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Isolation of Phytoecdysones from Japanese Ferns. I[1]

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In a preceding paper,[3] we have reported a systematic screening of Japanese ferns for steroids having insect moulting hormone activity, phytoecdysones, by means of bioassay, the *Sarcophaga* test. Thus, screening of the total of 283 species, 39 varieties, and 1 form of ferns belonging to 76 genera and 20 families has led to the discovery of 170 species, 22 varieties, and 1 form belonging to 48 genera and 13 families which exhibit the activity towards the insect. It is now evident that Pteridophyta especially show the activity in much higher frequency than the other plant groups. Therefore, our next interest has naturally been focused on survey of the active ferns in order to seek new phytoecdysones and, if possible, to clarify any taxonomical relationship present. The present paper summarizes our work on the isolation of phytoecdysones from Japanese ferns.[4]

The plant materials were extracted with methanol. The concentrates obtained after removal of the solvent were extracted with ethyl acetate. Phytoecdysones were isolated by repeated chromatography of the ethyl acetate extracts over neutral alumina and silica gel followed by crystallization.

So far, 16 species have been subjected to analysis and, as summarized in Table I, revealed to contain 7 kinds of phytoecdysones in which 4 kinds are new substances.

From the present findings coupled with the previous knowledge, the following conclusion can be drawn. Ecdysterone was isolated from all the ferns tested except for *Hicriopteris glauca* and *Pteridium aquilinum* var. *latiusculum*, in which, however, its presence was indicated by means of thin-layer chromatography of the extracts. Therefore, ecdysterone may properly be considered to be the most widely occurring phytoecdysone. Ecdysone, the first known zooecdysone, has so far been isolated only from ferns as far as the plant kingdom is concerned.[5] The present work added further examples. Ponasterone A which has hitherto been isolated only from gymnosperms,[5] was isolated also from fern sources in this work. Ponasterone A has not been found in the animal sources. Therefore, ecdysterone is most likely biosynthesized only through ecdysone in animals. On the other hand, however, the common occurrence of ecdysone, ponasterone A, and ecdysterone in certain ferns, *Osmunda asiatica* and *O. japonica*,

1) This paper constitutes Part XVIII in the series on Steroids. Part XVII: H. Hikino, T. Okuyama, H. Jin, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), 21, 2292 (1973).
2) Location: *Aoba-yama, Sendai.*
3) H. Hikino, T. Okuyama, H. Jin, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **21**, 2292 (1973).
4) Part of the material contained herein has been outlined in preliminary communications; T. Takemoto, Y. Hikino, T. Arai, M. Kawahara, C. Konno, S. Arihara, and H. Hikino, *Chem. Pharm. Bull.* (Tokyo) **15**, 1816 (1967); T. Takemoto, Y. Hikino, T. Arai, C. Konno, S. Nabetani, and H. Hikino, *ibid.*, **16**, 759 (1968); T. Takemoto, S. Arihara, Y. Hikino, and H. Hikino, *ibid.*, **16**, 762 (1968); T. Takemoto, Y. Hikino, H. Jin, T. Arai, and H. Hikino, *ibid.*, **16**, 1636 (1968); T. Takemoto, T. Okuyama, S. Arihara, Y. Hikino, and H. Hikino, *ibid.*, **17**, 1973 (1969).
5) *cf.*, H. Hikino and Y. Hikino, "Fortschritte d. Chem. Org. Naturst.," Vol. 28, ed. by W. Herz, H. Grisebach, and A.I. Scott, Springer-Verlag, Wien, 1970, pp. 256—312.

TABLE I. Phytoecdysones from Japanese Ferns

Family	Species	Phytoecdysone[a]						
		Ec	Pn	Pt	Es	Sh	Le	Po
Osmundaceae								
	Osmunda asiatica	○	○		○			
	O. japonica	○	○		○			
Gleicheniaceae								
	Hicriopteris glauca		○					
Pteridaceae								
	Pteridium aquilinum var. *latiusculum*		○	○				○
Aspidiaceae								
	Athyrium niponicum		○	○	○			
	A. yokoscense				○			
	Lastrea japonica				○			
	L. thelypteris			○	○			
	Matteuccia struthiopteris				○			
	Onoclea sensibilis	○	○	○	○			
Blechnaceae								
	Blechnum amabile		○		○			
	B. niponicum		○		○	○		
Polypodiaceae								
	Crypsinus hastatus				○			
	Lemmaphyllum microphyllum	○		○	○		○	
	Neocheiropteris ensata	○			○			
	Pleopeltis thunbergiana				○			

a) Abbreviations: Ec=ecdysone, Pn=ponasterone A, Pt=pterosterone, Es=ecdysterone, Sh=shidasterone, Le=lemmasterone, and Po=ponasteroside A

as presently found suggests that two alternative biosynthetic pathways from a hypothetical precursor, 25-deoxy-ecdysone, to ecdysterone *via* ecdysone and ponasterone A are operating in the plants. Pterosterone[6] is a new phytoecdysone which has only been isolated from the ferns presently investigated. While pterosterone was thought to be an indigenous substance to the Pteridophyta, one exception, *Vitex megapotamica*, was later reported to contain also the same substance.[5] Another novel phytoecdysone shidasterone,[7] which has been isolated from the fern *Blechnum niponicum*, is an interesting substance in that it is the example having no 20(*R*), 22(*R*)-dihydroxyl moiety and still shows an insect moulting hormone activity in the bioassay, the *Sarcophaga* test. Among the C_{27} phytoecdysones with the cholestane skeleton, lemmasterone[8] is the only exception possessing the C_{29} stigmastane skeleton as isolated from Japanese ferns. Lemmasterone is known to occur, other than this fern, in gymnosperms, *Podocarpus elatus* (as podecdysone A)[5] and *P. macrophyllus* (as makisterone C).[5] Since the fern *Lemmaphyllum microphyllum* contains not only lemmasterone but also the other C_{27} congeners, the multiplicity of the biosynthetic mechanism in this plant is suggested. Ponasteroside A,[9] which has been isolated from the fern *Pteridium aquilinum* var. *latiusculum*, is considered to be a unique example of a glycoside of a phytoecdysone, ponasterone A 3-β-D-glucoside. Of interest biologically is the fact that ponasteroside A still shows a strong insect moulting hormone activity in the *Sarcophaga* test. This phenomenon provides an interesting subject in the biochemical field since it has been considered to be one possibility that insect

6) T. Takemoto, S. Arihara, Y. Hikino, and H. Hikino, *Tetrahedron Letters*, **1968**, 375.
7) T. Takemoto, Y. Hikino, T. Okuyama, S. Arihara, and H. Hikino, *Tetrahedron Letters*, **1968**, 6095; H. Hikino, T. Okuyama, S. Arihara, Y. Hikino, T. Takemoto, H. Mori, and K. Shibata, to be published.
8) T. Takemoto, Y. Hikino, T. Arai, and H. Hikino, *Tetrahedron Letters*, **1968**, 4061.
9) T. Takemoto, S. Arihara, and H. Hikino, *Tetrahedron Letters*, **1968**, 4199; H, Hikino, S. Arihara, and T. Takemoto, *Tetrahedron*, **25**, 3909 (1969).

moulting hormones may be detoxicated in insects by formation of a glycoside linkage. Although we have been of the opinion that glycosides of phytoecdysones are also widely distributed in the plant kingdom, ponasteroside A is still the only known example.

The high accelerating effects on the protein anabolism in mouse liver of the phytoecdysones isolated from Japanese ferns have been reported elsewhere.[10]

Experimental

Materials——Collecting dates and locations of the plant samples are described below.

General Extraction Procedure——A whole plant material was heated under reflux with MeOH for 10 hr. The extraction was repeated 5 times, and the combined MeOH solution was concentrated to give the MeOH extract. After the extract was diluted with water, the mixture was continuously extracted with AcOEt. The AcOEt solution was concentrated and submitted to chromatography on neutral alumina. Fractions eluted with AcOEt–MeOH which contained all phytoecdysones were collected and subjected to repeated chromatography on silica gel. Fractions eluted with AcOEt–MeOH or $CHCl_3$–MeOH and contained respective phytoecdysone were combined and crystallized from MeOH (in the case of ponasterone A) or MeOH–AcOEt (in the cases of the other phytoecdysones). Identification of each known phytoecdysone was carried out by means of thin-layer chromatography (TLC) (*Rf* values in various developing solvent systems and characteristic color reactions with H_2SO_4 spray reagent), mp, the infrared (IR) spectrum, and, if possible, the nuclear magnetic resonance (NMR) spectrum.

The structures of the new phytoecdysones have been elucidated elsewhere.[6-9]

Properties of Phytoecdysones isolated from Ferns——From *Osmunda asiatica* Ohwi (Dec., Mt. Izumi, Rikuzen): Ecdysone, mp 243—244°; ponasterone A, mp 260—261°, ecdysterone, mp 242—243°.
From *O. japonica* Thunberg (Dec., Mt. Izumi, Rikuzen): Ecdysone, mp 241.5—242°; ponasterone A, mp 266—268°; ecdysterone, mp 239—240°.
From *Hicriopteris glauca* St. John (Jul., Inagawa, Settsu): Ponasterone A, mp 269—270°.
From *Pteridium aquilinum* Kuhn var. *latiusculum* Underwood (Oct., Mt. Izumi, Rikuzen): Ponasterone A, mp 257—259°; pterosterone, mp 231.5—232.5°, ponasteroside A, mp 278—279.5°.
From *Athyrium niponicum* Hance (Nov., Sekiyama, Rikuzen): Ponasterone A, mp 267—268°; pterosterone, mp 218—219.5°, ecdysterone, mp 243.5—244°.
From *A. yokoscense* H. Christ (Jun., Mt. Izumi, Rikuzen): Ecdysterone, mp 244—245°.
From *Lastrea japonica* Copeland (Sep., Sendai, Rikuzen): Ecdysterone, mp 236—237°.
From *L. thelypteris* Bory (Oct., Ayashi, Rikuzen): Pterosterone, mp 229—230°; ecdysterone, mp 239—241°.
From *Matteuccia struthiopteris* Todaro (May, Mt. Banzan, Rikuzen): Ecdysterone, mp 228—231°.
From *Onoclea sensibilis* Linné (Sep., Ōkura, Rikuzen): Ecdysone, mp 237—239°; ponasterone A, mp 266—268°; pterosterone, mp 229—230°; ecdysterone, mp 240—242°.
From *Blechnum amabile* Makino (Sep., Okunikkawa, Rikuzen): Ponasterone A, mp 268—269.5°; ecdysterone, mp 245—246.5°.
From *B. niponicum* Makino (Oct., Okunikkawa, Rikuzen): Ponasterone A, mp 264—266°; ecdysterone, mp 243.5—244.5°; shidasterone, mp 257—258°.
From *Crypsinus hastatus* Copeland (Oct., Shizuoka, Suruga): Ecdysterone, mp 243—244°.
From *Lemmaphyllum microphyllum* Presl (Aug., Nachi, Kii): Ecdysone, mp 241—242°; pterosterone, mp 211—212.5°; ecdysterone, mp 245—249°; lemmasterone, mp 258—259°.
From *Neocheiropteris ensata* Ching (Aug., Nachi, Kii): Ecdysone, mp 241—242.5°, ecdysterone, mp 246—250°.
From *Pleopeltis thunbergiana* Kaulfuss (Oct., Minō, Settsu): Ecdysterone, mp 243—245°.

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10) T. Otaka, M. Uchiyama, T. Takemoto, and H. Hikino, *Chem. Pharm. Bull.* (Tokyo), **17**, 1352 (1969).