIN" ISOLATED FROM *RHODODENDRON HYMENANTHES** AND "ANDROMEDOTOXIN" ISOLATED FROM *ANDROMEDA JAPONICA*, THUNBERG**

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INTRODUCTION

The poisonous properties of plants of family *Ericaceae* have been known for many years. The oldest report is Xenophon's (1) in about 400 B.C., in Anabasis, which is the poisoning of troops by the honey of flowers which have been identified as *Rhododendron ponticum*. Eykman (2) in 1882 isolated a toxic substance, which was precipitated by basic lead acetate, from *Andromeda japonica*, Thunberg, which he called "asebotoxin" according to the Japanese name of the plant.

Plugge (3-6), at the same period, isolated a colourless and transparent substance, m.p. 110-120°C, from *Andromeda japonica*, several species of *Andromeda* and other plants of *Ericaceae*, e.g. *Azalea indica*. He gave it the name "andromedotoxin." Afterwards in 1901 Hayashi and Muto (7) had tried to isolate pure andromedotoxin from *Andromeda japonica*. In recent time (1955) Murakami etc. (8) obtained presumably the same, but purer colourless substance, m.p. 130-135°C, from *Andromeda japonica*. In 1887 de Zaayer and Plugge isolated a toxic substance from *Rhododendron ponticum*, and gave it the name andromedotoxin, too. That was a white crystalline substance with m.p. 228-229°C and molecular formula $C_{31}H_{51}O_{10}$. Andromedotoxin obtained from *Rhododendron* in 1922 by Hardikar (9), was a nitrogen-free crystalline substance of 258°C m.p. and formula $C_{19}H_{32}O_6$. Horning, E.C. etc. (cited by Neil C. Moran etc., 1954) got a water-soluble, nitrogen-free, neutral crystalline substance, m.p. 258-260°C, $[\alpha]_D^{25}$ -8.4 (ethanol) closely resembling with the Hardikar's formula, from *Rhododendron maximum*. In 1927 Makino (10, 11) isolated rhodotoxin from *Rhododendron hymenanthos*. Makino's rhodotoxin was reported to have m.p. 245°C, and its formula was same with de Zaayer's andromedotoxin. In 1955 Takemoto etc. (14) obtained andromedotoxin, m.p. 258-260°C, $[\alpha]_D$ -8.6 from *Rhododendron maximum*, and rhodotoxin, m.p. 259°C, $[\alpha]_D$ -8.8, from *Rhododendron metternichii* and reported that rhodotoxin was identical with andromedotoxin.

* *Pieris japonica* D. Don.

** *Rhododendron Degronianum* Carriere form. *spontaneum* Nakai

Thus the melting points and analytical data of the hitherto isolated substances were not agreed. It was not known whether such differences were due to impurity of preparations or the substances were really different.

On the other hand, the first pharmacological studies of andromedotoxin isolated from *Andromeda* were made by Plugge (1882), and subsequent studies were carried out by Hayashi and Muto (1901), and Kohanawa (1955).

Crystalline andromedotoxin isolated from *Rhododendron* was studied first by de Zaayer (16) (1887), and subsequent studies were done by Archangelsky (1901), Hardikar (1922), Makino (1927, 1928) and Chu and How (cited by Neil C. Moran etc. 1931).

Plugge reported, that andromedotoxin causes a systolic standstill of frog hearts like digitalis and has a curare-like action on motor nerve endings. Hardikar made a more systematic evacuation of the actions of andromedotoxin, describing in mammals a depression of the respiration, and hypotention, a cardiotoxic action in frogs and rabbits, vomiting, and paralysis of skeletal muscle. Makino, working with a substance he termed "rhodotoxin," described effects essentially like those reported by Hardikar as to andromedotoxin.

While there is uniform agreement in the literature, that *Andromeda*, *Rhododendron* and *Kalmia* species contain a physiologically similar active principle or principles, descriptions of the isolated active agents are not in agreement.

Basing on the above literature, we think, we can classify these poisonous substances into three groups, andromedotoxin (ATA) isolated from *Andromeda japonica*, andromedotoxin (ATR) from *Rhododendrons* and rhodotoxin (RTR) from *Rhododendron hymenanthos*.

A series of experiments undertaken in attempt to compare these three substances pharmacologically and chemically have yielded some significant results.

MATERIALS AND METHODS

The isolation of rhodotoxin (RTR) from *Rhododendron hymenanthos*, Makino; and that of andromedotoxin (ATA) from *Andromeda japonica*, Thunberg were carried out by Plugge's revised method. RTR was purified easier than ATA.

ATA is m.p. 119–120°C, amorphous, colourless and transparent substance, on the other hand RTR is m.p. 258–260°C, white crystalline substance.

For the studies of lethal doses mice of 12–18 g body weight were used and were equally divided by sex. The substances were dissolved in sterile physiological saline solution, and were given subcutaneously in a volume of 0.25 ml/15g. The values of LD50 were calculated by van der Waerden's method.

Frogs (*Rana nigromaculata*) weighing 12–22 g were used in the studies of emetic and lethal doses. They were administered into the breast lymphsack with solutions of a volume of 0.5cc/10 g. As the solvent sterile Ringer's solution for cold-blooded animal was used.

For the blood pressure experiments cats and rabbits weighing from 2.3 to 3.9 kg were used. Under urethane (1 g/kg subcutaneously) anesthesia the usual operation for the recording of blood pressure of carotid artery with mercury manometer and of respiration with Marey's tambour connected to trachea was performed.

RESULTS

A. Lethal dose of RTR and ATA

The results of experiment on mice are given in Table 1. From these results following 24 hours' LD50 was found (by van der Waerden's method):

RTR: 0.148 ± 0.01 $\mu\text{g/g}$, ATA: 3.473 ± 0.132 $\mu\text{g/g}$

The toxicity of RTR is 23.4 times that of ATA.

TABLE 1. Death rate of mice during 24 hours after subcutaneous administration of RTR and ATA.

RTR			ATA		
Dose ($\mu\text{g/g}$)	Death time after administration (min)	Death rate (%)	Dose ($\mu\text{g/g}$)	Death time after administration (min)	Death rate (%)
1.0	7.6	100	4.5	12	100
0.5	10.4	100	4.0	13.7	70
0.25	17.5	100	3.5	13	60
0.1875	33.9	70	3.25	17.6	30
0.125	44.75	40	3.0	15	10
0.09375	—	0	2.0	—	0

Each group: 10 mice

The results of experiments on frogs are given in Table 2. The death time after administration is 2.5–6 hours in both RTR and ATA. The 24 hours' LD50 was:

RTR: 3.899 ± 0.125 $\mu\text{g/g}$, ATA: 5.074 ± 0.66 $\mu\text{g/g}$

TABLE 2. Death rate of frogs during 24 hours after administration into lymphsack of RTR and ATA.

Dose ($\mu\text{g/g}$)	10	7.5	5.5	5.0	4.1	3.7	3.4	3.1	2.8	2.5	2.0
Death rate of ATA (%)	100	70	—	50	—	—	—	—	—	20	0
Death rate of RTR (%)	—	—	100	90	70	50	20	10	0	—	—

Each group: 10 mice

The toxicity of RTR is 1.3 times that of ATA in frogs.

The lethal dose of intravenously administered RTR and ATA for cats and rabbits anesthetized with 1 g/kg of urethane, was 300–400 $\mu\text{g/kg}$. The animals died from 8 to 28 minutes after the administration.

B. Emetic action of RTR and ATA

Healthy adult cats, weighing from 2.3 to 4.14 kg were used. Sterile physiological saline solutions were used as the solvent, and 0.01% solution was given subcutaneously. The results are given in Table 3 and 4.

TABLE 3. Emetic action of RTR in cats.

Cat No.	Body weight (g)	Sex	Dose ($\mu\text{g/g}$)	First vomiting time (min)	Average first vomiting time (min)	Vomiting number	Average vomiting number
1	3750	♂	80	—	—	0	0
2	2980	♂				0	
3	2830	♂				0	
4	2700	♂				0	
5	2650	♂				0	
6	3270	♂	85	52	48	4	4.5
7	3000	♀		41		7	
8	2550	♂		52		3	
9	2500	♂		48		4	
10	2400	♂				0	
11	3730	♂	90	29	33.4	1	2.2
12	3500	♂		37		2	
13	3370	♂		39		2	
14	3250	♂		31		4	
15	2400	♂		31		2	

TABLE 4. Emetic action of ATA on cats after subcutaneous administration.

Cat No.	Body weight (g)	Sex	Dose ($\mu\text{g/kg}$)	First vomiting time (min)	Average first vomiting time (min)	Vomiting number	Average vomiting number
16	2350	♂	60	—	—	0	0
17	2930	♂		—		0	
18	2700	♀		—		0	
19	3220	♂		—		0	
20	2800	♂		—		0	
21	3700	♀	70	26	26	1	1
22	4140	♀		—		0	
23	2350	♀		—		0	
24	2800	♂		—		0	
25	2000	♂		—		0	
26	2300	♀	80	—	19	0	19
27	3100	♂		19		19	
28	2030	♀		—		0	
29	2650	♂		—		0	
30	3530	♀		—		0	
31	2840	♂	90	—	29	0	4
32	3000	♂		29		4	
33	3580	♀		—		0	
34	2400	♂		—		0	
35	2400	♂		—		0	
36	2425	♀	100	—	26	0	4.5
37	3520	♂		18		8	
38	2600	♀		—		0	
39	2450	♂		34		1	
40	2450	♀		—		0	

41	2680	♀	150	—	24	0	3
42	2950	♂		—		0	
43	2350	♀		—		0	
44	2770	♂		16		5	
45	2200	♀		32		1	
46	2400	♀	200	18	32	2	6
47	2750	♂		32		4	
48	3700	♀		22		12	
49	3650	♂		33		4	
50	3250	♀		60		8	
22	2000	♂	500	60	30	1	8.3
23	3890	♀		15		13	
25	2890	♂		15		11	
21	2900	♂	600	17	14	4	7.3 (dead)
23	3500	♀		11		13	
24	2400	♂		14		5	

From Table 3 and 4, ED50 (the dose which causes vomiting in 50 per cent of cases of test animal) was:

RTR: $83.386 \pm 0.864 \mu\text{g/kg}$, ATA: $123.3 \pm 15.2 \mu\text{g/kg}$

The results of experiments performed on emetic action on frogs of RTR and ATA are given in Table 5 and 6. ED50 of both substances calculated from them are:

RTR: $0.347 \pm 0.026 \mu\text{g/g}$, ATA: $1.180 \pm 0.120 \mu\text{g/g}$

The emetic action of RTR is 3.4 times that of ATA on frogs.

TABLE 5. Emetic reaction of frogs against administration of RTR.

Dose ($\mu\text{g/g}$)	Number of frogs used	Mouth-opening reaction (Nausea)			Vomiting		
		Number of positive cases	Time of first appearance (min)	Average number of reaction	Number of positive cases	Time of first appearance (min)	Average number of vomiting
0.255	10	2	38	5	0	0	0
0.362	10	8	32.7	9.5	6	35.6	6.3
0.500	10	6	23.7	11.7	10	33.4	2.8
0.590	10	10	25.6	35.6	6	16	2.3
0.707	10	10	15.6	30.2	2	27	1
1.083	10	10	18.5	8.5	0	—	0
1.919	1	1	17.0	7	0	—	0
2.565	1	1	11.0	4	1	—	2
3.415	5	5	8.4	3.6	0	—	0
3.749	3	3	14.2	5	0	—	0
4.132	3	3	12.2	4.7	0	—	0
4.545	4	4	10.5	6	0	—	0
5.000	5	5	13.4	8.8	0	—	0
5.500	5	5	13.0	8.2	0	—	0
6.555	1	0	—	0	0	—	0
9.300	1	1	13.0	3	0	—	0

TABLE 6. Emetic reaction of frogs against administration of ATA.

Dose ($\mu\text{g/g}$)	Number of frogs used	Mouth-opening reaction (Nausea)			Vomiting		
		Number of positive cases	Time of first appearance (min)	Number of reaction	Number of positive cases	Time of first appearance (min)	Number of vomiting
0.125	10	1	58	1	0	—	0
0.1875	10	5	23.9	8.2	0	—	0
0.375	10	8	15.375	28.25	0	—	0
0.75	10	9	23.88	18.77	1	44	1
1.000	10	5	16	18	1	23	7
1.25	10	7	21.5	5	2	39.25	2
1.3	10	10	19.9	17.9	5	37	2
1.375	10	10	12.6	23.2	10	20.1	3
1.45	10	10	19.4	18.2	4	19.25	3.25
1.50	10	8	18.25	6.75	2	53	1
1.75	10	10	17.85	19.6	2	25.75	1
2.0	10	10	10.15	21.1	0	—	0
2.5	10	9	14.1	12.5	1	26.5	1
5.0	10	8	11	1.5	1	16	2
7.5	10	8	10.1	1	0	—	0
10.0	10	8	8.81	1.375	0	—	0

C. Vasodepressor action of RTR and ATA in anesthetized mammals (20 $\mu\text{g/kg}$)

The results are given in Table 7 and 8.

TABLE 7. Vasodepressor action of ATA and RTR in anesthetized rabbits.

Agent	Rabbit No.	Body weight (g)	Sex	Normal blood pressure (mmHg)	Change of blood pressure after administration										
					Maximum depress. bl. pres. (mmHg)	Its ratio (%)*	Its time (min)	15 min		30 min		45 min		60 min	
								(mmHg)	(%)*	(mmHg)	(%)*	(mmHg)	(%)*	(mmHg)	(%)*
RTR	6	2300	♂	104	56	53.8	5	70	67.3	94	90.4	96	92.3	100	96.1
	7	2300	♂	93	34	36.5	5	42	45.1	60	64.5	69	74.2	78	83.9
	8	2900	♂	116	40	34.5	3	58	50.0	88	75.8	100	86.2	112	96.5
	9	3850	♂	120	36	30.0	2	44	36.6	78	65.0	106	96.7	106	96.7
	10	3000	♂	103	44	42.7	2	54	52.4	61	59.2	68	66.0	78	75.7
Average						39.5	3.4		50.3		70.7		80.8		89.8
ATA	11	2650	♂	100	58	58.0	5	78	78.0	87	87.0	90	90.0	92	92.0
	12	2800	♀	117	88	75.2	8	100	85.5	102	87.2	103	88.0	104	88.9
	13	2600	♂	106	38	35.8	5	58	50.0	86	81.1	98	92.5	103	97.2
	14	3740	♂	138	99	71.7	8	111	80.5	130	94.2	134	97.1	138	100.0
	15	3750	♂	115	81	70.4	2	100	87.0	104	90.5	104	90.5	104	90.5
Average						62.2	5.6		76.2		88.0		91.6		93.7

(%)*: (Ratio of blood pressure at each time after administration against normal blood pressure) × 100

TABLE 8. Vasodepressor action of ATA and RTR in anesthetized cats.

Agent	Cat No.	Body weight (g)	Sex	Normal blood pressure (mmHg)	Change of blood pressure after administration										
					Maximum depress. bl. pres. (mmHg)	Its ratio (%)	Its time (min)	15 min		30 min		45 min		60 min	
								(mmHg)	(%)*	(mmHg)	(%)*	(mmHg)	(%)*	(mmHg)	(%)*
RTR	51	2650	♂	133	62	46.6	3	106	79.2	112	83.7	112	83.7	103	77.4
	52	2450	♀	134	61	45.5	3	106	79.2	112	83.7	112	83.7	113	84.3
	53	3180	♀	153	86	56.1	2	124	81.0	132	86.3	134	87.6	128	83.7
	54	2670	♂	155	32	20.7	2	68	45.3	106	68.4	106	68.4	112	72.3
	55	3980	♀	160	64	40.0	2	84	52.5	96	60.0	108	67.5	132	82.5
Average						41.8	2.4		64.5		74.6		76.8		80.0
ATA	56	2900	♂	150	128	85.4	8	133	88.8	138	92.0	150	100.0		
	57	3600	♂	158	126	79.8	2	142	89.9	156	98.7	158	100.0		
	58	3400	♂	156	126	80.8	2	146	93.5	151	96.7	156	100.0		
	59	2670	♂	158	116	73.5	3	155	98.2	158	100.0				
	60	3500	♀	160	115	72.5	3	156	97.5	160	100.0				
Average						78.3	3.6		93.6		97.5		100.0		

Above mentioned experimental data may be summarized as Table 9.

TABLE 9. The pharmacological comparison of RTR and ATA.

		Animal	Administration	RTR	ATA	$\frac{\text{ATA}}{\text{RTR}}$
Toxicity	LD50 ($\mu\text{g/g}$)	Mouse	subcutaneous	0.148 ± 0.01	3.473 ± 0.132	23.4*
		Frog	into lymphsack	3.899 ± 0.125	5.074 ± 0.66	1.3
	MLD ($\mu\text{g/kg}$)	Cat	intravenous	400	400	1
		Rabbit	intravenous	270~400	300~400	1
Emetic dose	(ED50 ($\mu\text{g/g}$))	Frog	into lymphsack	0.347 ± 0.026	1.180 ± 0.12	3.4
		Cat	subcutaneous	83.3 ± 0.8	123.3 ± 15.2	1.47
	Mouth-opening sphere	Frog	into lymphsack	0.255~5.50	0.125~10	
	Vomiting sphere	Cat	subcutaneous	above 80	above 70	0.87
Vaso- depressor action	Maximum	Cat	20 $\mu\text{g/kg}$ intravenous	41.8%	78.2%	
	15 min after adm.			64.5%	93.6%	
	60 min after adm.			80.0%	100.0%	
	Maximum	Rabbit	20 $\mu\text{g/kg}$ intravenous	39.5%	62.2%	
	15 min after adm.			50.3%	76.2%	
	60 min after adm.			89.8%	93.7%	

* shows that the toxicity of RTR is 23.4 times that of ATA.

D. The comparison of physico-chemical characters of RTR and ATA

The results are given in Table 10.

TABLE 10. Comparison of physico-chemical characters of RTR and ATA.

		RTR	ATA
	Crystal form	Needle-shaped crystal	Amorphous
	Colour	White	Colourless and transparent
	Melting point	258—260°C	119—120°C
Precipitation by	Fe ₂ Cl ₃	—	—
	CuSO ₄	—	—
	HgCl ₂	—	—
	AgNO ₃	—	—
	Lead acetate	—	—
	Phosphomolybdic acid	—	—
	Scheibler's reagent	—	—
	Meyer's reagent	—	—
	Picric acid	—	—
	Tannic acid	—	—

Colour reaction	H ₂ SO ₄ conc.	dark red	dark violet red
	H ₂ SO ₄ dil.	rosy	rosy
	HCl conc.	red or rosy	red or rosy
	HCl dil.	rosy	rosy
	HNO ₃ conc.	yellow	yellow
	HNO ₃ dil.	yellowish	yellowish
	H ₃ PO ₄ conc.	mulberry	mulberry
	H ₃ PO ₄ dil.	mulberry or reddish	mulberry or reddish
Reduction of Fehling's solution		—	—
Rf-values of chromatogram (<i>n</i> -Butanol saturated with water) 18~20°C		0.96	0.89

DISCUSSION

Since the studies of Plugge, the poisonous principles of plants of family *Ericaceae* have been called "andromedotoxin" by Hayashi (7), Hardikar (9), N.C. Moran (12, 13), Takemoto et al. (14), de Zaayer (16), Tokuda (18) and Masaki (19). Makino (10, 11) is the only one who has termed the active substance which he had isolated from *Rhododendron hymenanthes* as "rhodotoxin." He reported that rhodotoxin was different from andromedotoxin isolated from *Rhododendron ponticum* by de Zaayer.

Following Prof. Hayashi's study, Prof. Masaki has studied on the poisonous honey based on andromedotoxin; he has been studying further on the difference and similarity between rhodotoxin and andromedotoxin. By chance when the present writer entered his Department of Pharmacology, he directed the writer to study on this theme. Some significant results as shown above have been obtained. Namely, studies on rhodotoxin (RTR) and andromedotoxin (ATA) in chemical and pharmacological aspects have proven that they are different substances.

On the other hand, it has become clear by comparison of data in Takemoto's report (14) and those of the writer on rhodotoxin, that the poisonous principles of 258–260°C isolated from *Rhododendron* are the same, that the reported melting point of Makino's rhodotoxin, 245°C, was due to impurity of preparation, and that the significance of reason for giving the poisonous principle of *Rhododendron hymenanthes* the special name, "rhodotoxin" is quite meaningless.

On the other side, the data from comparative studies of rhodotoxin isolated from *Rhododendron hymenanthes* (RTR) and andromedotoxin isolated from *Andromeda japonica* (ATA) are as shown above. That is to say, the pharmacological actions of RTR and ATA are alike, yet unlike in pharmacological intensity; in chemical reaction they are alike, yet unlike in melting point and Rf-value of the paperchromatograms.

So, it is the opinion of the writer that separate terms should be retained, because ATA isolated from *Andromeda* and RTR obtained from *Rhododendron* (including *Rhododendron hymenanthes*) are different substances. Calling them by the same name "andromedotoxin" is inconvenient and likely to cause confusion in the prosecution of researches.

So the writer proposes, in commemoration of Makino's work, to apply the name "rhodotoxin" to the white crystalline substance with m.p. 258–260°C isolated from *Rhododendron*

and other plants of *Ericaceae* excepting *Andromeda*. He further proposes to give the different substance obtained from *Andromeda* the term "andromedotoxin" in commemoration of Plugge's work; or to leave for that substance the common name "andromedotoxin," and to give this present substance derived from *Andromeda* a new name.

SUMMARY

1. In chemical characters of rhodotoxin (RTR) and andromedotoxin (ATA) isolated from *Andromeda japonica*, they have similar reactions against several reagents, but m.p. 120–119°C in ATA, 258–260°C in RTR, and Rf-value of paperchromatograms is 0.89 in ATA, 0.96 in RTR.

2. LD₅₀ on mice (subcutaneously) is 3.473 ± 0.132 $\mu\text{g/g}$ in ATA, and 0.143 ± 0.01 $\mu\text{g/g}$ in RTR. The toxicity on mice of RTR is 23.4 times that of ATA.

LD₅₀ on *Rana nigromaculata* (into lymphsack) is 5.074 ± 0.66 $\mu\text{g/g}$ in ATA, and 3.899 ± 0.125 $\mu\text{g/g}$ in RTR. The toxicity on frogs of RTR is 1.3 times that of ATA.

MLD on cats and rabbits anesthetized with urethane (intravenously) is about 400 $\mu\text{g/kg}$ in both ATA and RTR, the time of death after administration is from 7 to 28 min. The cause of death is respiratory paralysis in all cases.

3. The emetic action on cats of RTR (subcutaneously) occurs from 80 $\mu\text{g/kg}$, and the dose which causes vomiting in 50 per cent of cases of that animal (ED₅₀) is 83.3 ± 0.8 $\mu\text{g/kg}$; the action of ATA occurs from 70 $\mu\text{g/kg}$, and its ED₅₀ is 123.3 ± 15.2 $\mu\text{g/kg}$. The emetic action of RTR is 1.47 times that of ATA.

ED₅₀ on *Rana nigromaculata* (into lymphsack) is 0.347 ± 0.026 $\mu\text{g/g}$ in RTR, and 1.180 ± 0.120 $\mu\text{g/g}$. The emetic action on frogs of RTR is 3.4 times that of ATA.

So-called frog's mouth-opening corresponding with mammal's nausea, occurs from 0.25 $\mu\text{g/g}$, and always from 0.59 to 5.5 $\mu\text{g/g}$ with a few exceptions, in RTR.

Its action occurs from 0.125 $\mu\text{g/g}$ in ATA, and invariably from 1.3 to 10 $\mu\text{g/g}$ with a few exceptions.

4. The vasodepressor action in anesthetized cats by the administration of 20 $\mu\text{g/kg}$ of RTR reduces by 41.8% the normal blood pressure, with recovery to 64.5, 74.6, 76.8 and 80% of normality, respectively in 15, 30, 45 and 60 min. The administration of 20 $\mu\text{g/kg}$ of ATA diminishes by 78.3% the normal blood pressure, and recovery to 93.6, 97.5, 100 and 100% of usual state occurs respectively in 15, 30, 45 and 60 min.

In rabbits the administration of 20 $\mu\text{g/kg}$ of RTR reduces by 39.5% the normal blood pressure, and recovery to 50.3, 70.7, 80.8 and 89.8% of normal, respectively follows in 15, 30, 45 and 60 min.

The same dose of ATA reduces normal blood pressure by 62.2%, with recovery to 76.2, 88.0, 91.6 and 93.7% of usual state respectively in 15, 30, 45 and 60 minutes.

From the above results, it is confirmed that RTR (rhodotoxin) and ATA (andromedotoxin) isolated from *Andromeda japonica* are different substances,

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