membrane (Figures 2 and 3). On the other hand, nuclei fold in and show a dented appearance (Figure 2). The changes observed are in accordance with those reported by MAJNO et al.[6] and similar to those induced in several kinds of cells by treatment with proteolitic enzymes[12,13] or alkylating agents[2]. Since these substances act by modifying the chemical composition of the cell membrane, it might be suggested that histamine-induced changes could be the result of changes in the cell coat. Histamine could bind, perhaps, to the cell coat and react with negatively charged loci, as happens in plasma proteins[14]. This would result in a diminution of binding forces with a subsequent disruption of intercellular junctions. In order to test this hypothesis, studies aimed at eliciting histamine effects on the cell surface are now in progress at our laboratory. Capillary structures of animals treated with calcium dobesilate alone do not differ significantly from those of control animals (Figure 4). On the other hand, capillary responses to histamine in dobesilate-treated rats markedly differ from those of untreated animals. In fact, endothelial cells appear normal in all senses and no changes are seen in the morphology of capillary venules. Thus, it seems that calcium dobesilate is able to protect the experimental animal from histamine injury at the capillary level. The mechanism of this protection is not clear. Calcium dobesilate is completely devoid of any antihistamine activity in the isolated guinea-pig ileum, as well as in other biological preparations[15], but it has been shown to counteract increases in capillary permeability induced by a variety of causes[11]. We believe that any possible interpretation should take into account the possibility that histamine reaction with the cell coat could be the initial cause of changes in cell morphology. In other words, it would be reasonable to explore whether the endothelial cell contraction, as suggested by MAJNO et al.[6], could be the result of changes in cellular adhesiveness. Capillary protection by calcium dobesilate might well be the result of a direct effect on the cell coat with secondary changes on cellular adhesiveness[16,17].

Summary. The ultrastructure of blood capillaries and venules are studied in rat skin. After i.v. injection of histamine the luminal surface of the endothelial cells show protrusions of variable size. Numerous gaps have be found in the capillary wall, specially in the venules. These alterations are not observed in the animals that were treated with calcium dobesilate before the administration of histamine. In these cases the capillary structure are indistinguishable of the controls. The possible effects of the histamine and calcium dobesilate on the cell coat and cell junctions of the endothelial capillary cells are discussed.

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Cytotoxicity and Carcinogenicity of Pterosins and Pterosides, 1-Indanone Derivatives from Bracken (*Pteridium aquilinum*)

Since bracken fern (*Pteridium aquilinum*) was noticed to produce tumors in experimental animals[1], extensive surveys have been carried out to attain the isolation of the carcinogenic principle(s)[2,3]. We have also conducted the fractionation of the extracts of dried young leaves and rhizomes of bracken along with cytotoxicity and carcinogenicity tests. We have so far isolated and identified more than 30 compounds, in which sesquiterpenes having 1-indanone nucleus, named pterosins, and the glucosides, pterosides, have been shown to be characteristic constituents of the plant[4].

Although there is no simple efficient method for detecting carcinogenicity using small amount of samples such as separated fractions by chromatographies, we have tentatively employed cytotoxicity tests using HeLa cells for the first screening[5]. Nearly 500 fractions separated from the methanol extract of young leaves of bracken were checked by this method. Although several fractions with high cytotoxicity have not been isolated as pure forms, the toxicity of pterosins and pterosides, isolated as the characteristic constituents, will be collectively presented here.

HeLa cells were grown in Eagle's minimum essential medium supplemented with 10% calf serum. For testing toxic concentration and morphological changes, modified panel method was employed[5]. Each cup of panels contained round cover-glasses, and the cells grown on them, were treated with test compounds for 3 days. These cover-glasses were then fixed with Carnoy's fixative and stained with hematoxylin and eosin. Cell injuries were recorded as 0 through 4, where 0 mean no appreciable effect, 4 lethal effect and 1, 2, 3, gradual injuries between 0 and 4.

In Table I, chemical structures of the compounds and the degree of cell injuries are presented. The morphology

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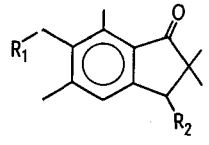
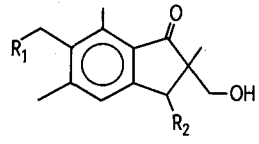
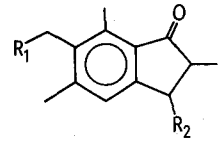
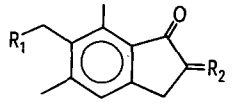
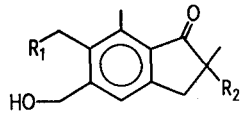
Table I. Structures and toxic effects of pterosins and pterosides on HeLa cells

Compound		R_1	R_2	μg/ml 320	100	32	10
	Pterosin Z	$-CH_2OH$	$-H$	4	4	3.5	2
	Pterosin I	$-CH_2OCH_3$	$-H$	4	4	0	0
	Pteroside Z	$-CH_2OGlu.$	$-H$	3.5	0	0	0
	(3R) pterosin D	$-CH_2OH$	$-OH$	–	0	0	0
	(3S) pteroside D	$-CH_2OGlu.$	$-OH$	2	0	0	0
	(2S) pterosin A	$-CH_2OH$	$-H$	2	0	0	0
	(2S) pterosin K	$-CH_2Cl$	$-H$	–	–	1	0
	(2R, 3R) pterosin L	$-CH_2OH$	$-OH$	4	0	0	0
	(2S) pteroside A	$-CH_2OGlu$	$-H$	0.5	0	0	0
	(2R) pterosin B	$-CH_2OH$	$-H$	4	2	0	0
	(2R) pterosin F	$-CH_2Cl$	$-H$	4	4	0.5	0
	(2R) pterosin E	$-COOH$	$-H$	–	3	2	1.5
	(±) pterosin O	$-CH_2OCH_3$	$-H$	4	4	2	0
	(2R) palmitylpterosin B	$-CH_2Opalmityl$	$-H$	–	0.5	0	0
	Acetyl-Δ^2-dehydropterosin B	$-CH_2OAc$	$-H$	4	4	4	–
	(2R) pteroside B	$-CH_2OGlu.$	$-H$	–	0	0	0
	(2S, 3S) pterosin C	$-CH_2OH$	$-OH$	2	0	0	0
	(2S, 3S) pterosin J	$-CH_2Cl$	$-OH$	–	–	1	0
	(2S, 3S) acetylpterosin C	$-CH_2OAc$	$-OH$	–	1	0	0
	(2R, 3R) pteroside C	$-CH_2OGlu.$	$-OH$	0	0		
	(2S) pterosin G	$-CH_2OH$	$\begin{matrix}CH_2OH\\H\end{matrix}$	4	0	0	0
	Pterosin N	$-CH_2OH$	$\begin{matrix}CH_3\\OH\end{matrix}$	3	0	0	0
	(2S) pterosin P	$-CH_2OH$	$-H$	4	2	0	0
	(2S) pteroside P	$-CH_2OGlu.$	$-H$	0	0	0	0

of HeLa cells injured by these indanone compounds (toxic degree 1 to 3) was more or less similar. The size of treated cells became more or less smaller. Some cells became spindle-shaped and some cells retained polygonal shape. The cytoplasm was clear, sometimes with vacuoles. The nucleus stained deeper with hematoxylin and tiny chromatin condensates were scattered throughout the nucleus. The nucleolus became rounded. Mitotic figures were decreased.

Since pterosin B was detected in abundance in the leaves, this compound was tested for production, if any,

Table II. Toxic effect on HeLa cells of extracts, and the fractions separated from the ethyl acetate supernatant after the procedure reported by EVANS[2]

Fractions	1000	320	100	32 μg/ml
Extracts				
Benzene extract of leaves	4	1	0	0
Ethyl acetate extract	4	1	0	0
Methanol extract	2	0	0	0
The fractions				
Ethyl acetate supernatant	4	1	0	0
Fraction 1 (Quinic acid)	0	0	0	0
Fraction 2 (Shikimic acid)	0	0	0	0
Fraction 3 (Mixture of pterosins and pterosides)	4	2	1	0

of chromosomal changes. Cells were treated at the concentration of 100 and 32 μg/ml for 24 or 48 h, swollen in hypotonic solution, fixed with methanol and acetic acid (3:1) mixture, spread on slides and stained with Giemsa solution. Mitotic index was decreased but morphologically no breakage was demonstrated.

Pterosin B and F were then tested for the effect on the incorporation rates of DNA, RNA and protein precursors by modified flying coverslip technique[5]. Cells were plated on cover-glasses which were treated with the compounds for 1 h and then treated with ^{3}H-TdR, ^{3}H-UR or ^{3}H-Leu. After 1 h further incubation, cover-glasses were fixed with Carnoy's fixative, treated with cold perchloric acid, washed with distilled water and dried. Radioactivities incorporated into cells attached on cover-glasses were counted in situ by windowless gas flow counter (Aloka JBC-104). In the Figure, the incorporated radioactivity was expressed as the percentage of the controls and plotted against the concentration of the compounds. In the case of pterosin B, the uptake of ^{3}H-Leu continued fairly well at the concentration to inhibit that of ^{3}H-TdR or ^{3}H-UR. Pterosin F also showed a similar pattern, but the incorporation of ^{3}H-Leu was fairly inhibited. The result indicated that Cl-containing compound is much more toxic and less specific.

EVANS[2] reported the isolation of an active principle from bracken, which was recently proved to be shikimic acid[6]. We have also tried the separation of the extract by the procedure reported by EVANS[2]. The ethyl acetate

Table III. Experimental results of feeding bracken samples to rats

Exp. No.	Samples[a]	Amount applied per day in diet	Strain of rats[b]	Initial No. of rats	No. of survivors at 120 days	No. of rats with tumors of					Total No. of tumors (period of tumor induction)
						Ileum	Colon	Bladder	Liver	Other tissues	
1	Dried powder of edible parts	30%	Wistar	16	12	2	1	0	0	0	3 (426–802 days)
2	Dried powder of young leaves	30%	Wistar	16	12	3	0	0	0	0	3 (456–650 days)
3	Dried powder of young leaves	30%	Fischer	16	15	7	0	0	0	0	7 (232–365 days)
4	Dried powder of rhizomes	30%	Wistar	16	12	5	1	0	0	1 lymphoma	7 (120–508 days)
5	Benzene extract	55 mg	Wistar	16	14	1	1	1	1	0	4 (580–718 days)
6	Ethyl acetate extract	26 mg	Wistar	16	14	0	0	0	0	1 mammary adenocarcinoma	1 (713 days)
7	Methanol extract	277 mg	Wistar	16	16	1	0	0	0	1 reticulum cell sarcoma	2 (460–626 days)
8	Residues after extraction	30%	Wistar	16	10	0	0	0	1	0	1 (487 days)
9	Phenindione[c]	13 mg–20 mg	Wistar	16	16	0	0	0	0	0	0
10	Pterosin B	4 mg	Wistar	16	14	0	0	0	0	0	0
11	Pteroside B	10 mg	Wistar	16	15	0	0	0	0	0	0
12	Control		Wistar	33	33	0	0	0	0	0	0
13	Control		Fischer	5	5	0	0	0	0	1 subcutaneous fibrosarcoma	1 (619 days)

[a] All dried samples of bracken (Exp. 1–4) and residues of extraction (Exp. 8) were mixed with the basal diet (CE–2, JCL, Tokyo) in the proportion by weight of 30% and made into pellets. Other samples were mixed with basal diet (daily 15 g per rat) at the dosis, respectively, as indicated in Table. [b] All rats were males, and 8 to 10 weeks in age at the beginning of treatment. [c] Phenindione (2-phenyl-1,3-indandione), an anticoagulant, was tested for the carcinogenicity because of the similarity of the structure to pterosins.

Incorporation rate of DNA, RNA and protein precurosors into acid-insolubles of HeLa cells treated with various concentrations of pterosin B and pterosin F.

supernatant[7] was fractionated into 3 portions, which were respectively identified by paper and thin-layer chromatography with quinic acid, shikimic acid and a mixture of pterosins and pterosides. The fractions did not show any noticeable cytotoxicity, except the fraction containing pterosins and pterosides, as shown in Table II.

Besides these cytotoxicity tests, long term experiments of feeding dried powder of young leaves and rhizomes, extracts prepared by successive treatment of the leaves with benzene, ethyl acetate, and methanol, pterosin B and pteroside B, major indanones in leaves and rhizomes respectively, were performed using rats (Wistar, or Fischer, male 8 to 10 weeks of age). The results are shown in Table III.

Further attempts to elucidate the carcinogenic principle(s) are now in progress.

Zusammenfassung. Über 30 Chemikalien aus getrockneten jungen Blättern und Wurzelstöcken des japanischen Farnkrautes *Pteridium aquilinum* wurden isoliert und als Sesquiterpen-Derivate mit 1-Indanonkern, Pterosine und deren Glukoside, Pteroside, identifiziert. Eine nur geringe Toxizität für HeLa-Zellen ohne Chromosomenschädigung wurde festgestellt. Langzeitfütterung von Pterosin B und Pterosid B ergab keine Tumorbildung in Wistar-Ratten, obwohl die rohen Blätter und Wurzelstöcke kanzerogen waren.

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