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Screening of Formosan Ferns for Phytoecdysones. I[1]

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Formosan ferns from 17 families, representing 50 genera, 115 species, 1 subspecies, and 3 varieties, have been screened by the bioassay for phytoecdysones. A total of 64 species and 2 varieties have been found to show distinct insect moulting hormone activity. Correlation between this hormone activity and taxonomy of ferns is discussed.

According to our earlier experience in screening plant materials of Japanese origin for the insect-moulting hormone activity, the probability of finding the activity appears to be high in ferns among the plant groups.[3] In fact, a systematic survey of Japanese ferns for phytoecdysones by means of bioassay has resulted in the discovery of 170 species, 22 varieties, and 1 form showing this activity towards insects out of 283 species, 39 varieties, and 1 form belonging to 76 genera (20 families) screened. Among the active Japanese ferns, 16 species have been subjected to analysis for active principles and were found to contain 7 kinds of phytoecdysones such as ecdysone, ponasterone A, pterosterone, ecdysterone, shidasterone, lemmasterone, and ponasteroside A.[1]

Taiwan is an island lying off the east coast of Asia, whose situation in the subtropical zone gives a warm temperate climate and heavy rainfall. Under these physical features, therefore, the flora of Taiwan is very rich, containing approximately 600 species of ferns.[4] Our next endeavor was then directed towards the screening of ferns of Formosan origin for the insect-moulting hormone activity. Since there has been some difficulty in collecting the plant material, a limited number of ferns, *i.e.*, 115 species, 1 subspecies, and 3 varieties, have so far been made available. Some species were collected at various seasons and/or locations so that a toal of 164 specimens were screened. The screening method adopted for the present bioassay was the *Sarcophaga* test.[5] The present paper deals with the result of screening ferns indigenous to Taiwan for phytoecdysones.

As shown in Table I, the present work on Formosan ferns gave a result similar to that on Japanese ferns. Thus, the screening has led to the discovery of 64 species and 2 varieties having this activity towards the insect,[6] while 51 species, 1 subspecies, and 1 variety exhibited no activity, corroborating the previous conclusion that ferns are sources of this hormonal activity with a higher probability. Among the ferns having this activity, 18 species and 2 varieties exhibited remarkable responses. The previous observation that samples of the same species but of different collecting dates and/or locations sometimes exhibited different responses,[3] was also noted in the present investigation. In particular, remarkable variation in the activity shown by *Diplazium donianum* was again observed. As in the previous work,[3]

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2) Location: *a*) *Taipei, Taiwan*; *b*) *Aoba-yama, Sendai.*
3) H. Hikino, T. Okuyama, H. Jin, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **21**, 2292 (1973).
4) W.-C. Shieh, "Jye-lei-chih-wu (Ferns and Fern Allies)," Provincial Taiwan Chung-Hsing University, Tai-chung, 1969.
5) T. Ohtaki, R.D. Milkman, and C.M. Williams, *Biol. Bull.*, **135**, 332 (1968).
6) Taxa each of which contained at least one active sample were regarded as positive.

TABLE I. The Insect Moulting Hormone Activity of Formosan Ferns

Family[a]	Species[b]	Chinese name	Date	Location[c]	Activity[d]
Equisetaceae	*Equisetum ramosissimum* DESFONTAINES subsp. *debile* HAUKE	Tai-wan-mu-cheih	Aug	NT, Mt. Ho-huan	−
Lycopodiaceae	*Lycopodium cernuum* LINNÉ	Quoh-shan-long	Feb	TP, An-keng	−
			Sep	TP, Yang-ming-shan	−
	L. clavatum LINNÉ (*L. clavatum* LINNÉ var. *nipponicum* NAKAI)	Shih-song	Feb	NT, Chi-tou	−
	L. complanatum LINNÉ	Tieh-shua-tsu	Aug	NT, Mt. Ho-huan	−
	L. fargesii HERTER	Zuey-yeh-shih-song	Sep	TP, Yang-ming-shan	−
	L. fordii BAKER	Fu's-shih-song	Sep	TP, Yang-ming-shan	−
	L. phlegmaria LINNÉ	Chwei-chi-shih-song	Sep	TP, Yang-ming-shan	−
Selaginellaceae	*Selaginella delicatula* ALSTON	Chuan-yuan-chian-bor	Jan	TC, Ku-kuan	−
			Aug	TP, Mt. Muh-tsu	−
			Aug	TP, Wu-lai	−
			Sep	TP, Yang-ming-shan	−
	S. doederleinii HIERONYMUS	Sheng-qen-chian-bor	Feb	TP, An-keng	−
	S. mollendorffii HIERONYMUS	Yih-yeh-chian-bor	Feb	TP, Ken-tin	−
			Aug	TP, Mt. Muh-tsu	−
	S. pseudo-involvens HAYATA	Nii-mih-yeh-chian-bor	Aug	TP, Mt. Muh-tsu	−
	S. tamariscina SPRING	Wann-nen-song	Feb	TP, Wu-lai	−
Marattiaceae	*Angiopteris lygodiifolia* ROSENSTOCK	Quan-in-tzoh-lien	Feb	NT, Chi-tou	−
			Aug	TP, Mt. Muh-tsu	−
			Aug	TP, Wu-lai	−
			Sep	TP, Yang-ming-shan	−
Osmundaceae	*Plenasium banksiaefolium* PRESL (*Osmunda banksiaefolia* KUHN)	Tsu-chy-guo-yeh-tsu-chin	Feb	TP, Wu-lai	++
			Aug	TP, Mt. Muh-tsu	+++
Schizaeaceae	*Lygodium japonicum* SWARTZ	Hai-chin-sah	Aug	TP, Wu-lai	−
			Dec	TP, Chih-nan-kung	−
	L. microstachyum DESVAUX	Shia-yeh-hai-chin-sah	Aug	TP, Mt. Muh-tsu	−
	L. scandens SWARTZ var. *microphyllum* BONGARD	Shiau-yeh-hai-chin-sah	Feb	TP, An-keng	−
Gleicheniaceae	*Dicranopteris dichotoma* BERNHARDI (*D. linearis* UNDERWOOD)	Mang-chih	Aug	TP, Mt. Muh-tsu	−
			Aug	TP, Hsin-pei-tou	−
Hymenophyllaceae	*Vandenboschia auriculata* COPELAND	Ping-jye	Feb	TP, Wu-lai	−
Pteridaceae	*Adiantum capillus-veneris* LINNÉ	Tie-shian-jye	Aug	TP, Mt. Muh-tsu	−
	A. caudatum LINNÉ	Pian-yeh-tie-shian-jye	Aug	NT, Mt. Ho-huan	−
	A. diaphanum BLUME	Chang-wei-tie-shian-jye	Aug	NT, Mt. Ho-huan	−
	A. flabellulatum LINNÉ	San-yeh-tie-shian-jye	Feb	PT, Ken-tin	+++
	A. hispidulum SWARTZ	Mau-yeh-tie-shien-jye	Aug	NT, Lu-shan	++
	A. philippense LINNÉ	Pan-ye-sing-tie-shien-jye	Aug	NT, Lu-shan	++
	Cheilanthes argentea KUNZE (*Aleuritopteris argentea* FÉE)	Chang-ping-fun-pei-jye	Aug	NT, Mt. Ho-huan	−
	C. hirsuta METTENIUS	Mau-suei-mii-jye	Aug	NT, Lu-shan	+
	Cibotium barometz J. SMITH	Chin-gou-mau-jye	Jan	TC, Ku-kuan	−
			Aug	TP, Mt. Muh-tsu	−
	Coniogramme fraxinea DIELS	Chuan-yuan-fong-liau-jye	Feb	NT, Chi-tou	−
	C. intermedia HIERONYMUS (*C. fraxinea* DIELS var. *intermedia* C. CHRISTENSEN)	Hwa-fong-liau-jye	Feb	TP, An-keng	+
	C. japonica DIELS	Zu-pen-fong-liau-jye	Feb	NT, Chi-tou	−
	Dennstaedtia scabra MOORE	Wan-jye	Aug	TP, Yang-ming-shan	−
	D. scandens MOORE	Tsyh-ping-wan-jye	Feb	TP, Wu-lai	−
	Histiopteris incisa J. SMITH	Lih-jye	Aug	TP, Mt. Muh-tsu	−
			Aug	TP, Yang-ming-shan	−
	Hypolepis alte-gracillima HAYATA	Tai-wan-ji-jye	Sep	TP, Yang-ming-shan	−
	H. punctata METTENIUS	Ji-jye	Sep	TP, Yang-ming-shan	−
	Microlepia hookeriana PRESL	Huk's-lin-gai-jye	Aug	TP, Yang-ming-shan	+
	M. marginata C. CHRISTENSEN	Pian-yuan-lin-gai-jye	Feb	TP, Ken-tin	+
			Aug	TP, Yang-ming-shan	−
	M. pilosula PRESL	Hor-mau-lin-gai-jye	Aug	TP, Hsin-pei-tou	−
			Aug	TP, Wu-lai	−
	M. speluncae MOORE	Zeh-dai-lin-gai-jye	Aug	TP, Hsin-pei-tou	++
			Sep	TP, Yang-ming-shan	+
	M. strigosa PRESL	Tsu-mau-lin-gai-jye	Sep	TP, Yang-ming-shan	−
	Onychium japonicum KUNZE	Zu-pen-chin-fun-jye	Jun	TY, Mt. Chiao-pan	−
	Pityrogramma calomelanos LINK	Fun-yeh-jye	Apr	TC, Ku-kuan	−
	Pteridium aquilinum KUHN var. *latiusculum* UNDERWOOD	Jye	Jan	TC, Ku-kuan	+++
	Pteris dispar KUNZE	Tien-tsau-fong-wei-jye	Feb	TP, Yang-ming-shan	++
	P. ensiformis BURME	Jian-yeh-fong-wei-jye	Jan	TC, Ku-kuan	−
			Aug	TP, Hsin-pei-tou	+
			Dec	TP, Chih-nan-kung	−
	P. fauriei HIERONYMUS (*P. quadriaurita* RETZIUS)	Fuh's-fong-wei-jye	Jan	TC, Ku-kuan	++
			Feb	TP, Wu-lai	++
			Aug	TC, Ku-kuan	++
	P. longipes DON	Ferng-lai-fong-wei-jye	Jan	TC, Ku-kuan	−
	P. semipinnata LINNÉ	Pan-pian-yii-lieh-fong-wei-jye	Aug	TP, Mt. Muh-tsu	−
			Aug	TP, Hsin-pei-tou	+
			Dec	TP, Chih-nan-kung	+
	P. tripartita SWARTZ	San-jau-fong-wei-jye	Aug	NT, Tien-chi	+
	P. vittata LINNÉ	Lin-gai-fong-wei-jye	Jan	TC, Ku-kuan	+
	P. wallichiana AGARDH	Wa's-fong-wei-jye	Feb	TP, Wu-lai	++
	Sphenomeris chusana COPELAND (*S. chinensis* MAXON)	Wu-jye	Feb	TP, An-keng	−
			Aug	TP, Mt. Muh-tsu	−
Davalliaceae	*Araiostegia parvipinnula* COPELAND	Tai-wan-shiau-mo-gai-jye	Jul	CY, Mt. A-li	++
	A. perdurans COPELAND	Shiau-mo-gai-jye	Jul	IL, Mt. Tai-ping	+++
	Davallia formosana HAYATA	Dah-yeh-gu-suei-pu	Jan	TC, Ku-kuan	++
	D. mariesii MOORE	Hai-jou-gu-suei-pu	Aug	TY, Mt. Chiao-pan	++
			Aug	NT, Lu-shan	++
	Nephrolepis auriculata TRIMEN	Shen-jye	Aug	TP, Mt. Muh-tsu	−
			Aug	TP, Wu-lai	−

Family[a]	Species[b]	Chinese name	Date	Location[c]	Activity[d]
	N. biserrata SCHOTT	Chang-yeh-shen-jye	Feb	TP, An-keng	+
	N. hirsutula PRESL	Mau-yeh-shen-jye	Aug	TP, Yang-ming-shan	−
			Aug	TP, Wu-lai	−
Plagiogyriaceae	*Plagiogyria formosana* NAKAI	Tai-wan-liu-tzu-jye	Jul	IL, Mt. Tai-ping	++
Cyatheaceae	*Cyathea hancockii* COPELAND	Han's-sah-lo	Feb	TP, Yang-ming-shan	++
	(*Gymnosphaera denticulata* COPELAND)		Sep	TP, Yang-ming-shan	++
	C. lepifera COPELAND	Pi-tong-shuh	Jan	TC, Ku-kuan	+
	C. podophylla COPELAND	Quei-sah-lo	Aug	TP, Yang-ming-shan	+++
	C. mentteniana C. CHRISTENSEN et TERDIEU-BLOT	Tai-wan-shu-jye	Sep	TP, Yang-ming-shan	+
	C. spinulosa WALLICH	Tai-wan-sah-lo	Feb	TP, Wu-lai	+++
Aspidiaceae	*Abacopteris triphylla* CHING	Sin-ye-jye	Sep	TP, Yuang-tung-szu	++
	(*Cyclosorus triphyllus* TARDIEU -BLOT)				
	Acrophorus stipellatus MOORE	Nii-lin-mau-jye	Feb	NT, Chi-tou	+++
	Athyrium anisopterum CHRIST	Suh-tii-gai-jye	Aug	NT, Tien-chih	+++
	A. arisanense TAGAWA	A-li-shan-tii-gai-jye	Jul	IL, Mt. Tai-ping	+++
	A. atkinsonii BEDDOME	Yade's-tii-gai-jye	Sep	TP, Yang-ming-phan	+++
	Ctenitis subglandulosa CHING	Leh-mau-jye	Feb	TP, Wu-lai	−
	Cyclosorus acuminatus NAKAI	Mau-jye	Aug	TP, Mt. Muh-tsu	+
			Aug	TP, Wu-lai	+
			Sep	TP, Yang-ming-shan	+
	C. parasiticus FARWELL	Mih-mau-mau-jye	Feb	TP, An-keng	++
			Aug	TP, Mt. Muh-tsu	+
			Aug	TP, Wu-lai	+
	C. taiwanensis H. ITO	Tai-wan-mau-jye	Feb	TP, Wu-lai	++
	Dictyoline griffithii MOORE	We's-sen-jye	Feb	TP, Wu-lai	+++
	var. *wilfordii* MOORE				
	(*D. griffithii* MOORE var. *pinnatifida* BEDDOME)				
	Diplazium dilatatum BLUME	Koang-yeh-jih-chy-shuang-gai-jye	Aug	TP, Yang-ming-shan	+
	(*Athyrium maximum* COPELAND)		Sep	TP, Yang-ming-shan	+
	D. donianum TARDIEU-BLOT	Shi-ping-shuang-gai-jye	Jan	TP, Yang-ming-shan	+++
	(*Athyrium aphanoneuron* OHWI)		Jul	TP, Yang-ming-shan	+
			Aug	TP, Yang-ming-shan	+++
	D. esculentum SWARTZ	Quoh-gou-tsai-jye	Feb	TP, An-keng	+++
	(*Athyrium esculentum* COPELAND)				
	D. kawakamii HAYATA	Chuan-shang's-shuang-gai-jye	Sep	TP, Yang-ming-shan	−
	(*Athyrium procerum* MILDE)				
	D. mettenianum C. CHRISTENSEN	Sheng-shan-shuang-gai-jye	Aug	TP, Yang-ming-shan	+
	(*Athyrium mettenianum* OHWI)				
	Dryopteris erythrosora O. KUNTZE	Hong-bau-lin-mau-jye	Sep	TP, Yang-ming-shan	++
	D. lepidopoda HAYATA	Hou-yeh-lin-mau-jye	Aug	NT, Mt. Ho-huan	++
	D. scottii CHING	Shu's-lin-mau-jye	Feb	NC, Chi-tou	−
	D. sieboldii O. KUNTZE	Ding-yeh-lin-mau-jye	Jan	TC, Ku-kuan	−
	D. varia O. KUNTZE	Nan-hai-lin-mau-jye	Feb	TP, Wu-lai	++
			Feb	TP, Wu-lai	++
	Lastrea beddomei BEDDOME	Shi-yeh-chin-shing-jye	Aug	NT, Tien-chih	++
	L. subochthodes TAGAWA	Sie-yeh-chin-shing-jye	Aug	NT, Mt. Ho-huan	+
	Phegopteris decursive-pinnata FÉE	Chyh-jour-jah-chin-shing-jye	Aug	TP, Mt. Muh-tsu	−
	(*Lastrea decursive-pinnata* J. SMITH)		Sep	TP, Yang-ming-shan	−
	P. subaurita TAGAWA	Al-yeh-jah-chin-shing-jye	Feb	TP, Wu-lai	++
			Aug	NT, Tien-chih	+
	Polystichopsis amabilis TAGAWA	Dah-fuh-yeh-al-jye	Aug	TP, Yang-ming-shan	−
	(*Rumohra amabilis* CHING)				
	P. quadripinnata SHIEH	Mau-bau-fuh-yeh-al-jye	Aug	NT, Tien-chih	−
	Polystichum nepalense C. CHRISTENSEN	Zuan-gu-al-jye	Aug	NT, Tien-chih	+
	P. parvipinnulum TAGAYA	Jien-yeh-al-jye	Jul	IL, Mt. Tai-ping	++
	Tectaria kwarenkoensis C. CHRISTENSEN	Hua-lian-san-tsa-jye	Feb	TP, Wu-lai	+
	(*T. kwarenkoensis* TAGAWA)				
	T. subtriphylla COPELAND	San-tsa-jye	Aug	PT, Ken-tin	++
Blechnaceae	*Blechnum niponicum* MAKINO	Zu-pen-wu-mau-jye	Jul	IL, Mt. Tai-ping	+++
	B. orientale LINNÉ	Wu-mau-jye	Aug	TP, Mt. Muh-tsu	−
			Aug	TP, Yang-ming-shan	+
			Sep	TP, Yang-ming-shan	−
			Dec	TP, Chih-nan-kung	−
	Woodwardia japonica SMITH	Zu-pen-gou-jii-jye	Aug	NT, Tien-chih	+++
	W. orientalis SWARTZ	Dong-fang-gou-jii-jye	Feb	PT, Ken-tin	+++
			Aug	TP, Mt. Muh-tsu	+++
			Aug	TP, Hsin-pei-tou	+++
Aspleniaceae	*Asplenium laserpitiifolium* LAMARCK	Dah-yeh-hei-ping-tie-jiau-jye	Feb	TP, Wu-lai	++
	A. loriceum CHRIST	Nan-hai-tie-jiau-jye	Feb	TP, Wu-lai	−
	A. nidus LINNÉ	Tai-wan-shan-su-hua	Jan	TC, Ku-kuan	++
	A. normale DON	Sheng-ya-tie-jiau-jye	Mar	NT, Lu-shan	−
	A. trichomanes LINNÉ	Tie-jiau-jye	Aug	NT, Tien-chih	++
Polypodiaceae	*Colysis elliptica* CHING	Tuo-yuan-shieu-jye	Jan	TC, Ku-kuan	+
	C. wrightii CHING	Lai's-shien-jye	Feb	TP, An-keng	+++
			Feb	TP, Wu-lai	+++
	Crypsinus hastatus COPELAND	San-yeh-fwu-jye	Jan	TC, Ku-kuan	+++
			Aug	TP, Wu-lai	+++
	C. quasidivaricatus COPELAND	Yi-shan-fwu-jye	Aug	NT, Tien-chih	++
	Lemnaphyllum microphyllum PRESL	Paw-shuh-jye	Feb	TP, Wu-lai	++
	Microsorium buergerianum CHING	Po's-shing-jye	Aug	TP, Yang-ming-shan	−
	M. fortunei CHING	Fwu's-shing-jye	Jan	TC, Ku-kuan	−
	M. membranifolium CHING	Mo-yeh-shing-jye	Feb	NT, Chi-tou	−
	Polypodium niponicum METTENIUS	Shwei-long-gu	Mar	NT, Lu-shan	−
	P. taiwanianum HAYATA	Jian-yeh-shwei-long-gu	Jul	IL, Mt. Tai-ping	+
	Pseudodrynaria coronans CHING	Yai-jiang	Jan	NT, Chi-tou	++
	Pyrrosia lingua FARWELL	Shih-woei	Feb	NT, Chi-tou	−
			Sep	TP, Yang-ming-shan	+
	P. polydactylis CHING	Tsi-yeh-shih-woei	Jul	TY, Mt. Chiao-pan	+++
Vittariaceae	*Vittaria anguste-elongata* HAYATA	Ji-shu-dai-jye	Aug	TC, Ku-kuan	−

a) The families are arranged in the sequence presented by J. Ohwi ("Flora of Japan" Shibundo, Tokyo, 1972), and genera and species are given in alphabetical order within the families.
b) The main source of the nomenclature is the manual of Shieh,[4] and certain different taxa have been supplemented. No attempt was made to apply to Formosan ferns about the same standards of taxonomic recognition accorded comparable taxa in the previous treatment of Japanese ferns,[3] though many inconsistencies are doubtless detectable. Since, in comparison of the present nomenclatures of the Formosan ferns with the previous ones of the Japanese ferns, there are consequently differences in several species, synonyms recorded in the previous work are also included in parentheses in order to avoid confusion.
c) Abbreviations: CY=Chia-yi, IL=I-lan, NT=Nan-tou, PT=Ping-tung, TC=Tai-chung, TP=Tai-pai, TY=Tao-yuan
d) Assays were carried out on *Sarcophaga peregrina*.[5] A test solution containing a methanol extract from a dried plant meterial (10 mg) was injected into each isolated larval abd omen. The results are expressed in terms of the activity indexes which are corresponding to the following average per cent puparium formation: $-=0$, $0<+\leqq 20$, $20<\#\leqq 40$, $40<\#\#\leqq 60$, $60<\#\#\#\leqq 80$, and $80<\#\#\#\#\leqq 100\%$.

it was again found that families such as Equisetaceae, Lycopodiaceae, Selaginellaceae, Marattiaceae, and Schizaeaceae, whose degree of differentiation is considered to be low from the point of taxonomy, do not contain active species, and yet, among these poorly differentiated families, Osmundaceae contains a strongly active species. Although the number of plant materials presently screened is rather limited for drawing any definite conclusion, it may be said that, as has been noted before, phytoecdysones are distributed in Cyatheaceae, Aspidiaceae, and Polypodiaceae with a high probability, though these still include some species showing no activity.

For more complete understanding of the distribution of phytoecdysones in Formosan ferns, further investigation is now under way.

Experimental

Materials——A total of 164 specimens, all growing wild, were selected. Samples from whole plants were submitted to the following extraction for bioassay. Sampling from a large fern was so made that the sample was representative of the whole plant, as completely as possible.

Preparation of Test Solutions——A dried plant material (1 g) in MeOH (20 ml) was heated under reflux for 2 hr. After filtration, the extract was concentrated under a reduced pressure to give a dried residue which was dissolved in 10% aqueous EtOH (1 ml), filtered, and the filtrate was used as the test solution.

Biological Assay——Assays were made with larvae of *Sarcophaga peregrina* as described by Ohtaki, *et al.*[5] The final instar larvae, which at 24 hr after the ligation formed puparia at their anterior ends but did not pupate behind ligature, were employed as the test insects.

An aliquot of the test solution (10 μl) was injected into each isolated larval abdomen which was kept at 26°. Ten individuals were used for each sample. The assays were evaluated 24 hr after injection when each abdomen was scored as having undergone complete, marked, slight, or no puparium formation; these responses being equated to 100, 75, 50, and 0%, respectively, for the purpose of calculating the weighed average percent puparium formation. The results in Table I are expressed in terms of the activity indices as $-$, $+$, $\#$, $\#\#$, $\#\#\#$, and $\#\#\#\#$, these indices corresponding to the following average percent puparium formation: $-=0$, $0<+\leqq 20$, $20<\#\leqq 40$, $40<\#\#\leqq 60$, $60<\#\#\#\leqq 80$, and $80<\#\#\#\#\leqq 100\%$.

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