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Screening Results of Plants for Phytoecdysones[1]

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1056 species of plants and 351 crude drugs were screened by the *Chilo* dipping test looking for the phytoecdysones. 13 species of Pteridophyta and 23 species of Gymnospermae and Angiospermae were newly found to show the insect-moulting activity. The chemotaxonomical relations were discussed.

Since the discovery of the four ponasterones A, B, C and D from plants in 1966 by Nakanishi and co-workers,[3] many compounds exhibiting the moulting hormone activity have been isolated from the plant kingdom, *e.g.*, ecdysterone,[4a] inokosterone,[4b] cyasterone,[4c] polypodine B,[4d] pterosterone,[4e] ecdysone,[5] makisterone A,[6] B, C and D.[7]

Some of the plants as a source of ecdysones are far superior to insects and crustacea not only for the much higher content but also for the simplicity of extractions.[8] In this connection, an increasing interest has been focused on plants during the past year.

Shortly after the discovery of ponasterones[3] and inokosterones[4b] an extensive survey for phytoecdysones was commenced in order to look for new active compounds and rich sources, and to see whether any chemotaxonomical relations were present.

The timely development of the rapid and simple *Chilo* dipping test[9] was remarkable for achievement of the extensive screening. The sources were chosen so that they were representatives of a wide taxonomical distribution; in addition 351 crude drugs were also screened. An independent screening of crude drugs and plants (*ca.* 180) has already been published by Takemoto and co-workers.[10]

1) This work was presented at the monthly meeting of the Pharmaceutical Society of Japan, the Kinki-Branch, Osaka, Feb. 17, 1968, this comprises "Studies on crude drugs and oriental crude drug preparations by bioassay 23".
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3) K. Nakanishi, M. Koreeda, S. Sasaki, M.L. Chang, and H.Y. Hsu, *Chem. Commun.*, **1966**, 915.
4) *a*) M.N. Galbraith and D.H. S. Horn, *Chem. Commun.*, **1966**, 905; *b*) T. Takemoto, S. Ogawa, and N. Nishimoto, *Yakugaku Zasshi*, **87**, 325 (1967); *c*) T. Takemoto, Y. Hikino, K. Nomoto, and H. Hikino, *Tetrahedron Letters*, **1967**, 3191; *d*) J. Jizba, V. Herout, and F. Šorm, *Tetrahedron Letters*, **1967**, 5139; *e*) T. Takemoto, S. Arihara, Y. Hikino, and H. Hikino, *Tetrahedron Letters*, in press [*Metabolism and Disease*, **5**, 44 (1968)].
5) J.N. Kaplanis, M.J. Thompson, W.E. Robbins, and B.M. Bryce, *Science*, **157**, 1436 (1967); G. Heinrich and H. Hoffmeister, *Experientia*, **23**, 995 (1967).
6) S. Imai, M. Hori, S. Fujioka, E. Murata, M. Goto, and K. Nakanishi, *Tetrahedron Letters*, in press.
7) S. Imai, S. Fujioka, E. Murata, Y. Sasakawa, and K. Nakanishi, *Tetrahedron Letters*, in press.
8) S. Imai, S. Fujioka, K. Nakanishi, H. Koreeda, and T. Kurokawa, *Steroids*, **10**, 557 (1967).
9) Y. Sato, M. Sakai, S. Imai, and S. Fujioka, *Appl. Ent. Zool.*, **3**, 49 (1968).
10) T. Takemoto, S. Ogawa, N. Nishimoto, S. Arihara, and K. Bue, *Yakugaku Zasshi*, **87**, 1414 (1967).

Experimental

Material——A total of 1056 species were collected during the period of October 1966 to October 1967, mainly at the Kyoto Herbal Garden, Takeda Chemical Industries, Ltd. (Table I). Whenever possible the various parts of the respective species were tested separately, so that a total of 1845 samples were screened. Most of the 351 crude drugs were purchased on the market but some were from the Takeda collection.

Table I. Tested Plants

Pteridophyta	47 species (24 genera, 7 families)
Gymnospermae	42 species (24 genera, 9 families)
Angiospermae	
Monocotyledoneae	118 species (93 genera, 22 families)
Dicotyleconeae	
Archichlamydeae	521 species (373 genera, 110 families)
Sympetale	328 species (224 genera, 38 families)
Total	1056 species (738 genera, 186 families)

Preparation of Test Solutions——Test solutions were prepared by adding 15 ml of methanol to 3 g of fresh material or crude drug, homogenizing the mixture at room temperature, filtering the homogenate and concentrating the filtrate. The dried residue was dissolved in methanol to make a 4% solution and 0.5 ml of this was employed as a test solution.

Biological Assay——The ligated final instar larvae of *Chilo suppressalis* which did not pupate after 24 hr were employed as test insects. Five insects were dipped for 5—10 sec in each solution and kept at $28 \pm 1°$ for 48 hr; each test was repeated 3 times. The results are expressed in terms of the number of insects which underwent sclerotization and tanning, *e.g.*, if 3 insects pupated this is expressed as 3/5.

Table II. Plants showing the Insect-Moulting Activity

Plant	Part	Activity[a]
Pteridophyta		
Polypodiaceae		
Adiantum capillus-veneris ("Horaishida")	L, S	2/5
Athyrium niponicum[b] ("Inuwarabi")	L, S	5/5
	Rh	3/5
A. Vidalii[b] ("Yamainuwarabi")	L, S	1/5
A. yokoscense ("Hebinonegoza")	Rh	5/5
Cornopteris decurrenti-alata ("Shikechishida")	Rh	3/5
Cyclosorus acuminatus ("Hoshida")	L, S	2/5
Dryopteris erythrosora ("Benishida")	L, S	2/5
D. Thelypteris[b] ("Himeshida")	L	4/5
	Rh	5/5
D. tokyoensis ("Tanihego")	L, S	1/5
D. varia var. setosa ("Itachishida")	L, S	1/5
Lastrea decursive-pinnata ("Gezigezishida")	L, S	1/5
L. oligophlebia ("Himewarabi")	L, S	1/5
	Rh	2/5
L. japonica[b] ("Hariganewarabi")	Rh	2/5
Lemmaphyllum microphyllum[b] ("Mamezuta")	L, S	3/5
Lepisorus Thunbergianus[b] ("Nokishinobu")	L	1/5
Polypodium japonicum ("Oshakuzidenda")	Rh	3/5
Polysticum polyblepharum[b] ("Inode")	L, S	2/5
P. tripteron[b] ("Jumonjishida")	Rh	2/5
Pteris cretica ("Obainomotoso")	L, S	1/5
P. multifida[b] ("Inomotoso")	L, S	1/5
Struthiopteris niponica[b] ("Shishigashira")	L	4/5
S. castanea ("Miyamashishigashira")	W	2/5
Osmundaceae		
Osmunda japonica[b] ("Zenmai")	Rh	4/5
Lycopodiaceae		
Lycopodium serratum ("Togeshiba")	L, S	2/5

a) *Chilo* dipping test L: Leaves S: Stems Rh: Rhizomes W: Whole
b) Those were reported by T. Takemoto, *et al.*[10] The Japanese names are indicated in parentheses.

Results

Table II lists the active Pteridophyta species while Table III lists species other than the Pteridophyta. Of the total of 24 species of Pteridophyta showing clear activity, 11 have previously been described by Takemoto and co-workers,[10] and therefore 13 are newly discovered species.

Table III. Plants showing the Insect-Moulting Activity

Plant	Part	Activity[a]
Gymnospermae		
Taxaceae		
Taxus cuspidata[b] ("Ichii")	L	4/5
T. cuspidata var. nana[c] ("Kyaraboku")	L	2/5
Torreya nucifera[c] ("Kaya")	L	1/5
Podocarpaceae		
Podocarpus macrophyllus[b] ("Inumaki")	L	5/5
P. chinensis[b] ("Rakanmaki")	L	5/5
Cephalotaxaceae		
Cephalotaxus haringtonia ("Inugaya")	L	1/5
Cupressaceae		
Cupressus fubris	L	1/5
Angiospermae		
Monocotyledoneae		
Liliaceae		
Paris tetraphylla ("Tsukubaneso")	L, S	4/5
Trillium Smallii ("Enreiso")	Rh	3/5
T. Tschonskii ("Miyamaenreiso")	Rh	3/5
Iridaceae		
Iris crocea	L	3/5
Dicotyledoneae		
Archichlamydeae		
Amaranthaceae		
Achyranthes japonica[c] ("Inokozuchi")	R	5/5
A. bidentata[c] ("Toinokozuchi")	R	5/5
Caryophyllaceae		
Gypsophila perfoliata	L	2/5
Lychnis miqueliana ("Fushigurosenno")	R	4/5
L. chalcedonica ("Amerikasenno")	L, S R	3/5 2/5
Ranunculaceae		
Helleborus niger ("Kurisumasurozu")	R	5/5
Malvaceae		
Hibiscus splendens	R	1/5
Napaea dioica	L	3/5
Stachyuraceae		
Stachyurus praecox ("Kibushi")	L Ba F	5/5 4/5 5/5
S. praecox var. Matsuzakii ("Hachijokibushi")	L, S R	5/5 1/5
Cistaceae		
Halimium halimifolium	L, S	3/5
Sympetalae		
Labiatae		
Ajuga decumbens ("Kiranso")	L, S R	3/5 4/5
A. incisa ("Hiiragiso")	L, S	3/5
A. nipponensis ("Junihitoe")	L, S R	4/5 4/5
Solanacea		
Withania furtescens	R	3/5
Compositae		
Artemisia Dracunculus ("Esutoragon")	L. S	2/5
Chrysanthemum indicum ("Kangiku")	L	1/5
C. Makino ("Ryunogiku")	L, S R	3/5 1/5
C. morifolium var. sinense ("Kiku")	L	3/5

a) *Chilo* dipping test b) Those were reported by S. Imai, *et al.*[8] c) Those were reported by T. Takemoto, *et al.*[4b,10,11] The Japanese names are indicated in parentheses.

Of the active species belonging to Gymnospermae and Angiospermae, 23 are new and 7 have been described earlier.[4b,8,10,11] The crude drugs were not promising as a source of phytoecdysones, and weak activity was observed in only three, *i.e.*, Zanthoxyli Fructus (蜀椒), Equiseti Herba (木賊), and Platycodi Radix (桔梗根).

11) T. Takemoto, Y. Hikino, H. Jin, and H. Hikino, *Yakugaku Zasshi*, **88**, 359 (1968).

Discussion

The plants were selected so that at least one species from as many as possible genera were included, and also care was taken not to concentrate on a few closely related plants at the outset; when an active species was detected, taxonomically close genera were tested as well. In Table IV, the active species discovered so far are listed in taxonomical order. The following deductions can be made from these findings.

Table IV. Plant–Families showing Insect–Moulting Activity

Pteridophyta
Psilotaceae
Lycopodiaceae[a)]
Selaginellaceae
⋮
Marattiaceae
Osmundaceae[b)]
Schizaeaceae
Gleicheniaceae[b)]
Hymenophyllaceae
Plagiogyriaceae[b)]
Cyatheaceae
Polypodiaceae[b)]
Parkeriaceae
⋮
Gymnospermae
Cycadaceae
Ginkgoaceae
Taxaceae[b)]
Podocarpaceae[b)]
Cephalotaxaceae[b)]
Pinaceae[b)]
Taxodiaceae[b)]
Cupressaceae[b)]
Ephedraceae
Angiospermae
Monocotyledoneae
⋮
Triuridaceae
Gramineae[b)]
Cyperaceae
⋮
⋮
Stemonaceae
Liliaceae[b)]
Amaryllidaceae
Dioscoreaceae
Iridaceae[b)]
Musaceae[b)]
Zingiberaceae
⋮
Dicotyledoneae
Archichlamydeae
⋮
Ulmaceae
Moraceae[b)]
Urticaceae
⋮
Polygonaceae
Chenopodiaceae[b)]
Amaranthaceae[b)]
Nyctaginaceae[b)]
Cynocrambaceae
Phytolaccaceae[b)]
Aizoaceae
Portulacaceae
Basellaceae
Caryophyllaceae[a)]
Magnoliaceae
⋮
Ceratophyllaceae
Ranunculaceae[a)]
Lardizabalaceae[b)]
Berberidaceae
⋮
Papaveraceae
Capparidaceae[b)]
Cruciferae
⋮
Platanaceae
Rosaceae[b)]
Leguminosae[b)]
Oxalidaceae
⋮
Zygophyllaceae
Rutaceae[b)]
Simaroubaceae
⋮
Tiliaceae
Malvaceae[a)]
Stercuriaceae
⋮
Tamaricaceae
Cistaceae[a)]
Violaceae
Flacourtiaceae
Stachyuraceae[a)]
Passifloraceae
⋮
Sympetalae
⋮
Polemoniaceae
Boraginaceae[b)]
Verbenaceae[b)]
Labiatae[b)]
Solanaceae[a)]
Scrophulariaceae
⋮
Cucurbitaceae
Campanulaceae[a)]
Compositae[a)]

a) new *b*) reported already

i) The probability of finding active phytoecdysones is high in Pteridophyta and Gymnospermae.

ii) Of the Angiospermae plants, those which belong to or are close to the Amaranthaceae (belonging to Archichlamydeae) and the Verbenaceae (belonging to Sympetalae) appear to be good sources.

iii) There are cases in which closely related genera of a family exhibit activity such as the genera *Adiantum*, *Athyrium*, *Dryopteris*, *Lastrea* and *Polypodium* of Polypodiaceae, and the genera *Arthemisia* and *Chrysanthemum* of Compositae. On the other hand, there are cases where one genus is active while another in the same family is not. For example, the genus *Lychnis* of Caryophyllaceae is active but the genera *Cucubalus*, *Dianthus*, *Saponaria* and *Silene*

are not. Similarly, whereas the genus *Ajuga* of Labiatae is active, 20 other genera such as *Agastache, Clinopodium, Coleus, Isodon, Leonurus, etc.* are inactive. Therefore the probability of finding an active plant from the same family is not necessarily high.

iv) The possibility of finding an active principle from plants belonging to the same genus is high. For example, several species of *Achyranthes, Stachyurus, Ajuga, Chrysanthemum, etc.* are all positive. However, in the case of *Helleborus niger*, other species belonging to the same genus are negative. It appears therefore that the results obtained to date are insufficient to permit one to judge on taxonomical grounds whether a plant contains a phytoecdysone or not. These polyhydroxy steroids are widely distributed in the plant kingdom but are not as ubiquitous as sugars and amino acids.

v) Activity was detected in the bark, leaves and fruits of *Trillium Smallii*. Although there are exceptions, the distribution of phytoecdysones in a particular plant is generally widespread.

vi) It is to be noted that the active plants discovered so far are limited to perennial herbaceous plants and woods, and none belonging to annual herbaceous plants have been found to be active.

Conclusion

According to our previous experience in screening plants and crude drugs for biological activity, the chance of finding activity from the latter is higher than that from the former. However, in the present screening for phytoecdysones, the fresh plants are sources giving higher probabilities. Especially, it was thought that crude drugs other than Achyranthis Radix[4b] and Cyathula Radix[4c] would be suitable sources but this was not the case.

In summary, the screening of 1056 species has shown activity in the following plants (including those already reported.)

Pteridophyta: A total of 24 species such as *Adiantum capillus-veneris, Athyrium yokoscense.*

Gymnospermae and Angiospermae: A total of 30 species, such as *Podocarpus macrophyllus, Taxus cuspidata, Trillium Smallii, Lychnis miqueliana, Helleborus niger, Stachyurus praecox, Ajuga decumbens,* and *Chrysanthemum morifolium* var. *sinense.*

The number of plants screened is only a small fraction of existing species, but it is hoped that future studies will enable one to deduce interesting results from a chemotaxonomical viewpoint. The active principles from the positive plants are being extracted and characterized One of the outcomes is the discovery and structural elucidation of the four makisterones, A,[6] B, C and D[7] from *P. macrophyllus* which are C_{28} and C_{29} steroids.

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