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Positive Inotropic Effect of Helenalin, a Sesquiterpene Lactone, on Guinea-pig Myocardium[1)]

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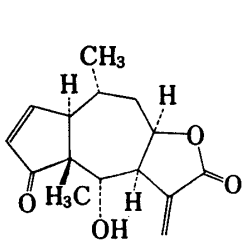
Helenalin, a pseudoguaianolide sesquiterpene lactone which has hitherto been known as a potent antitumor and cytotoxic substance was found in this study to have a positive inotropic effect (PIE) on the myocardium. Left atrial strips and papillary muscles isolated from the guinea-pig heart were driven at a frequency of 1.0 Hz in Krebs–Henseleit solution at 30°C. Helenalin produced an increase in the force of contraction (F_C) depending on its concentration in the range between 10^{-5}—10^{-3} M. The potency of helenalin, expressed as pD_2 value, was 4.69 and 4.11 in the left atria and papillary muscle, respectively. The PIE of 3×10^{-4} M helenalin was equivalent to 78% of that of 10^{-5} M norepinephrine. The PIE of a higher concentration of helenalin (above 3×10^{-4} M) was neither affected by reserpinization nor by propranolol (3×10^{-5} M), but the PIE at lower concentrations was inhibited by reserpinization or propranolol. We conclude that the PIE of helenalin is produced in two different ways; one is catecholamine-mediated and the other is an unknown direct effect.

Keywords——helenalin; sesquiterpene lactone; positive inotropic effect; isolated guinea-pig myocardium; catecholamine; reserpinization

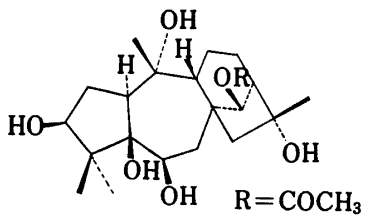
Introduction

Helenalin is one of the pseudoguaianolide sesquiterpene lactones isolated from the plant *Helenium* genus of the family Compositae.[2)] Some biological actions of helenalin such as antitumor[3)] and antimicrobial[4)] activities have been known. However, its action on the heart has never been reported.

In a previous paper,[5)] we reported the potent positive inotropic effect of grayanotoxins in the guinea-pig heart. Helenalin has in part a common chemical structure of perhydroazulene skeleton with grayanotoxins. Thus, our interest was stimulated as to whether this common structural feature is related to the development of positive inotropic action.



helenalin



grayanotoxin-I

Fig. 1. Chemical Structures of Helenalin and Grayanotoxin I

With regard to helenalin, it was clarified that in studies on the relation between chemical structure and cytotoxic activity, α-methylene-γ-lactone and cyclopentenone moieties are essential for the antitumor activity. The mechanism of cytotoxic activity is believed to be

due to the alkylation of the α-methylene-γ-lactone and cyclopentenone rings in the molecule by thiols of key enzymes of nucleic acid and chromation metabolites through Michael addition reaction.[6] Our interests were also stimulated how hitherto known mechanism of cytotoxic action of helenalin take part in the cardiac action.

In this study, we examined the cardiac action of helenalin using isolated atrial and ventricular preparations from the guinea-pig heart and found it caused positive inotropic effect.

Materials and Methods

Papillary muscles and atrial strips were isolated from the right ventricle and left atrium of the hearts of guinea pigs of either sex weighing about 300 g and were mounted vertically in a two-chambered vessel.[7] The bath solution was a modified Krebs–Henseleit solution of the following composition in mM: NaCl 115, $NaHCO_3$ 25, $CaCl_2$ 3.2, KCl 4.7, KH_2PO_4 1.2, $MgCl_2$ 1.2 and glucose 10. The temperature of the bathing solution was 30°C. The solution in a total volume of 45 ml was saturated with and circulated by a gas mixture of 95% O_2 and 5% CO_2. The pH of the solution was 7.5. The preparations at resting tension of 0.4 g were driven by a rectangular pulse of 5 msec duration at a strength 1.5 times above the threshold at a frequency of 1.0 Hz. The force of the muscle was measured by means of an isometric transducer (Nihonkohden SB-1T) connected with an amplifier and was oscillographically recorded on the paper and/or on a tape recorder (Nihonkohden RMG 5104). The parameters of the contraction curve were first recorded on the tape, and analyzed by a computer (Melcom 70, Mitsubishi Electric Co.) to determine the peak force (F_C), positive inotropic effect (ΔF_C), contraction speed (S_1) and time to peak tension (t_1). The concentration-F_C relation was examined by cumulatively adding helenalin solution to the organ bath (below the volume of 1/100 of bathing solution) when F_C of muscle preparations became constant at a given concentration. The half-maximally effective concentration for the positive inotropic effect (PIE) was determined by depicting the concentration-PIE curves assuming the magnitude of PIE at 10^{-3} M as 100% and was expressed by pD_2 value. Helenalin was isolated from *Helenium microcephalum* (Compositae)[8] according to the following procedure: the ground, air-dried whole plant material was extracted with chloroform, and then evaporated. The crude extract was chromatographed on silica gel eluted with benzene–chloroform, chloroform, chloroform–ethyl acetate, and acetone, successively. Helenalin was obtained from fractions eluted with chloroform–ethyl acetate at a mixing ratio of 9: 1. Helenalin was purified by recrystallization from chloroform–benzene. The purity was corroborated by measurements of melting point (170—172°C), thin layer chromatography (TLC), infrared spectra and ^{1}H nuclear magnetic resonance (^{1}H-NMR) spectra. Test solutions for pharmacological studies were freshly prepared before the experiment from helenalin by adding dimethyl sulfoxide (DMSO). DMSO was used at a final concentration lower than 1.27%. DMSO at this concentration caused no appreciable effect on the contractility of myocardium preparations (unpublished data).

Results

The influence of helenalin on the contractility of the myocardium was examined using atrial strips from the left atrium and papillary muscles from the right ventricle of the guinea-pig heart. Helenalin produced PIE on both kinds of preparations.

Concentration-effect Relationship

Helenalin was added cumulatively into the organ bath after establishing a steady-state contraction at a given concentration. The PIE of helenalin developed depending on its concentration (Figs. 2 and 3). Concentration dependency was clearly observed on atrial strips in the range between 10^{-5} and 3×10^{-4} M, and on the papillary muscle in the range between 3×10^{-4} and 10^{-3} M. Helenalin caused more potent PIE in atria than in ventricular muscle (Fig. 3). Helenalin concentration at the half-maximal PIE was measured by depicting concentration-PIE curves in both atrial and ventricular preparations, as shown in Fig. 4. The pD_2 values of helenalin in atrial strips and papillary muscles were 4.69 ± 0.06 and 4.11 ± 0.08, respectively. The difference in pD_2 values or protency of helenalin is statistically significant ($p<0.01$).

Changes in the contraction curves of the preparations with various concentrations of helenalin are shown in Fig. 5 and Table I. An increase in F_c by helenalin was associated with an increase in steepness of contraction (S_1). In the papillary muscle, a marked increase in S_1

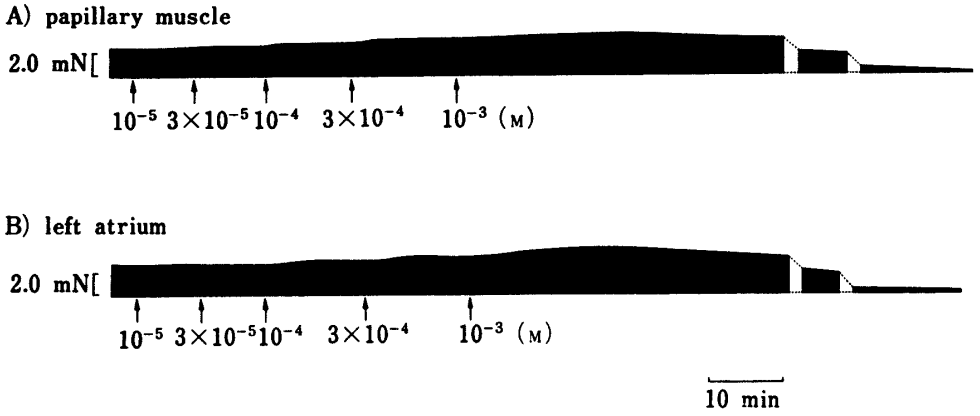


Fig. 2. Actual Recordings of Peak Force of Contraction of Guinea-pig Papillary (A) and Atrial (B) Muscles at Cumulatively Increasing Helenalin Concentrations

The papillary muscle and left-atrial strips bathed in Krebs-Henseleit solution at 30°C were driven at 1.0 Hz. Peak force of contraction (F_c) is expressed in millinewtons (mN).

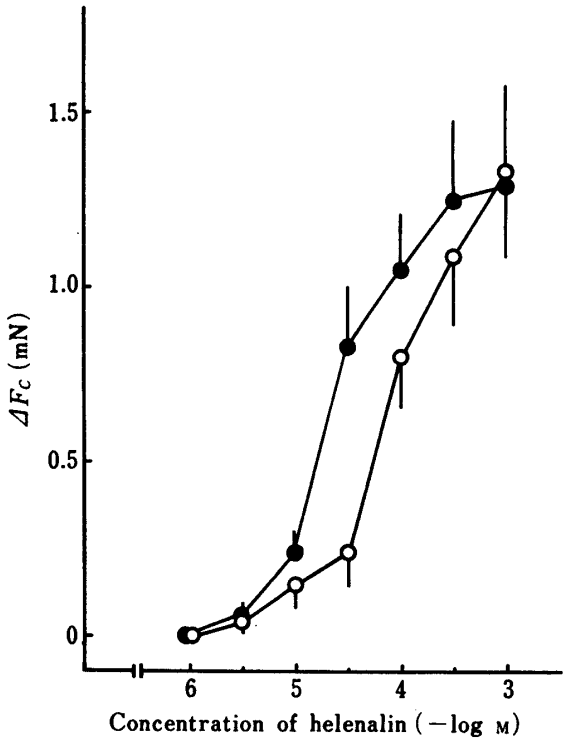


Fig. 3. Positive Inotropic Effect–Concentration Curves for Helenalin in Guinea-pig Myocardium

Plot of positive inotropic effect (PIE; ΔF_c) against helenalin concentration. The curves in this figure are drawn from data obtained in the same experiments as in Fig. 2. Papillary muscles (○); Atrial muscle strips (●). F_c in mN was expressed by means ± S.E.M. of 5 muscles.

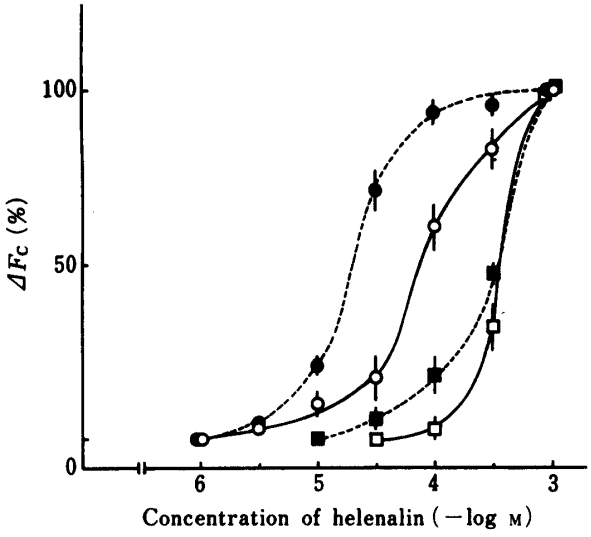


Fig. 4. Influence of Reserpinization on the Positive Inotropic Effect of Helenalin

Concentration-ΔF_c curves for helenalin in myocardial preparations from guinea pigs treated with and without reserpine (5 mg/kg, *i.p.* once a day for two days). Non-reserpinized papillary muscles (○) and left atrial strips (●); Reserpinized papillary muscles (□) and atrial strips (■). The curves are depicted assuming the PIE of 10^{-3} M helenalin in each preparation to be 100%. Symbols and bars represent means ± S.E.M. of 5 muscles.

(1.86 times) and shortening of the time to peak tension (t_1) were observed. In atrial strips, F_c increased in parallel with change in S_1 since t_1 was not changed.

The strength of PIE of 3×10^{-4} M helenalin was compared with that of 10^{-5} M norepinephrine as shown in Fig. 6. Helenalin at this concentration produced PIE equivalent to 78% of PIE of 10^{-5} M norepinephrine.

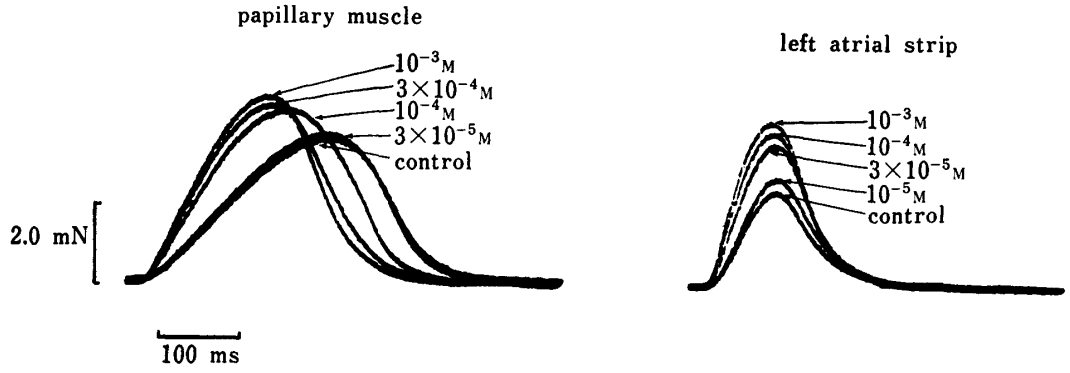


Fig. 5. Isometric Contraction Curves of Guinea-pig Myocardial Muscles as Affected by Cumulatively Increasing Concentrations of Helenalin

The papillary muscle in the right ventricle (0.9 mm in diameter and 3.2 mm in length) and a muscle strip from the left auricle (2.0×8.5 mm) were isolated from the heart of a non-reserpinized guinea pig. Driving rate; 1.0 Hz. Resting tension; 0.4 g. Krebs-Henseleit solution at 30°C.

TABLE I. Helenalin Concentration and Its Effects on the Parameters of Contraction

		Control	10^{-5}M	3×10^{-5}M	10^{-4}M	3×10^{-4}M	10^{-3}M
Papillary muscles	F_c (mN)	3.85±0.70	3.99±0.67	4.09±0.65	4.65±0.74	4.93±0.82	5.18±0.78
	(%)	100	104	106	121	128	135
	S_1 (mN/s)	31.2±5.3	40.7±4.6	42.1±4.6	50.1±5.3	54.1±6.1	58.1±9.0
	(%)	100	130	135	161	173	186
	S_2 (mN/s)	−35.5±7.5	−37.1±7.2	−38.4±7.2	−44.5±8.2	−48.2±9.4	−50.0±11.0
	(%)	100	105	108	125	136	141
	t_1 (ms)	118.8±9.2	116.4±8.0	113.4±8.6	108.2±7.4	102.6±7.7	100.8±5.2
	(%)	100	98	95	91	86	85
Atrial strips	F_c (mN)	2.75±0.54	2.99±0.55	3.58±0.60	3.80±0.60	3.84±0.58	3.87±0.57
	(%)	100	109	130	138	140	141
	S_1 (mN/s)	39.1±7.6	42.4±7.9	50.9±8.6	54.5±8.7	55.0±8.3	55.2±8.0
	(%)	100	108	130	139	141	141
	S_2 (mN/s)	−27.1±4.6	−30.1±5.1	E39.1±6.2	−43.8±6.6	−46.1±6.7	−48.7±6.9
	(%)	100	111	144	162	170	180
	t_1 (ms)	58.0±3.4	58.8±3.6	60.6±3.3	58.8±2.9	59.6±3.1	60.2±3.2
	(%)	100	101	104	101	103	104

Each parameter is represented by means ± S.E.M. (n=5). Abbreviations: F_c; force of contraction; S_1, maximum steepness of contraction; S_2, maximum speed of relaxation; t_1, time to peak tension from the onset of contraction. The other experimental conditions are shown in Figs. 3 and 5.

Positive Inotropic Effect of Helenalin in Reserpinized Guinea-pig Myocardium

Experiments were made to examine whether the PIE of helenalin depends on catecholamine or not. With the atrial and ventricular muscles isolated from reserpine-pretreated guinea pigs (5 mg/kg, *i.p.* for 2 d), the concentration-PIE curves for helenalin were shown in Fig. 4. The curves in normal (non-reserpinized) preparations were shifted to the right by reserpinization *i.e.*, the pD_2 values of helenalin tested in reserpinized left atrial strips and papillary muscles were 3.46 and 3.47, respectively. There was no difference in pD_2 values obtained from two kinds of reserpinized myocardial preparations. Comparing these pD_2 values with those in nonreserpinized cases, the concentration-PIE curve was shifted to the right with a magnitude of difference of 1.23 in the case of atrium. In the case of the papillary muscles, pD_2 value shifted by a magnitude of 0.64. By reserpinization, the PIE of helenalin in the case of atrium was inhibited more strongly than that in the case of papillary muscle. At concentrations above 10^{-3} M helenalin, the PIE was not affected by reserpinization.

Helenalin Effect in the Presence of Propranolol

The influence of β-blocking agents on the PIE of helenalin was examined with *l*-propranolol-pretreated papillary muscle of guinea pig. In the presence of 3×10^{-5} M *l*-propranolol,

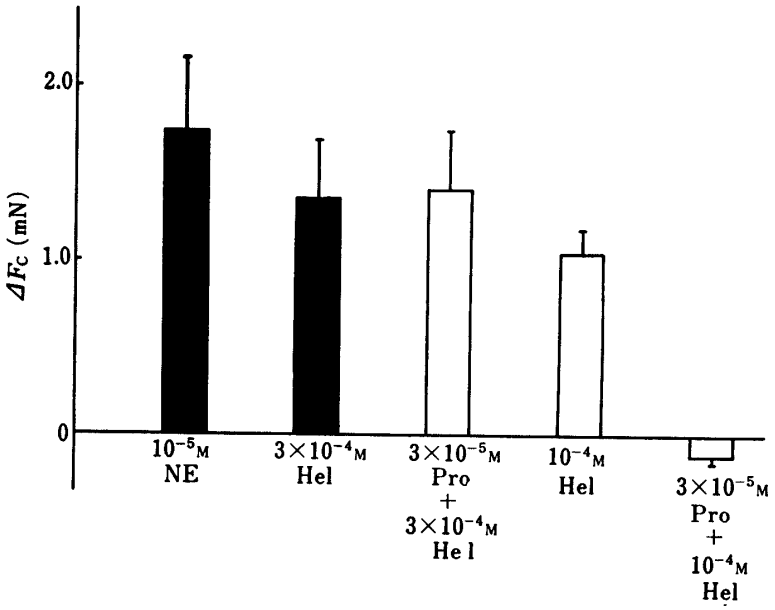


Fig. 6. Influence of Propranolol on the PIE of Helenalin on Guinea-pig Papillary Muscles

The maximum PIEs at a given helenalin concentration are compared with that in the presence of 3×10^{-5} M propranolol (Pro). The PIE of 3×10^{-4} M helenalin was not inhibited but that of 10^{-4} M was inhibited by propranolol at this concentration. Actual recordings in experiments on this subject are presented in Fig. 7. The strength of PIE of 3×10^{-4} M helenalin (Hel) is also compared with that of 10^{-5} M norepinephrine (NE). The PIE of helenalin amounted to 78% of that of NE (significant by paired t-test at $p=0.02$).

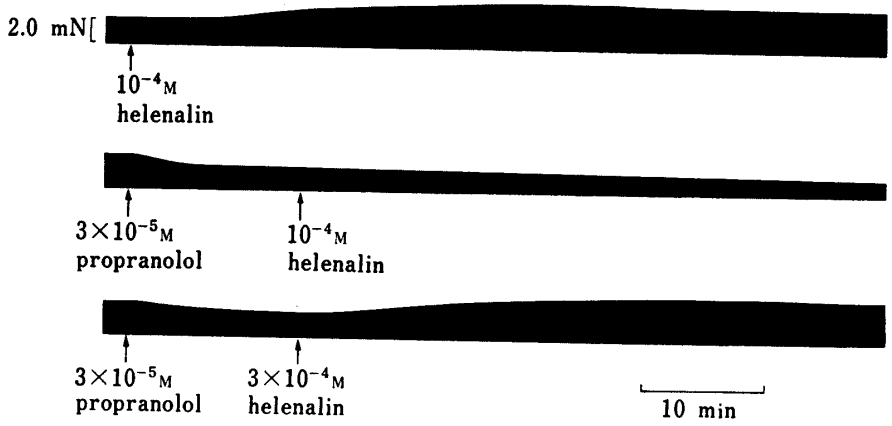


Fig. 7. Time Course of the Effects of Helenalin on the Change in Peak Force of Contraction of Guinea-pig Papillary Muscles with and without Propranolol

Driving rate; 1.0 Hz. The representative examples from the experiment in Fig. 6 are demonstrated.

the PIE of relatively low concentration (10^{-4} M) of helenalin was strongly inhibited but that caused by 3×10^{-4} M or more helenalin was not affected (Figs. 6 and 7). The development of PIE by helenalin in the presence of propranolol was highly critical with regard to the concentration at which F_c of the papillary muscle first increased. In accord with the result in the case of reserpinization, it will be concluded that catecholamine takes part in the PIE produced by relatively low concentrations of helenalin.

Negative Inotropic Effect of High Concentrations of Helenalin

Helenalin at a concentration higher than 10^{-3} M produced negative inotropic effect in the later stage (Fig. 2). The F_c of either atrial or ventricular muscle with high concentrations of helenalin decreased after reaching the maximum. The muscle finally stood still in a diastolic state (about 90 min). The negative inotropic effect of helenalin at the later stage was irreversible judging from the observation that the F_c could not be restored by washing out the muscle preparations 3 times with Krebs–Henseleit solution at intervals of 3 min.

Discussion

Throughout the present experiments, it became clear that helenalin has a positive inotropic effect on the myocardium of guinea pig. There has been no previous report on the PIE of helenalin or of its analogues. On the other biological activities than cardiac effect, a number of reports[9] have referred to antitumor or cytotoxic action. Antitumor activity of helenalin was reported to be due to inhibit the enzyme activities of deoxyribonucleic acid (DNA) synthetase and DNA polymerase as verified in experiments using Ehrlich ascites tumor cells.[6] We showed in this study that helenalin caused not only positive but also negative inotropic effect. The contractility was not replenished by washing out the preparations. This leads to an assumption that helenalin irreversibly inhibits the enzyme activities of myocardial cells. It might be further assumed that the α-methylene-γ-lactone and/or cyclopentenone moieties of helenalin react with some nucleophilies such as thiol groups of enzymes in the heart cells. Accordingly, this stable binding formed by alkylation might be related to the irreversible negative inotropic or cytotoxic effect in the later stage with high concentrations of helenalin. With respect to the metabolic effects of helenalin in Ehrlich ascites tumor cells, it was reported that phosphofructokinase and hexokinase activities were inhibited when helenalin was administrated to mice.[6] On catecholamine metabolism, there has been no report to account for the PIE of low concentrations of helenalin. However, vernolepin, which has an α-methylene-γ-lactone structure in common with helenalin, activated the enzyme activity of catechol-*O*-methyl-transferase (COMT) in cat erythrocytes.[10] This leads to an assumption that the negative inotropic effect of helenalin may be related to COMT activation. But this does not account for the irreversibility of the negative inotropy.

The present results show that the PIE of helenalin depends on catecholamine, because the PIE of low concentrations of helenalin on reserpinized heart was clearly inhibited. This was further supported by the finding that the PIE was not observed in the myocardium pretreated with propranolol. Helenalin at concentrations higher than 10^{-3} M produced PIE without the participation of catecholamine. The mechanism of this direct PIE is not clear at the present stage but the possibility cannot be ruled out that some part of the PIE depends on cyclic adenosine-5′-monophosphate (cAMP), since Hall *et al.*[6] demonstrated that in Ehrlich ascites tumor cells the cAMP level was elevated by helenalin to 3 times the control. In addition, we observed that 3×10^{-4} M helenalin increased the duration of action potential (unpublished data). These results support the view that the PIE of helenalin is mediated by an elevation of cAMP in the myocardium. Further experiments will be needed to clarify the mechanism of direct PIE of high concentrations of helenalin.

Helenalin and grayanotoxin have a common structural feature, perhydroazulene skeleton. In a previous report,[5] we reported the structure-activity relationship of 18 kinds of grayanotoxins, with the results that hydroxyl groups at 3β and 6β, methyl group at 10β and acyl group at 14 position of grayanan skeleton were essential for the development of PIE. From the stereochemical aspects, helenalin has no such active groups in the perhydroazulene skeleton. We assume from our preliminary experiments on structure-activity relationships that the α-methylene-γ-lactone is essential for the PIE development (unpublished data). Comparing

the cardiac action of helenalin with that of grayanotoxins (*e.g.*, grayanotoxin I and asebotoxin III), a distinct difference can be pointed out; in contrast to the action of helenalin, grayanotoxin I caused no negative inotropic effect. The inotropic effect of grayanotoxin I is reversible because the PIE easily returned to the control by washing out the myocardial preparation.[11] In addition, electrophysiological studies on the action of grayanotoxin I showed that grayanotoxin produced sodium-dependent depolarization in the giant axon of squid.[12] The PIE of grayanotoxin I was concluded to depend upon sodium ion.[11,13] Of helenalin, ionic mechanism to produce the PIE was not studied. Further experiments on the mechanism of action from an electromechanical aspect or on the structure–activity relationship will be made.

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References and Notes

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