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Structure-Activity Relationship of Lyoniol-A and Related Compounds in Association with the Excitatory Effect on Muscle Spindle Afferents[1]

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The action on muscle spindle afferents, sialogogous action and acute toxicity of lyoniol-A and eleven related compounds were determined. The excitatory action on muscle spindle afferents were measured as the increase in frequencies of the afferent discharge from de-efferented muscle spindles in anesthetized rats. LD_{50}- and salivation-tests were conducted using mice. Of the compounds studied, lyoniol-A, lyoniol-B, dihydrolyoniol-B, grayanotoxin-I and grayanotoxin-III were more potent than suxamethonium, an established muscle spindle excitant, in causing an increase in afferent discharges from muscle spindles. The action on muscle spindle afferents and acute toxicity of the compounds studied were roughly parallel. The relationship between structure and activity was discussed.

Some plants of Ericaceous family contain a variety of non-nitrogenous biological active compounds. Lyoniol-A is one of toxic diterpenoids isolated from *Lyonia ovalifolia* var. *elliptica*. The chemical structure of lyoniol-A has been established by Yasue, *et al.*[3,4] This compound has been demonstrated to induce abnormal postures in mammals and have pharmacologically complex activities.[5-8] Recently, it was found that lyoniol-A exerted a marked excitatory effect on the afferent activities of muscle spindles when applied *in vivo* in mammals[9] as well as *in vitro* in frogs.[10] From the results,[9,10] the effect on muscle spindle afferents was expected to be the main cause of abnormal postures resulting from an administration of lyoniol-A, and this compound was suggested to become a pharmacological tool available for an extensive study of the motor system.

In the present study, we have investigated the action on muscle spindle afferents and acute toxicity of lyoniol-A and eleven related compounds[11] in order to determine the optimal structural requirement for the pharmacological activity.

Method and Material

1) **Acute Toxicity in Mice**——Acute toxicity test (LD_{50}) and sialogogous activity test (ED_{50}) of twelve compounds were conducted in adult male mice (ddY, Shizuoka Farmer) weighing from 18 to 25 g following the intraperitoneal administration. The lethal effect was determined by means of "up and down method"[12]

1) Presented at the 92nd Annual Meeting of Pharmaceutical Society of Japan, Osaka, April, 1972.
2) Location: *Tanabe-dori, Mizuho-ku, Nagoya, 467, Japan.*
3) M. Yasue, T. Kato, and J. Sakakibara, *Chem. Pharm. Bull.* (Tokyo), **18**, 854 (1970).
4) M. Yasue, J. Sakakibara, and T. Kato, *Chem. Pharm. Bull.* (Tokyo), **18**, 2586 (1970).
5) T. Kishida, H. Ota, K. Tsujimura, M. Yasue, and Y. Kato, *Yokohama Medical Bull.*, **14**, 107 (1963).
6) H. Fukuda, K. Watanabe, and T. Ito, *Yakugaku Zasshi*, **89**, 382 (1969).
7) H. Fukuda, K. Watanabe, and T. Ito, *Japan. J. Pharmacol.*, **19**, 394 (1969).
8) H. Fukuda, K. Watanabe, and T. Ito, *Japan. J. Pharmacol.*, **22**, 457 (1972).
9) H. Ono, Y. Kudo, and H. Fukuda, *Japan. J. Pharmacol.*, **22**, suppl. 101 (1972).
10) Y. Kudo, K. Watanabe, and H. Fukuda, *Nippon Yakurigaku Zasshi*, **65**, 186 (1969).
11) T. Kato, J. Sakakibara, and M. Yasue, *Yakugaku Zasshi*, **91**, 1194 (1971).
12) K.A. Brownlee, J.L. Hodges, Jr., and Murray Rosenblatt, *J. Am. Stat. Assoc.*, **48**, 262 (1953).

using 10 to 15 mice for each compound (because only a small amount of each compound was available). Lethal criterion was done 1 hr after administration. The salivation response of the mice was defined as positive when saliva spreaded over upper and lower lips within 30 min after administration.

2) **Muscle Spindle Discharge**——Experiments were performed on male albino rats (Moriyama inbred strain) weighing from 200 to 300 g anesthetized with chloralose (25 mg/kg, *i.p.*) and urethane (1 g/kg, *i.p.*). The diagram of the method is shown in Fig. 1. After tracheal cannulation animals were artificially ventilated and warmed using a DC infrared lamp and a DC heating pad. The animal was fixed to a stereotaxic frame using two spinal clamps. Muscle spindles in gastrocnemius-soleus muscle were studied. The hind limb was denervated except for branches leading to the gastrocnemius-soleus muscle, and fixed with a clamp. A laminectomy was performed, and the lumbosacral cord was exposed and immersed in a liquid paraffin at body temperature. The ventral roots were cut below L_4 in order to eliminate motor innervation of the muscle used. The gastrocnemius-soleus muscle was isolated from the surrounding tissue, and then Achilles tendon was severed distally and usually loaded with a tension of 20 g.

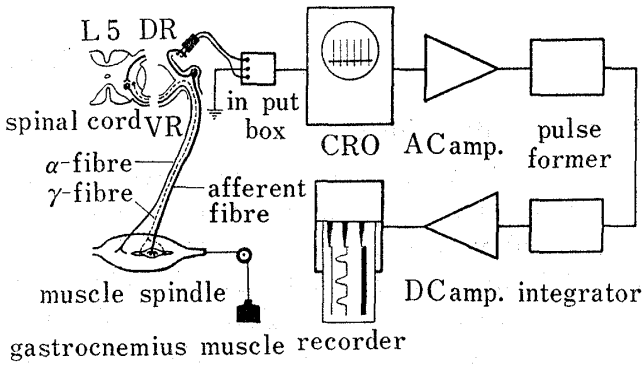

Fig. 1. Block Diagram of the Method which was used to study the Effect of Drugs on Afferents from De-efferented Muscle Spindles in Rats

Afferent nerve units were isolated by splitting the L_5 dorsal root filament. Action potentials were displayed on a cathode ray oscilloscope using bipolar silver electrodes and photographed on a moving film. The same potentials were then transformed into square waves and fed into an integrator, the output of which was recorded by an ink-writing oscillograph. Muscle spindle afferents were identified by 1) static and dynamic

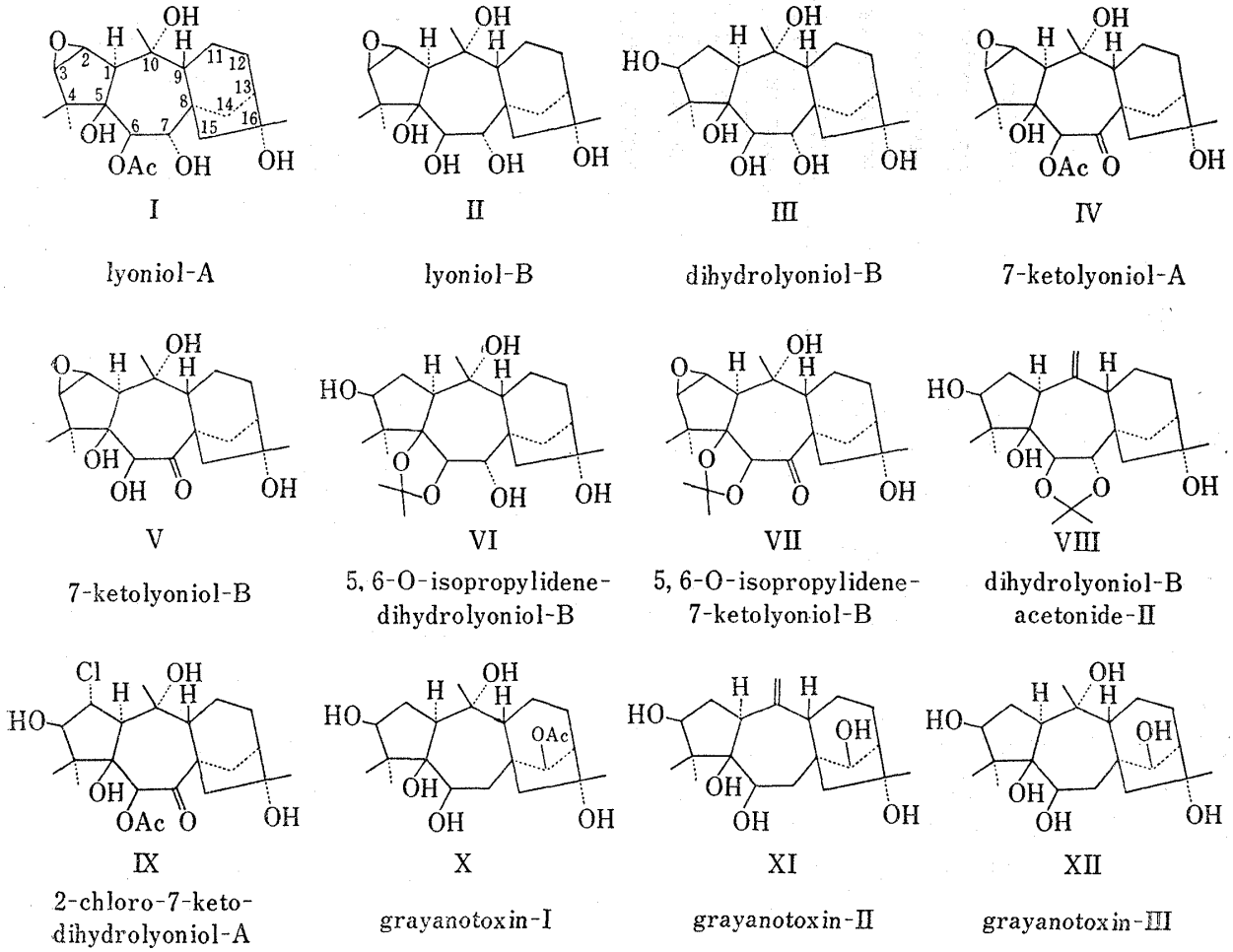

Fig. 2. Structures and Names of the Compounds

discharges during a fast stretch of the muscle, 2) sustained discharges during a loading of 20 g and 3) a pause of discharging after a release of the stretch.[13] The solution of each compound was injected into the femoral vein through a cannula.

3) **Chemicals**——Chemical structures and names of the compounds studied are given in Fig. 2. Drugs used were *d*-tubocurarine chloride (Wako Pure Chemicals) and suxamethonium chloride (Succin Injection, Yamanouchi). All were dissolved in 0.9% saline.

Result

Acute Toxicity in Mice

Fig. 3 shows the result of acute toxicity and sialogogous activity of the compounds studied. Symptoms caused by the compounds did not differ significantly, although LD_{50} and ED_{50} (salivation) varied with compounds. The most toxic compounds were lyoniol-B (deacetyl lyoniol-A) and grayanotoxin-III. Retching appeared and spontaneous acts decreased with doses below those causing salivation. Washing, grooming and abnormal gait were observed when the dose was increased to the extent of causing salivation. With further increasing doses, behavioural symptoms changed to ataxia. Symptomatology which led to the death

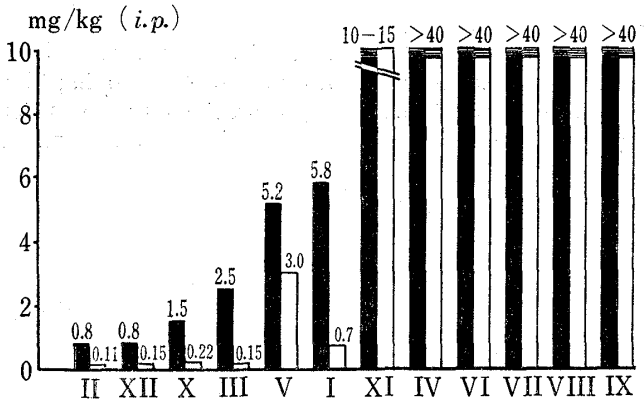

Fig. 3. Summary of LD_{50} and ED_{50} (salivation) in Mice

■ : LD 50
□ : ED 50 (salivation)

of animals was characterized by disturbance of the cardiac rhythm and depression of the respiration. Only 7-ketolyoniol-B caused excitatory behaviours.

Animals died whithin lethal criterion time of 60 min except for three animals which died between 60 and 90 min. All animals which did not die within 90 min survived over the observation time of 7 days.

Effect on the muscle spindle afferents

When the de-efferented gastrocnemius-soleus muscle *in situ* was subjected to tension, discharges were continuously obtained from the muscle spindles. In the following experiments,

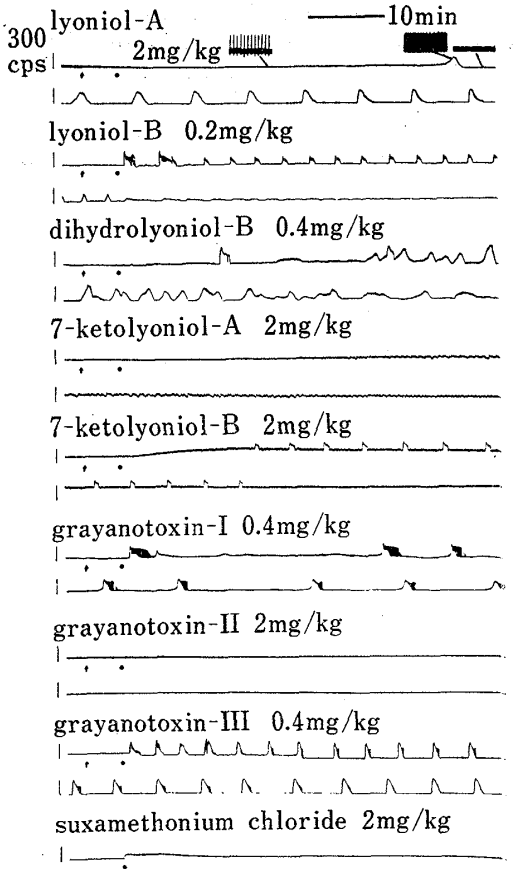

Fig. 4. Effect on the Afferent Activities of Muscle Spindles in Rats

The muscle was subjected to a tension of 20 g. Impulse frequencies of discharges from muscle spindles were recorded using an integrator and an ink-writing oscillograph. Oscilloscopic records of the effect of lyoniol-A were added. At the arrow, *d*-tubocurarine chloride (0.2 mg/animal, *i.v.*) was injected to prevent an undesirable movement. At the dot, drugs were given intravenously.

13) B.H.C. Matthews, *J. Physiol.*, 78, 1 (1933).

the muscle was always loaded with a tension of 20 g. Examples of the effect of the compounds are shown in Fig. 4.

a) **Lyoniol-A**——Lyoniol-A (0.5 mg/kg, *i.v.*) exerted no effect on the afferent activities of muscle spindles; a dose of 1 mg/kg, however, increased the rate of afferent discharge. With 2.0 mg/kg, discharges developed into irregularly repeated discharges of high frequencies lasting for 2 hr. Increase in the frequency of discharges obtained with lyoniol-A (2 mg/kg) was greater than those obtainable with mechanically stretching to a maximum or the use of suxamethonium.[9] The maximal frequency of the discharges induced by lyoniol-A was 200 to 300 cps.

b) **Lyoniol-B**——Lyoniol-B exerted an immediate effect and was about ten times as potent as lyoniol-A in causing an increase in the rate of discharge. An example of 0.2 mg/kg is shown in Fig. 4.

c) **Dihydrolyoniol-B**——A dose of 0.4 mg/kg caused a marked increase in the rate of discharge, but was less potent than lyoniol-B.

d) **7-Ketolyoniol-A and 7-Ketolyoniol-B**——7-Ketolyoniol-A (2 mg/kg) increased the rate of discharge significantly, but was not capable of producing discharges of high frequencies. This compound was less potent than lyoniol-A. Similarly, 7-ketolyoniol-B (2 mg/kg) increased the rate of discharge, being less potent than lyoniol-B.

e) **5,6-O-Isopropylidene-dihydrolyoniol-B, 5,6-O-Isopropyridene-7-ketolyoniol-B, Dihydrolyoniol-B Acetonide-II and 2-Chloro-7-ketodihydrolyoniol-A**——In a dose of 2 mg/kg, these compounds had no effect on the afferent activities of muscle spindles.

f) **Grayanotoxin-I**——A dose of 0.4 mg/kg immediately caused discharges of high frequencies. This compound resembled lyoniol-B in causing a strong and immediate effect.

g) **Grayanotoxin-II**——A dose of 2.0 mg/kg had no effect on the rate of discharge.

h) **Grayanotoxin-III**——A dose of 0.4 mg/kg caused discharges of high frequencies, being a little more potent than grayanotoxin-I.

Discussion

In a previous study,[9] lyoniol-A has been demonstrated to be a strong excitant of muscle spindle afferents, being more potent than suxamethonium, an established muscle spindle excitant.[14] The result of the present study indicated that biological activities of lyoniol-A and related compounds, measured as LD_{50}, the sialogogous effect and the effect on muscle spindle afferents, varied with compounds.

As shown in Fig. 3, the order of the acute toxicity was as follows: lyoniol-B, grayanotoxin-III>grayanotoxin-I>dihydrolyoniol-B>7-ketolyoniol-B, lyoniol-A. In other six compounds, the dose of 40 mg/kg was not lethal. The order of sialogogous effect was as follows: lyoniol-B>grayanotoxin-III, dihydrolyoniol-B>grayanotoxin-I>lyoniol-A>7-ketolyoniol-B>grayanotoxin-II>7-ketolyoniol-A.

The compounds caused increases of the afferent discharges from the muscle spindle in the following order: lyoniol-B>grayanotoxin-III>grayanotoxin-I>dihydroxylyoniol-B>lyoniol-A>7-ketolyoniol-B (Fig. 4).

A summary of the experimental result of six compounds, which clearly increased the afferent discharges, is shown in Fig. 5, thus allowing a comparison to be made of their effect on the afferent activities of muscle spindles and toxicities. In these compounds, the pharmacological activity was roughly parallel with the acute toxicity; namely, the two activities could not be dissociated.

Hydrolysis of acetoxy-group at C-6 position in lyoniol-A strengthened the activity of the compound (lyoniol-A→lyoniol-B), whereas substitution of carbonyl group for hydroxy-

14) R. Granit, S. Skoglund, and S. Thesleff, *Acta Physiol. Scand.*, **28**, 134 (1953).

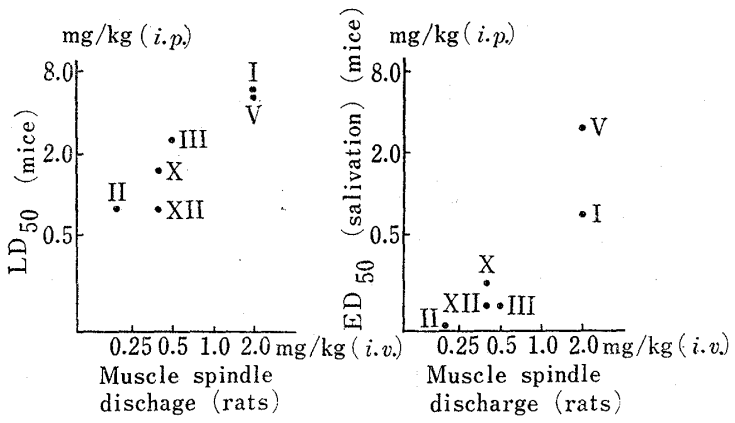

Fig. 5. A Comparison of the Effect on the Activities of Muscle Spindles with LD_{50} and ED_{50} (salivation)

group at C-7 weakened the activity (*e.g.* 7-ketolyoniol-A and 7-ketolyoniol-B). Furthermore, it is evident that the presence of epoxide ring in lyoniol-compound is important for the biological activity (lyoniol-B> dihydrolyoniol-B). The introduction of a dioxolon ring at C-5 and -6 or C-6 and -7 position decreased the effect remarkably. Hydrolysis of acetoxy-group at C-14 in grayanotoxin-I produced a stronger compound (grayanotoxin-III). Dehydration at C-10 of grayanotoxin-III weakened the effect (grayanotoxin-II). Grayanotoxin-III was more potent than dihydrolyoniol-B, indicating the contribution of hydroxy-group at C-14 to the activity.

Thus the result clearly indicates that hydroxy-groups at C-6, -7, -10 or -14 position are required for the biological activity of the grayanane compounds, and that, in general, the number and location of hydroxy-groups are important factors. We now expect possible strong activities of the compounds having hydroxy-groups at C-6, -7 and -14 positions, which have not yet been obtained.

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