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Pharmacological Studies of Troponoids. IV.[1] Antipyretic Analgesic, and Anti-inflammatory Activities of Aminotroponoids[2]

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The analgesic, anti-inflammatory and antipyretic activities of six aminotroponoid compounds were investigated.

Analgesic activities of 5-aminotropolone(I) and 2-aminotropone(II) are found to be equipotent to that of aminopyrine.

All the test compounds do not inhibit the development of the erythema induced by ultraviolet irradiation, but I and II show the strong inhibition (70—80%) of the enhancement of capillary permeability induced by acetic acid.

Compounds, I and II produce marked hypothermia in mice, rats, and guinea-pigs, and I and II show the antipyretic activities as same as aminopyrine.

The acute toxic symptoms of most of the test compounds are generally the extremities ataxia and the respiratory paralysis.

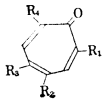
In a previous report,[4] the authors studied hypothermic, analgesic and anti-inflammatory acti vities of tirty troponoids that we have synthesized. And it has been recognized that analgesic activities of tropolone and 5-aminotropolone were equipotent with that of aminopyrine, and 5-aminotropolone showed marked hypothermic activities in mice.

In this paper, we further studied analgesic, and antipyretic activities of six aminotroponoids that have amino group on different positions.

Materials

5-Aminotropolone (I), 2-aminotropone(II), and 2,5-diaminotropone(III) were synthesized according to the methods reported previously.[5] 4-Aminotropolone(IV), 2-amino-7-bromotropone (V), and 5-amino-7-bromotropolone (VI) were supplied by Seto laboratory[6] (Chemical Research Institute of Non-aqueous Solution, Tohoku University).

TABLE I. Chemical Structures of Test Compounds



No.	Compounds	R_1	R_2	R_3	R_4
I	5-aminotropolone	OH	H	NH_2	H
II	2-aminotropone	NH_2	H	H	H
III	2,5-diaminotropone	NH_2	H	NH_2	H
IV	4-aminotropolone	OH	NH_2	H	H
V	2-amino-7-bromotropone	NH_2	H	H	Br
VI	5-amino-7-bromotropolone	OH	H	NH_2	Br

1) Part III: H. Ozawa, Y. Ohizumi, and S. Murai, *Yakugaku Zasshi*, **91**, 1307 (1971).
2) This work was reported at the Hokubu Area, Regional Meeting XXI of the Japaness Pharmacological Society, Fukushima, July 1970.
3) Location: *Aobayama, Sendai.*
4) H. Ozawa, S. Seto, S. Murai, and Y. Ohizumi, *Yakugaku Zasshi*, **91**, 550 (1971).
5) H. Ozawa, S. Seto, Y. Ohizumi, and S. Murai, *Yakugaku Zasshi*, **92**, 19 (1972).
6) Location: *Katahiracho, Sendai.*

I, II, and III were dissolved in 0.9% NaCl solution, and IV, V, and VI were suspended in 0.3% carboxymethylcellulose solution. In the experiments of the hypothermic action and antipyretic action, the test compounds were dissolved in pyrogen-free 0.9% NaCl solution.

Animals

Female mice (*dd*-strain), rats (Wistar strain), and guinea-pigs (Hartley strain) were used.

Experimental

Measurement of Analgesic Activity a) Acetic Acid Method——The experiment was carried out according to the method of Koster, *et al.*[7] Mice (body weight 20±2 g) were given the test compounds subcutaneously. After 30 min each animal was given an intraperitoneal injection of a 0.6% solution of acetic acid in a volume of 0.1 ml/10 g. Each dose group consisted of ten mice. These were placed in a five-compartment observation cage and the number of squirming for each animal was counted for 20 min after the injection of acetic acid.

The experiment was performed at room temperature (21±1°). ED_{50} was determined by the method of Litchfield and Wilcoxon.[8]

b) Pressure Method——The experiment was carried out according to the method of Takagi, *et al.*[9] Before the experiment, normal pain threshold of mice was measured twice and the mice which did not show the normal pain threshold ranging 40—80 mmHg were removed out and the selected mice(18—22 g) were used finally in a group of five. The test compounds were administered subcutaneously and the change of the pain threshold was measured at 15 min interval for 90 min.

c) Hot Plate Method——The experiment was performed by the method of Takagi *et al.*[10] Before the experiment, mice were placed on the copper plate heated at 60° and the mice showing the jump were picked out. Furthermore, the jumping time of the mice picked out was measured twice at 15 min interval. On these two measurements, mice(20—22 g) showing the jumping time of 4—6 sec were used finally in a group of ten.

Potentiation of Hexobarbital Sleeping Time in Mice——Mice (20±2 g) were given the test compounds (100 mg/kg) subcutaneously. After 30 min each animal was given an intraperitoneal injection of hexobarbital (70 mg/kg, 0.1 ml/10 g). The mean sleeping time was measured as the period between the disappearance and the restoration of the righting reflex. The room temperature was maintained at 22°. Each dose group consisted of six mice.

Measurement of the Motor Activity——The wheel cage apparatus was used for the measurement of the spontaneous activity, as described by Takagi, *et al.*[11] Mice (20±2 g) were placed in the apparatus for 30 min and the mice which showed about 500 revolutions per 30 min were picked out and used in a group of six. The test compounds were administered subcutaneously and the motor activity was measured at 30 min intervals for 150 min.

Measurement of Anticonvulsion Activity——Mice (20—22 g) were used in a group of five. At 15 min after an intraperitoneal administration of the test compounds (100 mg/kg), pentetrazol (80 mg/kg) was administered subcutaneously. Manifestation time of the tonic extension was measured and compared with controls. In another measurement, the test compounds (100 mg/kg) were administered orally. After 30 min, strychnine HNO_3 (1.38 mg/kg) was administered intraperitoneally. The lethal rate after 24 hr was measured and compared with controls.

Inhibition of the Enhancement of Capillary Permeability by Acetic Acid——The measurement was carried out according to the method of Whittle.[12] Mice (20—23 g) were used in a group of ten. Mice were given the test compounds subcutaneously. After 30 min, each animal was given an intravenous injection of 0.1 ml of a 4% solution of Pontamine Sky Blue. Furthermore, at 35 min after the administration of the test compounds, each animal was given an intraperitoneal injection of a 0.6% solution of acetic acid in a volume of 0.1 ml/10 g. After 20 min the mice were killed by the dislocation of the neck and the viscera were exposed and irrigated with distilled water over a Petri dish. The collected washing fluid were filtered and made up to 10 ml in a test tube. 0.1 ml of 0.1 N sodium hydroxide solution was added to each tube and the absorbance was read at 590 mμ.

Inhibition of Ultraviolet Erythema Formation——The experiment was carried out according to the method of Winder, *et al.*[13] Guinea-pigs (250—350 g) were used in a group of four. At three hours before

7) R. Koster, *Federation Proc.*, **22**, 249 (1969).
8) J.T. Litchfield and F. Wilcoxon, *J. Pharmacol. Exptl. Therap.*, **96**, 99 (1949).
9) K. Takagi, T. Kameyama, and K. Yano, *Yakugaku Zasshi*, **78**, 553 (1968).
10) K. Takagi and T. Kameyama, *Yakugaku Zasshi*, **77**, 871 (1957).
11) H. Takagi, T. Ban, H. Takashima, and T. Takashima, *Nippon Yakurigaku Zasshi*, **56**, 1421 (1960).
12) B.A. Whittle, *Brit. J. Pharmacol.*, **22**, 246 (1964).
13) C.V. Winder, J. Wax, V. Burn, and M. Beer, *Arch. Intern. Pharmacodyn.*, **116**, 261 (1968).

the experiment, each animal was depilated with a depilatory containing barium sulfide on the both flanks of the body. The depilated skin was exposed to ultraviolet irradiation (sun lamp, Fuji X ray Co., Ltd., 600 W) for 150 sec through three circular hole (diameter 10 mm) at the distance of 13 cm from the light. At 15 min after the irradiation, the test compounds were injected intraperitoneally. The degree of the erythema formation was scored with the naked eye at two hours after the exposure.

Hypothermic Action——Mice (19—23 g), rats(200—290 g), and guinea-pigs(250—300 g) were used in a group of six. Before the experiment, normal rectal temperature of animals was measured and mice and rats showing the rectal temperature 37.5—38.5° and guinea-pigs showing 38.0—38.8° were selected and used in this expermiment. The test compounds were administered subcutaneously and the rectal temperatures were measured at 30 min interval for a further 5—6 hr. The rectal temperatures of mice were measured by a thermister(Natsume seisakusho) at the distance of 2.5 cm inside from the anus and those of rats or guinea-pigs were measured by a clinical thermometer (Jintan Co., Ltd.) at the distance of 4 cm inside from the anus. The measurement were performed at the room temperature ($21 \pm 1°$).

Antipyretic Activity——Guinea-pigs (250—320 g) were used on a group of five. (the control group consisted of ten guinea-pigs). The experiment was carried out according to the method of Kobayashi, *et al.*[14] Before the experiment, the initial rectal temperatures of guinea-pigs were measured and the animals showing their temperatures over 38° were selected. With the selected animals, further measurements were done twice and animals showing the ranges of their temperatures from 38.0° to 38.8° were used finally in this experiment. TTG (10 μg/kg) was injected intravenously through the saphenous vein, and immediately after the injection of TTG, the test compounds were injected intraperitoneally or administered orally. The rectal temperatures were measured at 30 min interval for three hours, subsequently at one hour interval thereafter. The room temperature was maintained at $21 \pm 1°$ and the rectal temperatures were measured by a clinical thermometer at the distance of 4 cm inside from the anus.

Acute Toxicity——Mice (20—23 g) were used. The test compounds were injected subcutaneously, intraperitoneally (by the method of Litchfield and Wilcoxon) and intravenously (by the method of up and down[15]). LD_{50} was calculated from the lethal rate of the mice at 24 hr after the administration.

Result

Measurement of Analgesic Activity

a) Acetic Acid Method——The ED_{50} of I, II, III, and V against the squirming are found to be nearly equipotent with that of aminopyrine, but the potency of VI that is a bromo derivatives of I at 7 position is weak, as compared with the ED_{50} of other test compounds. IV do not reduce the squirming at all. The results obtained are shown in Table II.

Table II. Effect of Troponoids and other Analgesics against Squirming induced by Acetic Acid in Mice

Compound	Inhibition of squirming		Compound	Inhibition of squirming	
	ED_{50}(mg/kg)	95% Confidence limits		ED_{50}(mg/kg)	95% Confidence limits
I	45	25— 80	V	42	23— 76
II	29	15— 58	VI	115	93—142
III	36	18— 72	AP[a]	42	26— 67
IV	—	—	SA[b]	217	121—386

Drugs were administered subcutaneously. ED_{50} was calculated by Litchfield-Wilcoxon method.
a) aminopyrine *b*) salicylic acid

b) Pressure Method——I and II among the test compounds shown in Table I raise the pain threshold of mice clearly against the pressure pain. The pain threshold and the time course of II are almost as same as that of aminopyrine. But analgesic acitivities of III, IV, V, and VI are not detected. The change of the pain threshold of I, II, aminopyrine, and salicylic acid are shown in Fig. 1.

14) S. Kobayashi and H. Takagi, *Japan J. Pharmacol.*, **18**, 80 (1968).
15) K.A. Blownlee, J.L. Hodges, Jr. and Murray Rosenblatt, *J. Am. Stat. Assoc.*, **48**, 262 (1953).

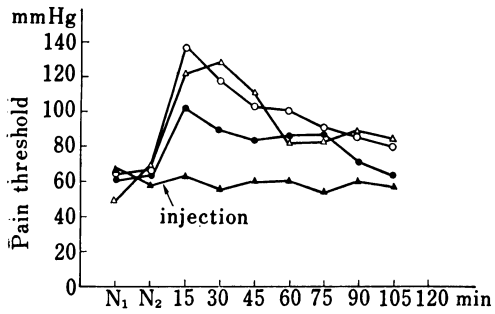


Fig. 1. Threshold Elevating Effects of I, II, Aminopyrine, and Salicylic Acid to the Pressure Pain in Mice

(N_1, N_2=normal activity, five animals for each point)
Drugs were administered subcutaneously at arrow.
●—●: I 150 mg/kg *s.c.*
○—○: II 150 mg/kg *s.c.*
△—△: aminopyrine 150 mg/kg *s.c.*
▲—▲: salicylic acid 150 mg/kg *s.c.*

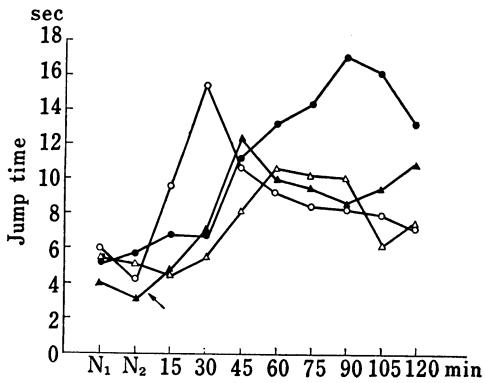


Fig. 2. Effects of I, II, Aminopyrine, and III on the Hot Plate Method in Mice

(N_1, N_2=normal activity, ten animals for each point)
Drugs were administered ar arrow.
●—●: I 150 mg/kg *s.c.*
○—○: II 150 mg/kg *s.c.*
▲—▲: III 150 mg/kg *s.c.*
△—△: aminopyrine 150 mg/kg *s.c.*

c) **Hot plate method**——I, II, and III show the marked prolongation of the jumping time, and the activities of I and II are more potent than that of aminopyrin. It is found that the time courses of I and II are clearly different from each other. The peak of the jumping time of I is observed at 90 min after administration, on the other hand, that of II is obtained at 30 min after administration. The results obtained are shown in Fig. 2.

Potentiation of Hexobarbital Sleeping Time in Mice——The potentiating activity of III is the most potent among these test compounds and the ratio of the potentiation of III is about 8.7 times as much as compared with the control. Also I and II significantly potentiated the sleeping time induced by hexobarbital. Activities of I, II, and III are more potent than that of aminopyrine, but the potency of III is about 1/80 of that of chlorpromazine. The results obtained are shown in Fig. 3.

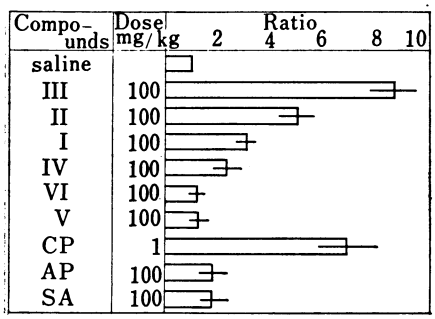


Fig. 3. Effects of Troponoids and Other Agents on the Sleeping Time induced with Hexobarbital in Mice

Hexobarbital (70 mg/kg) was administered intraperitoneally 30 min after subcutaneously administration of test compounds. Abscissa bars represents standard errors of the mean.
CP: chlorpromozine
AP: aminopyrine
SA: salicylic acid

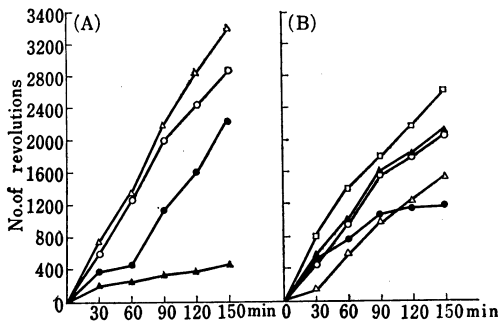


Fig. 4. Inhibition of other Agents (A) and Troponoids (B) on Revolution Activity in the Wheel Cage on Mice

(ten animals for each point)
(A) ○—○: saline
●—●: aminopyrine 100 mg/kg *s.c.*
△—△: acetylsalicylic acid 100 mg/kg *s.c.*
▲—▲: chlorpromazine 2.5 mg/kg *s.c.*
(B) ○—○: II 100 mg/kg *s.c.*
△—△: V 100 mg/kg *s.c.*
●—●: I 100 mg/kg *s.c.*
▲—▲: VI 100 mg/kg *s.c.*
□—□: III 100 mg/kg *s.c.*

Measurement of Motor Activity——The compound, III which shows the most potent activities among the test compounds in Fig. 3, and IV do not reduce the revolutions of the wheel cage. I, II, and their 7-bromo derivatives (V and VI) reduce than that of aminopyrine. these results are shown in Fig. 4.

Measurement of Anticonvulsion Activity——None of six test compounds inhibit the development of the convulsion induced by pentetrazol or strychnine at all, and the development time of tonic extention is not different between the test compounds and controls. The compounds, I and II make slightly the LD_{50} of strychnine low in mice.

Inhibition of the Enhancement of Capillary Permeability by Acetic Acid——Among the test compounds, I and II show markedly the inhibitory activities of the enhancement of capillary permeability in mice. Among I, II, and aminopyrine, the inhibitory activities are not different after oral administration, however, after subcutaneous administration, I and II inhibit clearly the enhancement of capillary permeability more potent than aminopyrine. The inhibition rate of II (150 mg/kg) is about 70—80% as compared with controls. There exsists no significant difference between the subcutaneous and the oral inhibition of I and II. Furthermore, I and II show dose-dependent inhibition. The results obtained are shown in Fig. 5.

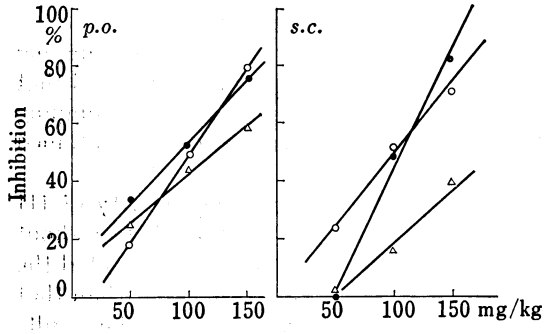


Fig. 5. Inhibition of I, II, and Aminopyrine against the Enhancement of Capillary Permeability induced with Acetic Acid in Mice

(ten animals for each point)
p.o.: oral administration, *s.c.*: subcutaneous administration
●—●: I ○—○: II △—△: aminopyrine

Inhibition of Ultraviolet Erythema Formation——Acetylsalicylic acid (300 mg/kg) and aminopyrine (150 mg/kg) used as controls completely inhibit the development of the erythema formation, but the test compounds (150 mg/kg) do not show the inhibition of the erythema formation.

Hypothermic Action——I and II show considerable dose dependent hypothermic actions, but the other test compounds show a little hypothermic actions (hypothermic action of I in mice was described previously by the authors[4]). The intensity and the time courses of the fall induced by aminopyrine and II are similar to each other, and after subcutaneous injection of I (150 mg/kg), the degree of the fall of the rectal temperature is about 6°. It is found that the time courses of the fall of the body temperature are apparently different between I and II.

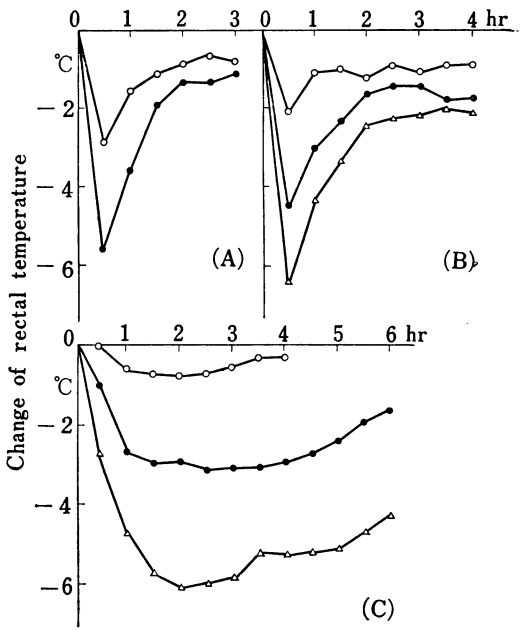


Fig. 6. Hypothermic Actions of Aminopyrine (A), II (B), and I (C) in Mice

(A) aminopyrine
○—○: 50 mg/kg *s.c.*, ●—●: 100mg/kg *s.c.*
(B) II
○—○: 25 mg/kg *s.c.*, ●—●: 50 mg/kg *s.c.*,
△—△: 100 mg/kg *s.c.*
(C) I
○—○: 50 mg/kg *s.c.*, ●—●: 100 mg/kg *s.c.*,
△—△: : 150 mg/kg *s.c.*

The maximal fall of I is produced at 2 hr after the administration and the degree of the restoration of the rectal temperature is very slow. The degree of the fall of I (100 mg/kg) is kept over 1.5° at six hours after the administration. On the other hand, the maximal fall of II is observed at 0.5—1 hr after the administration and also the rectal temperature rapidly recovers. The results of hypothermic actions of I, II, and aminopyrine in mice are shown in Fig. 6.

On the hypothermic actions in rats, I and II also show the fall of the rectal temperature considerably in the same manner as that of mice. The difference of the time courses between I and II in mice are also found in rats. The intensity of the fall induced by I and II in rats are slightly weak in comparison with that in mice. The results obtained in rats are shown in Fig. 7.

Antipyretic Activity——Intravenously injections of TTG (10 μg/kg) produce the pyretic activity of 2° that have two peaks at one hour and three hours in guinea-pigs, whereas the pyretic activities produced by the injections of TTG are reduced by the oral administration of I and II. The antipyretic activity of II administered orally is equipotent with aminopyrine. The time courses of antipyretic activities of I and aminopyrine show also two peaks as seen in control, and these two peaks are similar to the time courses observed in the injection of TTG alone, as shown in Fig. 8 (A). The body temperatures of guinea-pigs are not influenced by the oral administration of I, II, and aminopyrine, as shown in Fig. 8 (B).

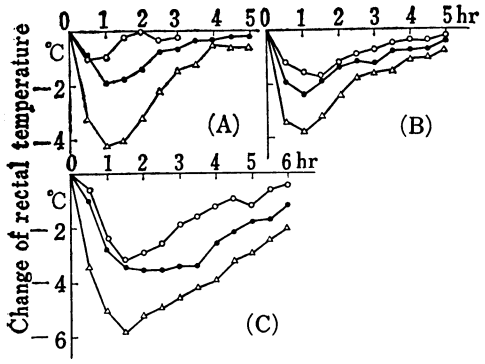


Fig. 7. Hypothermic Actions of II (A), Aminopyrine (B), and I (C) in Rats

(six animals for each point)
(A) II, (B) aminopyrine, (C) I
○——○: 50 mg/kg *s.c.*
●——●: 100 mg/kg *s.c.*
△——△: 200 mg/kg *s.c.*

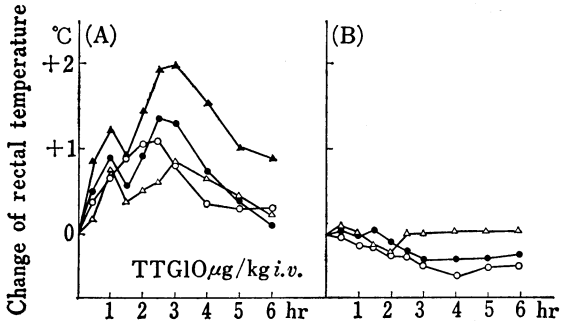


Fig. 8. Antipyretic Actions (A) and Hypothermic Actions (B) of I, II, and Aminopyrine in Guinea-pigs

(six animals for each point)
(A) antipyretic actions
▲——▲: TTG 10 μg/kg *i.v.*
●——●: TTG 10 μg/kg *i.v.*+I, 100 mg/kg *p.o.*
○——○: TTG 10 μg/kg *i.v.*+II, 100 mg/kg *p.o.*
△——△: TTG 10 μg/kg *i.v.*+aminopyrine 100 mg/kg *p.o.*
(B) hypothermic actions
△——△: aminopyrine 100 mg/kg *p.o.*
●——●: I, 100 mg/kg *p.o.*
○——○: II, 100 mg/kg *p.o.*

As shown in Fig. 9, the hyperthermic actions of TTG are not inhibited by intraperitoneal injections of 50 mg/kg of aminopyrine, but are completely inhibited by injections of 100 mg/kg of aminopyrine. Usually, aminopyrine does not show the hypothermic actions after intraperitoneal injections in guinea-pigs. On the other hand, after intraperitoneal injections of 75 mg/kg, I inhibits almost the hyperthermic actions of TTG. However, the administration of I (100 mg/kg) produce conversely significant hypothermic actions. The hypothermic actions produced by injections of I in guinea-pigs show the fall of 2°. The results obtained are shown in Fig. 10.

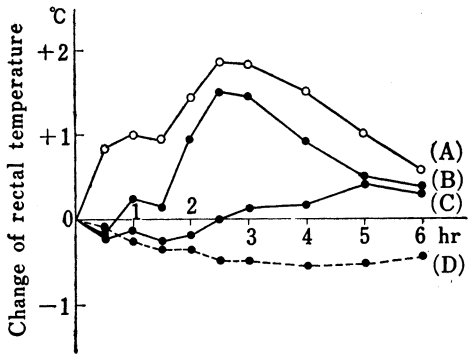


Fig. 9. Antipyretic Action and Hypothermic Action of Aminopyrine in Guinea-pigs

(six animals for each point)
○—○ (A): TTG 10 μg/kg *i.v.*
●—● (B): TTG 10 μg/kg *i.v.*+aminopyrine 50 mg/kg *i.p.*
●—● (C): TTG 10 μg/kg *i.v.*+aminopyrine 100 mg/kg *i.p.*
●—● (D): aminopyrine 100 mg/kg *i.p.*

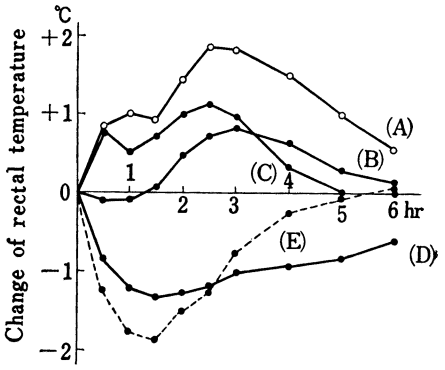


Fig. 10. Antipyretic Action and Hypothermic Action of I in Guinea-pigs

○—○ (A): TTG 10 μg/kg *i,v.*
●—● (B): TTG 10 μg/kg *i.v.*+I, 75 mg/kg *i.p.*
●—● (C): TTG 10 μg/kg *i.v.*+I, 50 mg/kg *i.p.*
●—● (D): TTG 10 μg/kg *i.v.*+I, 100 mg/kg *i.p.*
●—● (E): I, 100 mg/kg *i.p.*

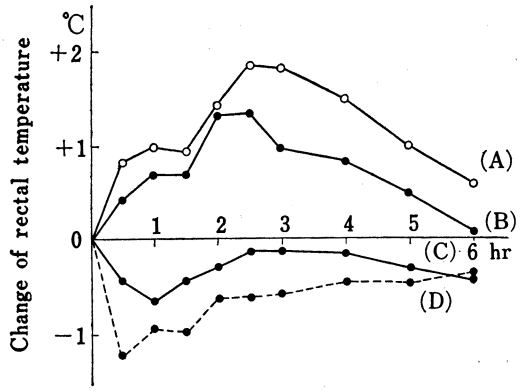


Fig. 11. Antipyretic Action and Hypothermic Action of II in Guinea-pigs

○—○ (A): TTG 10μg/kg *i.v.*
●—● (B): TTG 10 μg/kg *i.v.*+II, 50 mg/kg *i.p.*
●—● (C): TTG 10 μg/kg *i.v.*+II, 100 mg/kg *i.p.*
●—● (D): II, 100 mg/kg *i.p.*

As shown in Fig. 11, the hyperthermic activity of TTG is completely inhibited by the injection of II (100 mg/kg), and also intraperitoneal injection of II alone show the hypothermic activity which is weaker than that of I.

Acute Toxicity——Most of the test compounds show generally the signs of the extremities ataxia and the respiratory paralysis in mice. In lower dose, mice come to the death slowly without the agony. I produces nausea 1—2 min after the intraperitoneal or subcutaneous injections. The nauses of I is recognized in mice and rats, but not in guinea-pigs. The intravenous injections of II (200 mg/kg) develop the strong respiratory paralysis. The respiration is stopped quickly 30 sec after injections and the cyanosis is shown, but after 2 min the respirations develope again, and after 7 min the mice restore the righting reflex and return to the state of normal behavior. The LD_{50} of the test compounds is shown in Table III.

Discussion

As the results of acetic acid method, pressure method, and hot plate method, we recognize that the analgesic activities of I and II are as same as that of aminopyrine. The compound, III shows as same analgesic activity as aminopyrine in acetic acid method and hot plate method, but negative in pressure method. The reason for the scattering results obtained in the experiment with III is not clear. The analgesic activity of III might be

Table III. Acute Toxicity in Mice

Compound	Route of administ. LD_{50} mg/kg		
	s.c.	*i.p.*	*i.v.*
I	531 (474—573)	230 (210—253)	177
II	175 (158—194)	176 (166—186)	333
III	361 (314—415)	174 (160—181)	192
IV	400	265 (221—318)	—
V	360 (308—421)	343 (296—401)	—
VI	400	350 (294—416)	—
AP[a)]	375 (332—424)	250 (236—268)	135

LD_{50} (*s.c.* and *i.p.*) was measured at 24 hr after administration and was calculated by Litchfield-Wilcoxon method.
LD_{50} (*i.v.*) was calculated by up and down method.
( ) 95% confidence limits
a) aminopyrine

due to the ganglion blocking action of III[1)] like tetraethylammonium, if tetraethylammonium seemed to be wide sense analgesia.

Troponoids inhibit markedly the enhancement of the capillary permeability induced by acetic acid, but do not inhibit the development of erythema by ultraviolet irradiation. Aminopyrine inhibits both of them. The ultraviolet erythema formation method has a fault that a part of anti-inflammatory agents (steroid hormone, oxyphenbutazone, *etc.*) show negative activity, but it is generally considered that this is an effective screening method of anti-inflammatory agents which have strong parallelism with a clinical dose. Also the enhancement of capillary permeability is not inhibited by narcotic analgesics and steroid hormones.

Kobayashi[16)] described that II (13%) and aminopyrine (14.1%) showed a slight inhibition for the carrageenin edema in rat's paw. It is suggested that from these results, troponoids have weak anti-inflammatory activity, while troponoids might inhibit the different stage of inflammation from aminopyrine. Takagi, *et al.*[17)] suggested that most of the enhancement of capillary permeability induced by acetic acid is due to the direct action of acetic acid to the vessel, but at the beginning of the enhancement, it might be related to the mediator. The relation between the action of acetic acid and the inhibition by troponoids should be investigated much deeply, but the possibility is assumed that troponoids inhibit only the early stage enhancement of capillary permeability.

Previously, the authors reported the hypothermia of troponoids in mice. In this paper, the authors reported the influence for the body temperature in mice, rats, and guinea-pigs.

Among the test compounds, only I and II show hypothermic actions. There is the apparent difference between the time courses of hypothermia of I and that of II. The time courses of II and of aminopyrine were very similar to each other.

This difference between the time courses of I and II is the most clear in mice, and the difference is recognized also in rats and guinea-pigs. It seems that the difference between I and II is due to difference of major action sites. The compound, I might inhibit not only central body temperature regulating system (body thermostat) but also the mechanism of heat production.

The method used guinea-pigs for antipyretic activity was reported by Kobayashi, *et al.* and they recognized a fever of 1.5° by the administration of TTG. The authors recognized a fever of 2° and the double-peaked fever. In Kobayashi's report, the double-peaked fever was not recognized by intravenous injection of TTG (10 μg/kg). The author's results might

16) Shinsaku Kobayashi, personal communication.
17) H. Takagi, Y. Iizuka, Meeting of Kanto Branch, Japanese Pharmacological Soceity, Tokyo, June 1966.

be due to a small number of animals (10 animals as control) or to difference of the administration route. The authors administrate TTG from the saphenous vein. It is characterized in author's experiment that the time courses of I, II, and aminopyrine on the antipyretic actions should also double-peaked courves. The compound, I produces not only the antipyretic action but marked hypothermic action after intraperitoneal injections of large dose (100 mg/kg).

Under the same conditions, aminopyrine does not produce the hypothermic action. This result also suggests that I influences strongly the mechanism of heat production.

It is intresting in the structure-activity relationship that among various aminotroponoid compounds, IV (4-aminotropolone) shows a little pharmacological activity. In the author's preliminary experiments, 3-aminotropone inhibits the squirming as same as aminopyrine, but 4-aminotropone does not inhibit the squirming at all. From these results, it is concluded that the amino group at 4 position or bromine group at 7 position abolish or diminish the analgesic activities.

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