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Pharmacological studies of lumisantonin derivatives, with special reference to anti-inflammatory effect and to histamine-release inhibitory action

Hidemasa Yamasaki* Kazuji Kondo† Teruo Uda‡
Takashi Yamamoto** Koiti Endo††

*Okayama University,
†Okayama University,
‡Okayama University,
**Okayama University,
††Okayama University,

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Abstract

A number of derivatives and related compounds of lumisantonin were submitted to evaluatien for the action of histamine-release inhibition and antiinflammatory effect, as they structurally resemble guaiazulene in which these actions had been proved. Nineteen compounds of these suppressed 50 per cent or more of the increase in urinary excretion of histamine due to ovomucoid injection. Five of them markedly inhibited all the edemas in the rat hind paws induced by local inoculation of dextran, hyaluronidase, histamine, and 5-hydroxytryptamine. Among these compounds, #32(methyl pyrophotosantoninate) showed a superior effect of inhibition than guaiazulene on all of these edemas, although the effects of two drugs were comparable in the case of oral administration. The members showing the edema inhibition likewise evidently protected passive cutaneous anaphylaxis in guinea pigs by the intraperitoneal administration; the effect of #32 was more marked than guaiazulene. This effect could be observed when applied to the skin with an ointment containing the compouhd in a concentration of more than 0.03 per cent 24 hours before. In vitro histamine releases from the minced lung tissue of sensitized guinea pig elicited by antigen and sinomenine were both inhibited by these compounds. These findings indicate that the main sites of the histamine-release inhibition and of the anti-inflammatory effect of these compounds are in the local tissue. Compound #32 failed to show any analgesic effect in mice, but possessed a considerable antipyretic action in rats. Some of the compounds in the tests depressed guinea-pig ileal strip while guaiazulene increased peristalsis, but any of these actions was not recognized with #32 even in a high concentration. Most of the members effective in inhibiting edemas as well as histamine release proved to be less toxic than guaiazulene. #32 was well tolerated in the doses of 6g/kg orally and of 4g/kg intraperitoneally by mice. The growth curves for three weeks of rats practically did not deviate from that of the controls by daily administration of 1g/kg of #32 by stomach tube and there were no gross and microscopical abnormalities in the main organs and blood.

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PHARMACOLOGICAL STUDIES OF LUMISANTONIN DERIVATIVES, WITH SPECIAL REFERENCE TO ANTI-INFLAMMATORY EFFECT AND TO HISTAMINE-RELEASE INHIBITORY ACTION*†

Hidemasa YAMASAKI, Kazuji KONDO, Teruo UDA,
Takashi YAMAMOTO and Koiti ENDO

Department of Pharmacology, Okayama University Medical School, Okayama
(Director : Prof. H. Yamasaki)

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Anti-inflammatory and anti-allergic effects of guaiazulene (1, 4-dimethyl-7-isopropylazulene) were previously confirmed through a series of experiments.[1,2] In these experiments we found this compound to have an inhibitory action on the release of tissue histamine by various histamine releasers including antigen. Therefore, it was deduced that this action had an important bearing on the mechanism of the therapeutic effects. This compound and a few other derivatives of azulene are widely used in clinics for combatting a variety of inflammatory and allergic symptoms.

Since lumisantonin has structurally azulene construction, it is interesting to anticipate a possibility that its derivatives would have guaiazulene-like effects. For this reason, we have studied a number of lumisantonin derivatives, with a synthetic co-operation of Dr. I. SATODA and his co-workers in the Nippon Shinyaku Research Laboratory, and as the result found some of these to have anti-inflammatory and anti-allergic effects rather superior to those of guaiazulene yet less toxic. This paper deals with the results of these studies.

MATERIALS AND METHODS

Compounds tested. Lumisantonin (Fig. 1) and its 72 derivatives as well as related compounds served as materials. The code number and chemical name of these compounds are listed in Table 1. Fig. 2 illustrates the structural formulas of some of these compounds whose histamine-release inhibitory action proved to be relatively marked as the results of the experiments to be described later. Most of these compounds were insoluble in water and soluble in alcohol, but some proved to be insoluble in alcohol.

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† Preliminary communication in *Folia pharmacol. japon.* 56, 1249, 1960 (in Japanese).

Table 1. Lumisantonin derivatives and related compounds tested.

No.	Chemical name
	Lumisantonin derivatives[3-5]
1	Lumisantonin
38	Lumi-6-*epi*-santonin
19	Lumisantonin oxime
39	Lumi-6-*epi*-santonin oxime
10	Lumisantoninic acid
2	Dihydrolumisantonin
45	Dihydrolumi-6-*epi*-santonin
26	Dihydrolumisantonin oxime
20	Dihydrolumisantonin semicarbazone
11	Diydrolumisantoninic acid
21	Sodium dihydrolumisantoninate
22	Dihydrolumisantoninic acid benzylthiouronium salt
23	Tetrahydrolumisantonin
61	Tetrahydrolumisantonin acetate
49	Tetrahydrolumisantoninic acid
57	Dehydrodihydrolumisantoninic acid
56	Methyl dehydrodihydrolumisantoninate
	Pyrolumisantonin derivatives[3,5]
12	Pyrolumisantonin
13	*iso*-Pyrolumisantonin
42	Dihydropyrolumisantonin
41	Dihydro-*iso*-pyrolumisantonin
35	Dehydrobromination product of lumisantonin hydrobromide
63	*iso*-Dihydropyrolumisantonin
36	Tetrahydropyrolumisantonin
64	Dihydro-*iso*-pyrolumisantonin oxide
67	Dihydroxytetrahydropyrolumisantonin
7	Lumisantonin hydrochloride
37	Lumisantonin *iso*-hydrochloride
8	Lumisantonin *iso*-hydrobromide
43	*iso*-Pyrolumisantonin dibromide
62	Bromopyrolumisantonin
29	Chlorotetrahydropyrolumisantonin
44	10-Oxotetrahydropyrolumisantonin
	***iso*-Dihydrolumisantonin derivatives[3-5]**
9	*iso*-Dihydrolumisantonin
53	*iso*-Dihydrolumi-6-*epi*-santonin
30	Dihydro-*iso*-dihydrolumisantonin

No.	Chemical name
	Guanian-6 : 12-olide derivatives[3,6,10]
34	*iso*-Photosantonic lactone
6	*iso*-Photosantonic lactone acetate
40	*iso*-Photo-6-*epi*-santonic lactone acetate
65	α-Dihydro-*iso*-photosantonic lactone
50	β-Dihydro-*iso*-photosantonic lactone
51	γ-Dihydro-*iso*-photosantonic lactone
47	α-Dihydro-*iso*-photosantonic lactone acetate
66	β-Dihydro-*iso*-photosantonic lactone acetate
71	α-Tetrahydro-*iso*-photosantonic lactone
3	3-Oxoguai-4,10(1)-dien-6 : 12-olide
16	3-Oxoguai-4,10(1)-dien-6 : 12-olide phenylhydrazone
17	3-Oxoguai-4-en-6 : 12-olide
18	3-Oxoguai-4-en-6 : 12-olide semicarbazone
58	3-Oxo-6-hydroxyguai-4-en-11-oic acid
59	Sodium 3-oxo-6-hydroxyguai-4-en-11-oate
25	3-Oxoguaian-6: 12-olide
27	Guaian-6 : 12-olide
28	3-Acetoxyguaian-6 : 12-olide
	Photosantoninic acid derivatives[3,4,7,11]
5	Photosantoninic acid
69	Photosantoninic acid oxime
4	Sodium photosantoninate
33	Dimethyl photosantoninate
60	Dimethyl dehydrophotosantoninate
15	Pyrophotosantoninic acid
32	Methyl pyrophotosantoninate
68	Methyl pyrophotosantoninate oxime
14	Pyrophotosantoninic acetic anhydride
52	Tetrahydrophotosantoninic acid
55	Dimethyl tetrahydrophotosantoninate
72	Dimethyl tetrahydrophotosantoninate diacetate
73	Tetrahydropyrophotosantoninic acetic anhydride diacetate
70	*iso*-Pyrophotosantoninic acid
54	Methyl *iso*-pyrophotosantoninate
	Photosantonic acid derivatives[8,9]
46	Photosantonic acid
48	Bromophotosantonic acid
	Others[11]
24	Azulene
31	Oil of *Artemisia Kurramensis* Q.

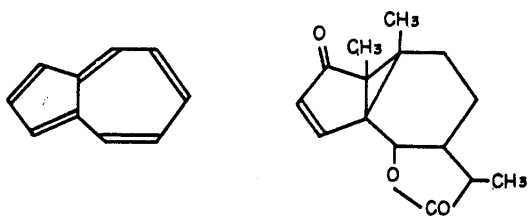


Fig. 1. Structural formulas of azulene and lumisantonin.

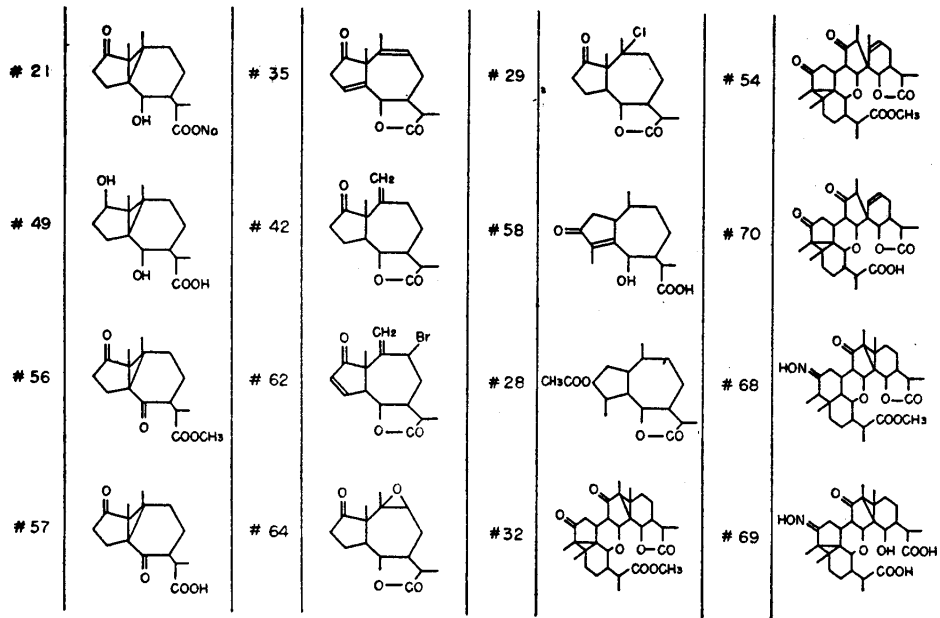


Fig. 2. Structural formulas of lumisantonin derivatives and related compounds effective in inhibiting histamine release *in vivo*.

Method of testing inhibitory effect on the increased urinary excretion of histamine induced by ovomucoid in rats. The methods were the same as employed by YAMASAKI *et al*[1]., and KONDO.[12,13] 40 mg/kg of ovomucoid dissolved in physiological saline solution was intraperitoneally injected into female albino rats. An increase in the urinary excretion of histamine after this injection is a reflection of the release of tissue histamine.[12,13] The relative potency of the compounds inhibiting this increase was sought by the intraperitoneal administration of 50 mg/kg of each made 2 hours prior to the ovomucoid injection. Control rats received a similar treatment with 2.5 c.c./kg of physiological saline solution containing 1 per cent gum arabic used as solvent instead of the compounds. In six control rats, after the ovomucoid injection the percentage of histamine

excreted in excess of the mean of the histamine excretion in every 30 minutes before the ovomucoid injection was 312 (301–323). First of all, those compounds that depressed this percentage below 1/2 in the average of three rats were selected. Ovomucoid was prepared by MÖRNER's method.[14]

Method of testing inhibitory effect on the edemas of rat hind paw induced by dextran, hyaluronidase, histamine and 5-hydroxytryptamine. The techniques of the edema induction and of quantitative expression of the intensity of edemas were reported elsewhere by UDA,[15] one of us. The dose used in the induction of edema was 3 mg for dextran, 20 μg for histamine, 0.1 μg for 5-hydroxytryptamine (5-HT), and 60 V. U. M. for hyaluronidase. 100 mg/kg of the compounds to be tested, suspended in physiological saline solution containing 1 per cent gum arabic, was injected intraperitoneally, and edema was produced in one of the hind paws one hour afterwards by injecting each of the above mentioned phlogogenic agents beneath the skin of dorsa of the paw. The intensity of the edema of the paw, 1, 2 and 3 hours after the injection of the agents was compared with that of the control edema at respective time of the other paw induced 24 hours previously, and the average inhibition rate for 1–3 hours was calculated. In this experiment some of the compounds were given by mouth as well.

Method of testing anti-allergic effect. Passive cutaneous anaphylaxis in guinea pigs was used as the aim of this test. 0.05 c.c. of the serum (Ab-N 20 μg) obtained from the rabbit sensitized by ovalbumin recrystallized four times was inoculated into the center of the depilated abdominal skin of guinea pigs. Four hours later 50 mg/kg of ovalbumin and 5 c.c./kg of 1 per cent Pelikan India ink-normal saline solution containing 1 per cent gelatin or 2.5 c.c./kg of 1 per cent aqueous solution of Trypan blue were concomitantly injected intraventricularly. In the case of India ink one hour after the injection and in Trypan blue after 10 minutes, the animals were sacrificed by a blow on the back of the head. The area colored with injected dyes was measured inside the local skin, and the intensity of coloration and inflammatory changes were also examined. 100 mg/kg of the drugs to be tested was injected intraperitoneally in a form of the above mentioned suspension one hour prior to the injection of antigen. With the purpose to determine the efficacy of external application, compound # 32 combined with a mixture of equal volumes of dehydrated lanolin and white vaseline was smeared on the local skin 24 hours previous to the inoculation of antibody. Most of other experimental methods were described elsewhere by YAMASAKI *et al.*[1]

RESULTS

A. Inhibition of histamine release

In the first step of the screening tests of these compounds the effect of the

suppression of histamine release *in vivo* was taken as a criterion. Those compounds that depressed below 1/2 the increase in the urinary output of histamine after the intraperitoneal injection of ovomucoid in rats, which was 312 in average as percentage increase in excess of mean controls, with the dose of 50 mg/kg, are shown in Fig. 3. The effect of guaiazulene accompanies in this figure for comparison. The structural formulas of these effective lumisantonin

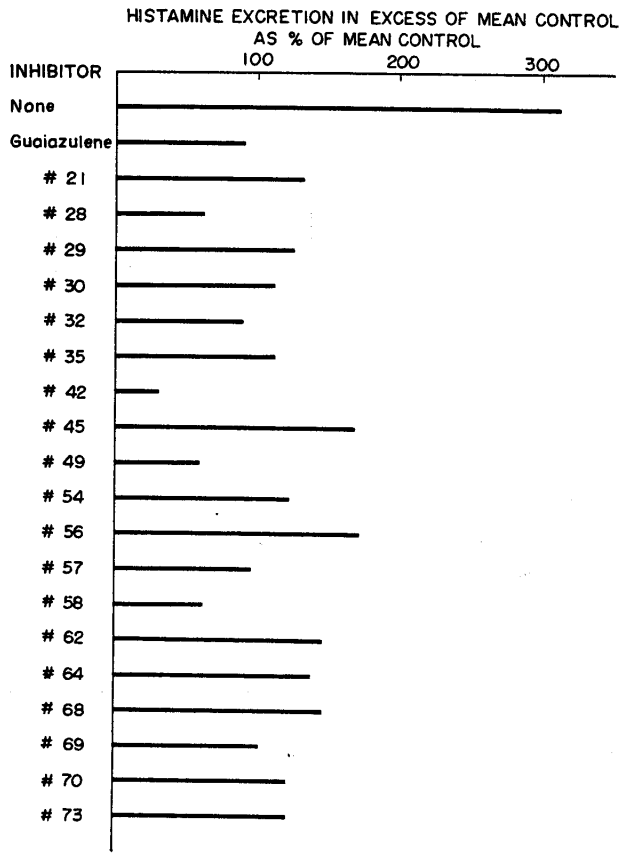


Fig. 3. Inhibitory effect of lumisantonin derivatives and related compounds on the increase in urinary excretion of histamine induced by intraperitoneal injection of 40 mg/kg of ovomucoid in the rat. The compounds (inhibitors) were administered in dose of 50 mg/kg intraperitoneally one hour prior to ovomucoid injection.

derivatives are presented in Fig. 2. Of these compounds, #32, #42, #49, #57, #58, and #69 depressed the increased excretion of histamine below 1/3 in more than two out of three animals.

B. Inhibition of anaphylactoid edema of rat hind paw

Seventeen compounds that proved to be effective in the preceding experi-

ments were selected for further examination of their efficacy in suppressing the anaphylactoid edema induced in a hind paw of rats by dextran, hyaluronidase, histamine, and 5-HT, with intraperitoneal administration of the compounds in dose of 100 mg/kg. In Fig. 4 are shown the mean values of percentages of inhibition as determined at 1, 2 and 3 hours after the induction of edema as the result of triplicate experiments. The compounds tested yielded a relatively good

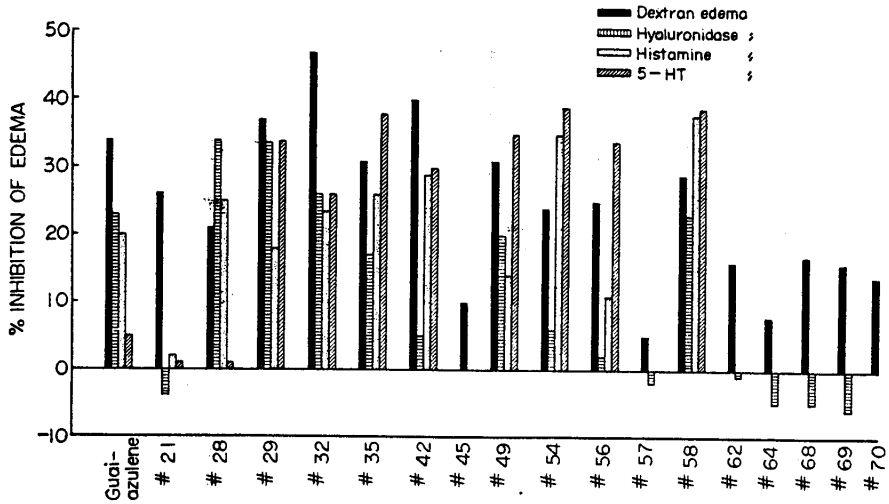


Fig. 4. Inhibitory effect of lumisantonin derivatives and related compounds on the edemas of rat hind paw induced by local injection of dextran, hyaluronidase, histamine and 5-hydroxytryptamine. 100 mg/kg of the compounds was injected intraperitoneally one hour before edema induction.

effect in inhibiting dextran edema, but the effect on other edemas was variable. The ones that yeilded a fairly favorable effect on all the four kinds of edema were #29, #32, #35, #49 and #58. The effect of #32 proved to be superior to that of guaiazulene in every kind of edemas and was quite apparent even at a glance (Fig. 5).

The results comparing the effect of oral administration of #32 with that of guaiazulene are illustrated in Table 2*a*. In both compounds the effect of oral administration proved to be weaker than that of the intraperitoneal. When given three hours prior to the induction of edema the oral effect was slightly more marked than when administered two hours before. The edema inhibiting effect of these two compounds given in doses of 10–50 mg/kg orally for 5 consecutive days before the edema induction was not superior to that obtained by a single administration of 100 mg/kg as shown in Table 2*b*. These results indicate that both #32 and guaiazulene are not easily absorbed by the digestive

a. Control *b*. # 32

Fig. 5. Inhibition of dextran edema of rat hind paw. *a*, control; *b*, after intraperitoneal administration of 100 mg/kg of # 32.

Table 2. Inhibitory effect of # 32 and guaiazulene on dextran edema by oral administration. Average of duplicate experiments.

a. Effect of single dose of 100 mg/kg

Compd.	Time of edema induction after medication (h)	Percent inhibition of edema				
		1h	2h	3h	5h	Av. (1-3h)
# 32	2	16	16	16	14	*16*
	3	26	26	20	16	*24*
GA	2	9	15	15	13	*13*
	3	13	23	27	22	*21*

b. Effect of repeated administration for 5 days

Compd.	Dose per day (mg/kg)	Percent inhibition of edema				
		1h	2h	3h	5h	Av. (1-3h)
#32	10	4.5	3.5	2	3	*3*
	50	13	13	17	14	*14*
GA	10	0.5	3.5	1	−1.5	*1.7*
	50	9	20	17	15	*15*

canal.

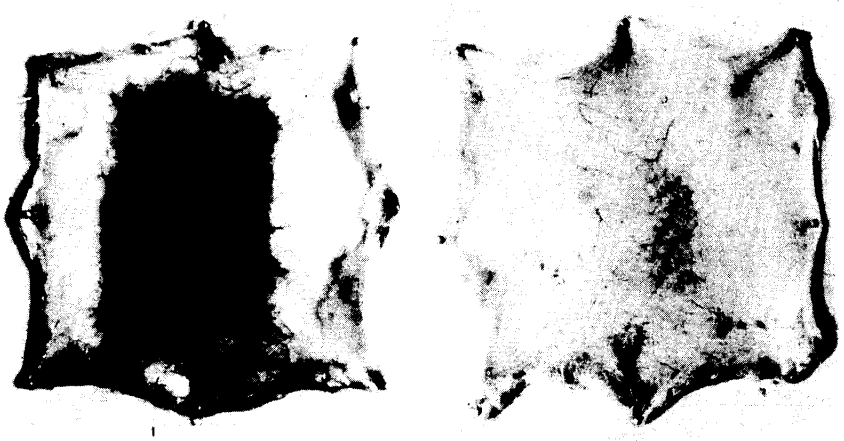
C. *Effect on passive cutaneous anaphylaxis (PCA)*

The eight lumisantonin derivatives that yielded a relatively marked effect on three or more kinds of edema in the above experiments as well as guaiazulene were submitted to the test for their preventive effect on PCA in guinea pigs by the intraperitoneal administration of 100 mg/kg dose. The results are shown in Table 3, where the intensity of the coloration with dyes in the inner surface of the skin is represented as follows. The coloration visible through a cellophane

Table 3. Inhibitory effect of lumisantonin dervatives on passive cutaneous anaphylaxis in guinea pigs. Each compound was administered intraperitoneally in 100 mg/kg one hour before antigen injection.

Compd.	† Dye	Area of coloration ($cm^2 \pm$S.E.)	Intensity of coloration	Inflammatory changes
Control	I	20.2, 23.7, 26.4, 19.3, 20.2, 19.4, 23.9, 20.1 20.3, 28.5, 17.3, 20.8, 26.4, 18.5, 18.6 ····· Av. *21.6±0.3*	4	3
〃	T	26.4, 17.8, 18.0, 16.8 ····· *19.8*	4	3
GA	I	15.1, 8.3, 10.4, 3.5, 1.1, 8.8 ····· *7.9±2.3*	1	1
〃	T	13.7, 12.5, 6.5 ····· *10.9*	2	2
#28	I	5.4, 14.8, 7.7, 20.8, 9.8, 9.8 ····· *11.4±2.3*	2	3
#29	I	18.2, 12.6, 19.3, 13.8, 16.8, 6.1 ····· *14.7±2.7*	2	2
#32	I	3.0, 2.4, 1.8, 3.8, 4.5, 4.8 ····· *3.4±0.9*	1	2
〃*	I	10.1, 17.8, 14.0, 15.2, 17.7, 17.0 ····· *15.3±1.3*	3	2
〃	T	7.2, 1.9, 11.4 ····· *6.8*	2	1
#35	I	14.5, 10.8, 14.2, 2.9, 11.1, 11.9 ····· *10.9±1.7*	3	2
#42	I	6.6, 12.6, 13.0, 3.8, 20.2, 3.0 ····· *9.9±2.7*	2	2
#49	I	15.5, 7.8, 6.5, 10.7, 9.8, 15.5 ····· *11.0±2.6*	2	2
#54	I	13.5, 13.4, 4.3, 18.2, 19.5, 8.4 ····· *12.9±2.4*	3	2
#58	I	17.6, 18.5, 15.7, 13.5, 17.7, 14.7 ····· *16.3±0.8*	3	2

* By stomach tube, the same dose; † I : India ink test, T : Trypan blue test.



a. Control *b*. #32

Fig. 6. Inhibition of passive cutaneous anaphylaxis in the guinea pig by #32. *a*, control; *b*, after intraperitoneal injection of 100 mg/kg of #32 prior to antigen injection. India ink test.

paper at a distance of 20 cm as 4, that visible in the same way at a distance of 10 cm as 3, that is not visible by this method but clearly visible macroscopically

as 2, and that is extremely weak as 1. As for the intensity of inflammatory reaction, the ones that showed marked hemorrhage, congestion and edema were classified as 3, those showing these changes slightly as 2, and the ones that revealed congestion only as 1.

Everyone of the compounds tested clearly diminished the coloration area, which was in control 19.8 cm^2 average for Trypan blue test, and 21.6 cm^2 for India ink test, and also demonstrated corresponding effects of inhibition of the intensity of coloration and of inflammatory reactions. Among these compounds the one that yielded the most suppressive effect was compound #32, and this drug diminished the coloration area down to 1/3 to 1/4 of the control. This effect is even more potent than guaiazulene. Fig. 6 reveals this marked effect of compound #32. Although the effect is much weaker in the case of oral administration, still it can distinctly be seen (* in Table 3). Following compound #32, the PCA-preventing effect diminished in the order of compounds #42, #35, #49, #28, #54, #29, and #58.

Ointment containing compound #32 in the concentrations of 1: 1, 000, 1: 3, 000 and 1: 10, 000 was applied on the 4 cm^2 area of the depilated skin of the abdomen of guinea pigs for 24 hours, and PCA was induced by inoculating antibody into the skin at the center of the area. As shown in Table 4, #32 in concentrations over 1 : 3, 000 revealed a distinct inhibition of PCA, and at 1: 1, 000 the coloration area was diminished down to about one half of the control in the animals receiving the application of the base of ointment only.

Table 4. Effect of local application of #32 on passive cutaneous anaphylaxis in guinea pigs. #32 containing ointment was smeared on the depilated skin of abdomen 24 hours prior to induction of PCA. Antibody was inoculated into the skin of the center of pretreated area. India ink test.

Concn. of compd.	Area of coloration ($cm^2 \pm$ S. E.)	Intensity of coloration	Inflammatory changes
Control (Base alone)	21.3, 19.6, 19.3, 20.0, 24.1, 21.3, 23.9 ·· Av. *21.4±0.7*	4	3
1: 1000	10.1, 10.3, 10.5, 9.1, 10.2, 14.2, 18.0 ·· *11.4±1.1*	2	2
1: 3000	16.2, 17.5, 19.0, 17.6, 16.8, 19.0 ····· *17.7±0.5*	2	2
1:10000	22.3, 21.2, 19.2, 18.1, 19.0, 19.8 ····· *19.8±1.3*	4	3

D. Inhibition of histamine release from sensitized guinea-pig lung by antigen and chemical histamine releasers

A certain number of lumisantonin derivatives clearly suppressed the increase in urinary excretion of histamine in rats caused by ovomucoid injection. This is an indication of the inhibitory action of these compounds on *in vivo* histamine release due to ovomucoid.[13] However, ovomucoid is the releaser specific to this

Table 5. Inhibition of *in vitro* histamine release by lumisantonin derivatives. Histamine was released from chopped lung tissue of a sensitized guinea pig by egg-albumin (antigen) or chemical histamine releasers. Concentration of releasers: egg-albumin, 2 mg/c. c.; sinomenine, 5 mg/c. c.; decylamine 0.05 mg/c. c. and *0.025 mg/c. c. Tyrode medium, at 37.5° C.

Releaser	Inhibitor	Concn. of inhibitor (%)	Percentage histamine released		$(a-b)/(a)$ ×100
			Releaser alone (a)	Releaser +inhibitor (b)	
Antigen	#32	1	8.5	10	−15
			5.8	5.6	3.4
			9.9	11	0.1
		0.1	5.8	2.2	62
			13	5.1	60
			9.9	5.1	50
		0.01	5.8	3.2	45
			13	7.8	46
			8.5	4.9	42
		0.001	5.8	4.2	28
			8.5	6.1	28
			9.9	6.6	33
		0.0001	5.8	5.5	5.1
			8.5	7.8	8.2
			9.9	8.4	15
		0.00001	5.8	5.5	5.1
			8.5	9.6	−11
			9.9	9.5	4.0
Antigen	#35	1	5.4	3.0	44
		0.1	5.4	2.8	48
		0.01	5.4	2.3	57
		0.001	5.4	2.2	59
		0.0001	5.4	4.8	11
		0.00001	5.4	4.8	11
Antigen	#42	1	9.9	4.1	58
		0.1	13	6.0	53
		0.01	13	7.6	41
		0.001	13	9.1	30
		0.0001	13	11	15
Antigen	#58	1	6.3	3.1	51
		0.1	13	8.2	36
			6.3	3.8	40
		0.01	13	12	7.6
			6.3	5.0	21
		0.001	13	12	7.6
			6.3	5.7	9.5

		0.0001	13	12	7.6
			6.3	5.7	9.5
		0.00001	6.3	6.0	4.7
Sinomenine	#32	0.1	6.7	4.6	31
			19	7.8	59
		0.01	6.7	4.1	40
			12	5.8	50
			19	9.0	53
		0.001	14	9.2	34
			14	9.0	36
		0.0001	14	12	14
		0.00001	14	13	7.0
Decylamine	#32	0.1	41	36	12
		0.01	41	37	9.7
		0.001	41	41	0
		0.0001	41	41	0
		0.1*	17	15	8
		0.01*	17	17	0
			19	19	0

species, and therefore, it is necessary to carry on further investigation on influences of these compounds on the histamine release by other chemical substances and by allergic causes in order to correlate the anti-inflammatory and anti-allergic effects of these compounds to histamine release.

Thus, by the methods previously reported by UCHIDA,[16] experiments were

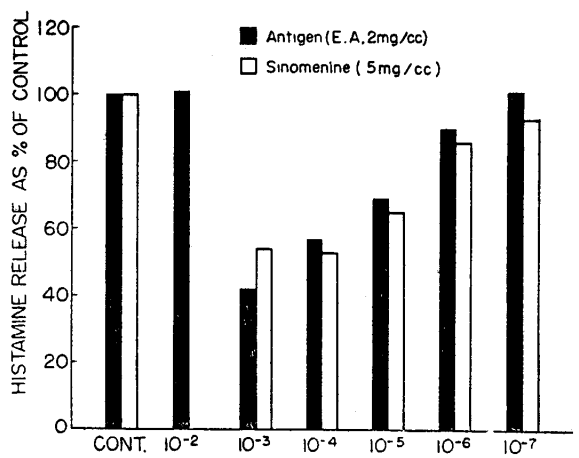


Fig. 7. Inhibition by # 32 of *in vitro* histamine release due to antigen and sinomenine from chopped lung of the sensitized guinea pig. Abscissa, concentration of # 32. Tyrode medium at 37°C.

conducted to examine effects of these compounds on histamine release from minced tissue of lung of anaphylactically sensitized guinea pigs, induced by antigen (2 mg/c.c. of egg albumin) and also by chemical releasers such as sinomenine hydrochloride[17] (5 mg/c.c.) and decylamine hydrochloride (0.025–0.05 mg/c.c.).

The results are presented in Table 5. The anaphylactic release of histamine was clearly inhibited by compounds, #32, #35, #42, and #58. The effect of #32 was particularly prominent, showing it at the concentration as low as 10^{-6} (Fig. 7). This inhibitory effect increased along with the increase in concentration up to 10^{-3}, but on further increase in the concentration it rather grew weaker. Such a decrease in the inhibitory action may signify that this compound itself releases histamine at a high concentration. Such a dual action on histamine release was reported with guaiazulene[1] and antihistamines[18].

Compound #32 showed a similar degree of inhibitory action on the histamine release by both sinomenine and antigen but a weaker inhibition on the release by decylamine.

E. Toxicity

1. Acute toxicity : Seven lumisantonin derivatives that had shown relatively marked anti-inflammatory and anti-allergic effects were submitted to the experiment of acute toxicity in mice weighing 15–20 g. 0.5 c.c./10 g of 1 per cent gum arabic suspension containing graded doses of the compounds was administered orally and intraperitoneally. The mortality rate determined 48 hours after the administration of the compounds is presented in Table 6. The toxicity of compounds #29, #32, #49, and #54 proved to be lower than others and these were well tolerated in the dose of 6 g/kg of oral administration and 4 g/kg of intraperitoneal injection. The dose of 6 g/kg is the limit of administration in the form of the suspension. Since LD_{50} of guaiazulene administered in the same form

Table 6. Acute toxicity of lumisantonin derivatives. no. deaths/no. tests, determined 48 hours after medication.

Compd.	Oral (mg/kg)						Intraperitoneal (mg/kg)						Subcutaneous (mg/kg)		
	6000	4000	2000	1000	500	200	6000	4000	2000	1000	500	200	2000	1000	500
#28	4/4	2/3	0/3				4/4	3/3	1/3	0/3					
#29	0/3						2/3	0/3							
#32	0/3	0/3					2/6	0/4	0/4						
#35		2/3	0/3					3/3	0/3						
#42			3/3	1/3	0/3	0/3				3/3	1/3	0/3	2/3	0/3	0/3
#49	0/3	0/3					2/3	1/3	0/3						
#54	0/3						0/3	0/3							
#58		3/3	1/3					3/3	1/3	0/3					

was 1, 220 mg/kg in oral administration and 525 mg/kg in intraperitoneal injection,[1] six compounds excluding #42 in the table are less toxic than guaiazulene. Subcutaneous toxicity of compound #42 which is soluble in olive oil was tested but it was lower as compared with the case of oral administration.

In mice given a large dose (6 g/kg) of compounds #32, #49 and #54 intraperitoneally only the symptoms observable were a diminution of active movement and gradual prostration, and death occurred in some mice 24–48 hours afterwards. With toxic doses of other members some manifestations due to central stimulation such as acceleration of respiration, erection of tail, and generalized tremor could be observed; and with 1 g/kg of #42, 2 g/kg of #58 and with 4 g/kg of #28, #29 and #35 tonic and clonic type of convulsion was recognized. In severe convulsion resultant asphyxia killed the animals. The properties of some of these compounds as convulsant seem to have been transmitted from santonin from which they were derived. Compounds #32 and #54, however, rather resemble guaiazulene in that they induce motor paralysis with large doses. These two compounds have two lumisantonins in a molecule and this fact seems to be responsible for the difference in toxic symptoms from most of other monolumisantonin derivatives.

2. Subchronic toxicity : Through a stomach tube 4 g/kg of compound #32 was administered once to 2 male and one female Wistar rats 30 days old of the same litter and their growth curves were compared with that of untreated other rats of the same litter for the period of 3 weeks. There could be seen not any difference between the two groups. Of 10 Wistar rats (32 days old, 6 males and 4 females) of the other same litter, 2 males and one female served as control and to the others 250 mg, 500 mg and 1000 mg/kg of #32 were administered daily through a stomach tube for 3 weeks. These litters receiving such medications showed apparently no abnormality and their growth curves were practically the same with controls as illustrated in Fig. 8. These rats were sacrificed 3 days after the last administration and submitted to autopsy. As the result, macroscopically no abnormality could be observed in all organs and also microscopically no pathologic changes in the heart, lungs, liver, spleen, pancreas, kidneys and adrenals. Blood taken just before autopsy showed no difference from that of controls in the hemoglobin content, erythrocyte and white-cell counts as well as classification findings nor any abnormally formed elements.

F. Other actions

1. Antipyretic and analgesic actions : The effect of compound #32 in lowering the body temperature of albino rats weighing 150–200 g in which fever was elicited by subcutaneous injection of 0.5 c.c. of 5 per cent Witte peptone incubated, was examined by BULLER, MIYA and CARR's method[19] taking the

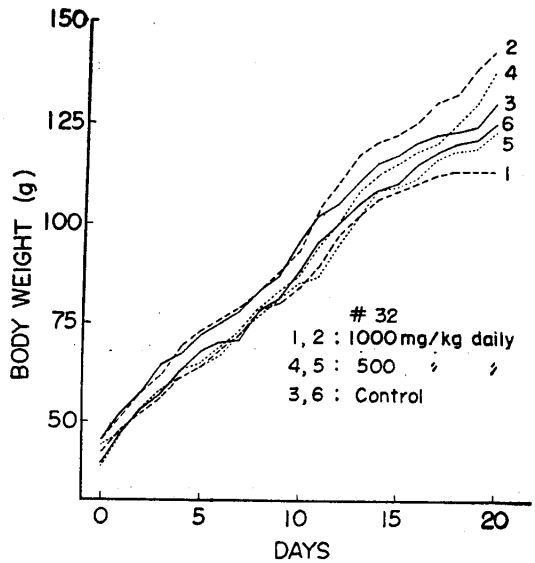


Fig. 8. Growth curves of Wistar rats. Indicated daily doses of #32 were given through a stomach tube.

effect of aminopyrine as the control. The compound to be tested was administered intraperitoneally or orally 4 hours after the peptone injection. Room temperature was kept at 20–22°C. The results are as shown in Fig. 9. In the case of intraperitoneal injection of #32 with the doses of over 10 mg/kg antipyretic action was definitely observed. Although its effect with larger doses was weaker than that of aminopyrine, with doses of 10–50 mg/kg it was comparable to the latter. In the case of oral administration, however, the effect of #32 was quite inferior to aminopyrine.

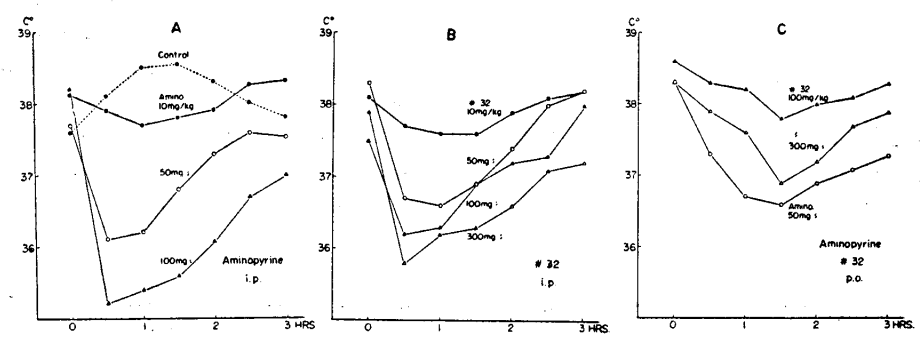


Fig. 9. Antipyretic effects of #32 and aminopyrine in the rats fevered by peptone. *A*, aminopyrine (intraperitoneal); *B*, #32 (intraperitoneal); *C*, aminopyrine and #32 (by mouth). Averages of triplicate experiments.

As for the test of analgesic effect of this compound SANUKI and OHNO's hot-plate method[20] (temperature of the plate, 55°±0.5°C) and HAFFNER-HESSE's method[21] were employed, but by either method no analgesic effect could be detected (Fig. 10).

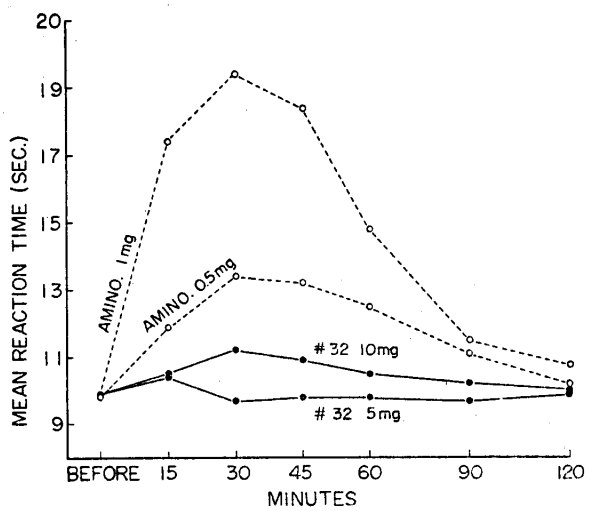


Fig. 10. Analgesic effect of #32 and aminopyrine in mice with SANUKI and OHNO's hot-plate technique. Plate temperature, 55°±0.5°C; 12 mice to a group; indicated doses are in terms of per 10 g body weight intraperitoneally.

2. Effect on intestine in vitro: Compounds #29, #32, #35, #42, #49, #54, and #58 below the concentration of 10^{-3} did not give any clear-cut influence on the tone of the ileum strip of guinea pig suspended in a Tyrode solution nor did they bring about gradual acceleration of peristalsis as demonstrated by guaiazulene. Only compound #28 at the concentration above 5×10^{-4} elicited contraction of the ileum. This was not antagonized by atropine or neoantergan. Pretreatment with #29, #35, and #42 at the concentration above 5×10^{-4} inhibited ileum spasm due to histamine (10^{-7}), acetylcholine (10^{-7}), and $BaCl_2$ (2×10^{-4}) in every case, and this inhibitory action was strongest in #42.

DISCUSSION

The inhibitory effect on the increase in urinary histamine excretion of rat due to ovomucoid was clearly detected in a considerable number of the lumisantonin derivatives tested. In addition, some of them suppressed histamine release from the lung of sensitized guinea pig induced by such chemical releasers as sinomenine and decylamine. The fact that these compounds all inhibit histamine release by a variety of releasers believed to have a different mechanism of action

and that such an inhibition can be observed not only *in vivo* but also *in vitro* indicates that it is undoubtedly local action at tissue level and in all probability at cell level. On this point this action may be said to be similar to the action of guaiazulene that had been previously reported.[1]

STERN and MILIN[22], who made observations on azulene compounds suggestive of their histamine-release inhibitory action, presumed that this action becomes effective probably through activation of the pituitary-adrenal system, judging from histologic pictures of hypophysis and adrenals. They have, however, overlooked the fact that the histamine-release inhibitory action and anti-allergic action of azulene compounds can be observed by local application as well as *in vitro* experiments with excised tissue, as already described precisely in our report on guaiazulene.[1] The action of lumisantonin derivatives as observed in the present series of experiments can be considered the same as that of guaiazulene in so far as the foregoing findings are concerned.

Most of these compounds that had suppressed histamine release did markedly inhibit anaphylactoid edema induced locally in the hind paw of rats by dextran. It is recognized that apart from histamine release 5-HT release[15,23,24] and hyaluronidase activation[15] are also responsible for the occurrence of this dextran edema. According to UDA,[15] one of us, in the inhibition of dextran edema due to guaiazulene is included the inhibition of histamine and 5-HT release as well as anti-hyaluronidase action. Therefore, it must be borne in mind that in the action of lumisantonin derivatives aside from histamine release, effects on other edema-producing factors are also involved. Actually these compounds exhibited inhibition of various degrees on edemas induced by local inoculation of hyaluronidase, 5-HT, and histamine other than dextran.

From the standpoint of clinical therapeutics, an inflammatory pathologic states of practically important pattern may be due to allergic causes. Those compounds that exhibited inhibitory effect on anaphylactoid edemas proved to possess also an undeniably clear-cut preventive action on passive cutaneous anaphylaxis (PCA). Since this allergic tissue reaction can be thought to be a vascular response to integrated stimulation including unidentified mediators[25,26] besides released histamine, taking cognizance of the aforementioned findings, these compounds must be thought to possess an action as to modify a certain basic mechanism of reaction of tissue cells including capillary endothelium, in response to these phlogogenic stimulation. It is possible that the action similar to this is included in the mechanism of inhibition of histamine release from cells.

STERN *et al.*,[22,27] ZIERZ and KIESSLING,[28] and UDA[2] reported that guaiazulene was effective in preventing anaphylactic shock in guinea pigs. UDA[2] in his experiment called an attention to the fact that guaiazulene in this instance has an action to slow down or make less firm the union of antigen and antibody.

This action seems to be related to the effect of guaiazulene on PCA. According to UDA, this action, however, constitutes only a minor part of the anti-anaphylactic effect of guaiazulene, hence it can not be considered as practically so important. Concerning lumisantoin derivatives, their influence on antigen-antibody interaction remains still unclarified. However, judging from a marked inhibitory effect on non-allergic inflammatory edema and on histamine release, after all an importance should be placed on their direct action on tissue cells or capillary endothelium.

Of the lumisantonin derivatives that served as the materials in the present experiments, the one that exhibited the strongest inhibition on PCA was compound #32, methyl pyrophotosantoninate. This compound also revealed inhibitory effect on dextran-, hyaluronidase-, histamine- and 5-HT edemas, all being superior to guaiazulene. Moreover, among the members of these compounds whose toxicity is lower than guaiazulene that of #32 is particularly minimal. For example, in the rats administered with as a large dose as 1 g/kg per day for 3 weeks there could be recognized no pathologic findings, this proving that the compound is quite a less toxic substance the like of which has no parallel among other known anti-inflammatory drugs. The fact that this drug applied in the form of ointment at the concentration above 1 : 3,000 acted as to prevent PCA likewise substantiates in itself clinical utility value of local application of this compound. From the experimental results concerning its inhibitory effect on dextran edema, it is thought that the effect of oral administration of this compound is considerably inferior to that of intraperitoneal injection as in the case with guaiazulene. We are now engaged in modification of physical properties of the compound so as to make it more readily absorbed by intestinal tract.

Compound #32, when administered parenterally, exhibited antipyretic effect comparable to aminopyrine. This effect is undoubtedly more marked than that of guiazulene as observed in our previous experiments[1] with the same methods. In the case of oral administration its effect was much less than that of aminopyrine. This seems in all probability to be due to the difference in ease with which it is absorbed, depending upon the solubility of the substance. The property of #32 differing from guaiazulene is in the point that this compound does not increase peristalsis of isolated ileum. In spite of the fact that some members of these derivatives showed a tendency to induce papaverine-like depression or some spasm on ileum strip, compound #32 even at a high concentration did not reveal such an action. This seems to be an advantage over the other members in its oral or systemic administration.

Be it as it may, from these results we anticipate therapeutic usefulness of some of lumisantonin derivatives in a comparable degree as with guaiazulene. It appears that compound #32 is more promising than guaiazulene in the use of

local application for the treatment of some mucosal and cutaneous disorders of inflammatory or allergic nature including gastritis and X-ray burns of skin for which azulene compounds are indicated

SUMMARY

A number of derivatives and related compounds of lumisantonin were submitted to evaluatien for the action of histamine-release inhibition and anti-inflammatory effect, as they structurally resemble guaiazulene in which these actions had been proved. Nineteen compounds of these suppressed 50 per cent or more of the increase in urinary excretion of histamine due to ovomucoid injection. Five of them markedly inhibited all the edemas in the rat hind paws induced by local inoculation of dextran, hyaluronidase, histamine, and 5-hydroxytryptamine. Among these compounds, ♯32 (methyl pyrophotosantoninate) showed a superior effect of inhibition than guaiazulene on all of these edemas, although the effects of two drugs were comparable in the case of oral administration.

The members showing the edema inhibition likewise evidently protected passive cutaneous anaphylaxis in guinea pigs by the intraperitoneal administration; the effect of ♯32 was more marked than guaiazulene. This effect could be observed when applied to the skin with an ointment containing the compound in a concentration of more than 0.03 per cent 24 hours before.

In vitro histamine releases from the minced lung tissue of sensitized guinea pig elicited by antigen and sinomenine were both inhibited by these compounds. These findings indicate that the main sites of the histamine-release inhibition and of the anti-inflammatory effect of these compounds are in the local tissue.

Compound ♯32 failed to show any analgesic effect in mice, but possessed a considerable antipyretic action in rats. Some of the compounds in the tests depressed guinea-pig ileal strip while guaiazulene increased peristalsis, but any of these actions was not recognized with ♯32 even in a high concentration. Most of the members effective in inhibiting edemas as well as histamine release proved to be less toxic than guaiazulene. ♯32 was well tolerated in the doses of 6 g/kg orally and of 4 g/kg intraperitoneally by mice. The growth curves for three weeks of rats practically did not deviate from that of the controls by daily administration of 1 g/kg of ♯32 by stomach tube and there were no gross and microscopical abnormalities in the main organs and blood.

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