

Screening of Japanese Ferns for Phytoecdysones. I<sup>1)</sup>HIROSHI HIKINO, TORU OKUYAMA, HISANORI JIN,  
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Japanese ferns from 20 families, representing 76 genera, 283 species, 39 varieties, and 1 form, have been subjected to screening tests by means of bioassay for the presence of phytoecdysones. A total of 170 species, 22 varieties, and 1 form have been found to show the insect moulting hormone activity. The taxonomical relationship is discussed.

After the structural elucidation of ecdysone and ecdysterone, the insect moulting hormones (zooecdysones) from animal sources, steroids identical with or similar to the zooecdysones have unexpectedly been found to be present also in plant sources.<sup>3)</sup> This discovery has stimulated extensive surveys of plant materials in various parts of the world which have shown that active substances are widely distributed in the plant kingdom, and further have resulted in the isolations of a number of closely related congeners, now termed phytoecdysones.<sup>4)</sup> We have been carrying out screening tests on plant materials by means of bioassay, and from our earlier experience it has been thought that the crude extracts of ferns especially show the insect moulting hormone activity in higher frequency than those of the other plant groups. Although preliminary screening results on some of Japanese ferns have been previously announced,<sup>5,6)</sup> we now wish to report more extensive and systematic survey of Japanese ferns for phytoecdysones, which has been performed in order to seek new substances and to examine chemotaxonomical relationships.

Japanese islands lying off the east coast of Asia, long extend from northeast to southwest. The climate ranges from subarctic to subtropical with a heavy rainfall. These physical conditions make the flora of Japan very rich. Concerning ferns, some 400 species, belonging to 83 genera and 23 families, are recorded in Japan,<sup>7)</sup> from which samples were selected so that they cover a wide taxonomical distribution as complete as possible. However, since many species are rare, the present investigation was limited to 251 species, 32 varieties, and 1 form belonging to 71 genera and 20 families (with the supplement of 32 species and 7 varieties as well as of 5 genera<sup>7)</sup>), and consequently no representatives were chosen from 8 genera and 3 families. Whenever possible the plant materials were collected at various seasons and/or locations so that a total of 871 specimens were screened.

- 1) This paper forms Part XVII in the series on Steroids. Part XVI: H. Hikino, Y. Ohizumi, and T. Takemoto, *Chem. Pharm. Bull.* (Tokyo), **20**, 2454 (1972).
- 2) Location: *Aoba-yama, Sendai.*
- 3) K. Nakanishi, M. Koreeda, S. Sasaki, M.L. Chang, and H.Y. Hsu, *Chem. Commun.*, **1966**, 915; T. Takemoto, S. Ogawa, and N. Nishimoto, *Yakugaku Zasshi*, **87**, 325 (1967).
- 4) *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.
- 5) T. Takemoto, S. Ogawa, N. Nishimoto, S. Arihara, and K. Bue, *Yakugaku Zasshi*, **87**, 1414 (1967).
- 6) S. Imai, T. Toyosato, M. Sakai, Y. Sato, S. Fujioka, E. Murata, and M. Goto, *Chem. Pharm. Bull.* (Tokyo), **17**, 335 (1969).
- 7) There are divided views on taxonomy of Japanese ferns. The classification in this work basically follows the manual of J. Ohwi ("Flora of Japan," Shibundo, Tokyo, 1972). Since this manual is not necessarily complete, certain different or newer taxa have been supplemented so that the classification may lack unity partially.



Family	Species <sup>(a,b)</sup>	Japanese name	Date	Location <sup>(c,d)</sup>	Activity <sup>(e)</sup>	Family	Species <sup>(a,b)</sup>	Japanese name	Date	Location <sup>(c,d)</sup>	Activity <sup>(e)</sup>
Hymenophyllaceae	<i>Histiopteris glauca</i> ST. JOHN	Urajiro	Mar	ST, Minō	##	<i>C. japonica</i> DIELS	Iwaganeseō	May	RZ, Sakunami	-	
			Jul	ST, Inagawa	##		RZ, Sakunami	-			
	<i>Crepidomanes latealatum</i> COPELAND		Aug	KI, Shingū	##			Sep	TS, Akka	-	
			Sep	YT, Murō	##			Oct	TS, Mt. Taniyama	-	
	<i>Gonocormus minutus</i> van den BOSCH	Aohoragoke	Apr	SR, Shizuoka	-			Jul	YZ, Nenuro	-	
			Sep	KI, Kumano	-			RZ, Shirasawa	-		
	<i>Hymenophyllum barbatum</i> MIQUEL	Uchiwagoke	Oct	KI, Owase	-			Jul	KI, Mt. Ōdaigahara	-	
			Nov	OS, Yakushima	-			Oct	TS, Mt. Ishidate	-	
	<i>Mecodium flexile</i> COPELAND	Kōyokokeshinobu	Oct	SR, Shizuoka	-			Oct	RZ, Mt. Izumi	-	
		Okokeshinobu	May	ST, Minō	-			Nov	UZ, Sekiyama	-	
<i>M. polyanthos</i> COPELAND	Hosobakokeshinobu	Oct	KI, Owase	-		Aug	ST, Minō	-			
		Nov	KI, Katsunura	-		Aug	YT, Nara	-			
<i>M. wrightii</i> COPELAND	Kokeshinobu	Oct	KI, Owase	-		Oct	KI, Kozagawa	-			
		Oct	KI, Kozagawa	-		Oct	TS, Mt. Sembon	-			
<i>Salenodasium obscurum</i> COPELAND	Onihoragoke	Aug	SR, Shizuoka	-		Nov	OS, Yakushima	-			
	Tsuruhoragoke	Oct	YT, Yoshino	-		Nov	OS, Yakushima	-			
<i>V. radicans</i> COPELAND var. <i>orientalis</i> H. ITO	Haihōragoke	Oct	KI, Owase	-		Nov	OS, Yakushima	-			
		Nov	OS, Yakushima	-		Nov	OS, Yakushima	-			
<i>V. radicans</i> COPELAND var. <i>nascana</i> H. ITO	Ryukyuhoragoke	Oct	IZ, Itō	-		Apr	YS, Kibune	-			
		Nov	KI, Owase	-		Oct	KI, Owase	-			
Pteridaceae	<i>Adiantum capillus-veneris</i> LINNÉ	Hōraishida	May	IZ, Itō	+		Nov	OS, Yakushima	-		
			Nov	SM, Kagoshima	+		Nov	OS, Yakushima	-		
<i>A. flabellatum</i> LINNÉ	Okinawakujakushida	Sep	SR, Shizuoka	##		May	KI, Nachi	-			
	Hakoneshida	Aug	YT, Shimookitayama	-		Oct	IZ, Itō	-			
<i>A. monochlamys</i> EATON		Aug	AW, Mt. Kiyosumi	+		Nov	OS, Yakushima	-			
		Oct	TS, Mt. Ishidate	+		Oct	KI, Owase	-			
<i>A. pedatum</i> LINNÉ	Kujakushida	Oct	SR, Shizuoka	+		Oct	TS, Ioki	-			
		Oct	IZ, Itō	+		Nov	OS, Yakushima	-			
<i>Aleuritopteris argentea</i> FÉE	Humeurajiro	Jun	RZ, Shirasawa	+		Nov	OS, Yakushima	-			
		Sep	YT, Nara	+		Nov	OS, Yakushima	-			
<i>Cheilanthes chusana</i> HOOKER	Ebigarashida	Aug	RZ, Sekiyama	##		Aug	YT, Nara	-			
	Takawarabi	Nov	UZ, Higashine	##		Oct	IZ, Itō	-			
<i>Coniogramme fraxinea</i> DIELS var. <i>intermedia</i> C. CHRISTENSEN	Iwaganomezumai	Aug	SR, Shizuoka	##		Nov	SM, Kagoshima	-			
		Nov	SR, Shizuoka	##		Sep	SR, Shizuoka	-			
<i>C. japonica</i> DIELS	Iwaganeseō	Mar	IZ, Itō	-		Nov	OS, Yakushima	-			
		Nov	RC, Akka	-		Nov	OS, Yakushima	-			
		Sep	RC, Akka	-		Aug	YT, Murō	-			
		Nov	RC, Akka	-		Oct	SR, Shizuoka	-			
		Mar	ST, Minō	-		Oct	YT, Murō	-			
				-		May	UZ, Higashine	-			
				-		Jun	RZ, Shirasawa	-			
				-		Oct	RZ, Shirasawa	-			

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Pteridaceae	<i>Pteridium aquilinum</i> KUHN var. <i>latiusculum</i> UNDERWOOD	Warabi	Oct	RZ, Mt. Izumi	##	Davalliaceae	<i>Davallia mariesii</i> MOORE	Shinobu	Sep	ST, Sengari	##
	<i>Pteris cretica</i> LINNÉ	Obanoinomotosô	Nov	RZ, Mt. Izumi	##					Oct	TS, Ioki
Pteridaceae	<i>P. cretica</i> LINNÉ var. <i>albocincta</i> HORT.	Matsuzakashida	Apr	IZ, Itô	+	Hamatidae	<i>Hamata repens</i> DIELS	Kikushinobu	Aug	SR, Shizuoka†	+
			Sep	ST, Minô	+					Sep	SR, Shizuoka†
Pteridaceae	<i>P. dispars</i> KUNZE	Amakusashida	Oct	SR, Shizuoka	+	Nephrolepisaceae	<i>Nephrolepis auriculata</i> TRIMEN	Tamashida	Oct	SR, Shizuoka†	+
	<i>P. gracillanum</i> WALLICH*	Ashigatashida	Oct	TS, Mt. Taniyama	+					Oct	IZ, Itô
Pteridaceae	<i>P. inaequalis</i> BAKER	Obanoinomotosashida	Nov	OS, Yakushima	+	Plagiogyriaceae	<i>Plagiogyria adnata</i> BEDDOME	Takasagokijino	Oct	OS, Yakushima	+
	<i>P. inaequalis</i> BAKER var. <i>aeguala</i> TAGAWA	Obanohachijôshida	Oct	SR, Shizuoka†	+					Oct	KI, Owase
Pteridaceae	<i>P. multifida</i> POIRET	Inomotosô	Oct	KI, Owase	+	Pteridaceae	<i>P. euphlebia</i> METTENIUS	Ôkijino	Oct	KI, Kozagawa	+
	<i>P. quadrivaria</i> RETZES	Hachijôshida	Nov	MS, Kawasaki	+					Mar	ST, Minô
Pteridaceae	<i>P. ryukyuensis</i> TAGAWA	Ryukyuinomotosô	May	KI, Katsunura	+	Pteridaceae	<i>P. japonica</i> NAKAI	Kijinooshida	Nov	OS, Yakushima	+
	<i>P. semipinnata</i> LINNÉ	Ôamakusashida	Oct	KI, Owase	+					Mar	ST, Minô
Pteridaceae	<i>P. setulos-costulata</i> HAYATA*	Togchachijôshida	Oct	KI, Katsunura	+	Cyatheaceae	<i>P. stenoptera</i> DIELS	Shimayamasotetsu	May	YI, Murô	+
	<i>P. tokioi</i> MASAMUNE*	Hiragcanakusashida	Nov	OS, Yakushima	+					Aug	AW, Mt. Kiyosumi
Pteridaceae	<i>P. vitata</i> LINNÉ	Moejimashida	Nov	OS, Yakushima	+	Cyatheaceae	<i>Cyathea boninensis</i> COPELAND	Hego	Oct	SR, Shizuoka	+
	<i>P. wallichiana</i> AGARDH	Nachishida	Nov	OS, Yakushima	+					Oct	SR, Shizuoka
Pteridaceae	<i>P. yakushimavaris</i> KURATA*	Yakushimahachijôshida	Sep	SR, Shizuoka†	+	Cyatheaceae	<i>C. metteniana</i> C. CHRISTENSEN et TARDIEU*	Chabohego	Oct	TS, Mt. Sembon	+
	<i>Sphenomeris chinensis</i> MAXON	Horashinobu	Nov	OS, Yakushima	+					Oct	OS, Yakushima
Pteridaceae	<i>S. chinensis</i> MAXON var. <i>littoralis</i> OHWI	Hamahorashinobu	Nov	OS, Yakushima	+	Aspidaceae	<i>Asplenium stipellatus</i> MOORE	Taiwanhimewarabi	Nov	OS, Yakushima	+
			Nov	OS, Yakushima	+					Nov	OS, Yakushima
Davalliaceae	<i>Davallia mariesii</i> MOORE	Shinobu	Mar	ST, Minô	+	Aspidaceae	<i>Atsuhakino</i> OHWI	Atsuhakino	Nov	OS, Yakushima	+
			Aug	AW, Mt. Kiyosumi	+					Nov	OS, Yakushima
			Aug	YI, Yoshino	##				Oct	SR, Shizuoka†	##

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<i>A. decurrens-alatum</i> COPELAND		Shikochishida	Nov	YT, Yoshino	+	<i>A. nipponicola</i> OHWII		Onihitegawarabi	Sep	SR, Shizuoka	+
<i>A. doebelinii</i> OHWII		Shimashiroyamashida	Nov	OS, Yakushima	+	<i>A. okadaii</i> OHWII		Iyokujaku	May	YT, Murō	+
<i>A. dubium</i> OHWII		Hirashida	Nov	OS, Yakushima	+	<i>A. ataphorum</i> KOZUMI		Taninuwarabi	Oct	KI, Owase	+
			Nov	OS, Yakushima	+	<i>A. petri</i> OHWII		Hirohamiyamanokogirishida	Nov	OS, Yakushima	+
			Aug	AW, Mt. Kiyosumi	+	<i>A. procerum</i> MILDE		Aoigawarabi	Nov	OS, Yakushima	+
			Sep	YT, Nara	+	<i>A. pyrenosorum</i> H. CHRIST		Miyanashtakeshida	Nov	OS, Yakushima	+
			Oct	SR, Shizuoka	+				Aug	ST, Minō	+
			Oct	KI, Kozagawa	+				Oct	KI, Kozagawa	+
			Oct	TS, Ioki	+				Nov	RZ, Sekiyama	+
<i>A. flavida</i> C. CHRISTENSEN		Hosobashikeshida	Nov	OS, Yakushima	+	<i>A. spinulosum</i> MILDE		Miyamainuwarabi	Aug	KI, Katsura	+
<i>A. graminifolius</i> MILDE		Hosobashikeshida	Sep	RZ, Avashi	+	<i>A. splanigerum</i> OHWII		Kiyotakishida	Jun	RZ, Mt. Tahaku	+
			Oct	RZ, Sakunani	-				Sep	RZ, Shirasawa	+
			Oct	MS, Kawasaki	-				Sep	RC, Akka	+
			Oct	SR, Shizuoka	-	<i>A. subrigescens</i> HAYATA		Otomeinuwarabi	Sep	ST, Minō	+
<i>A. graminifolius</i> MILDE var. <i>simplexifolium</i> OHWII		Hitosubashikeshida	Oct	SR, Shizuoka	-	<i>A. unifurcatum</i> C. CHRISTENSEN		Ohimewarabimodoki	Nov	OS, Yakushima	+
<i>A. hachijoense</i> OHWII		Shiroyamashida	Sep	SR, Shizuoka	+	<i>A. vidalii</i> NAKAI		Yamainuwarabi	Oct	SR, Shizuoka	+
			Oct	KI, Owase	+				May	RZ, Sakunani	+
<i>A. lehyui</i> DIEHL		Ohimewarabi	Sep	OS, Yakushima	+				Aug	IG, Kōchidani	+
<i>A. iscanium</i> ROSENSTOCK		Hosobainuwarabi	Oct	SR, Shizuoka	+	<i>A. virescens</i> OHWII		Kokumōkujaku	Sep	RZ, Sekiyama	+
<i>A. iscanium</i> ROSENSTOCK var. <i>angustisectum</i> TAGAWA*		Togariibanuwarabi	Nov	OS, Yakushima	+				Nov	UZ, Higashine	+
<i>A. japonicum</i> COPELAND		Shikeshida	Nov	OS, Yakushima	+				Sep	SR, Shizuoka	+
			Jun	RZ, Mt. Tahaku	-				Oct	KI, Owase	-
			Aug	RZ, Mt. Izumi	-	<i>A. wardii</i> MAKINO		Hirohanoinuwarabi	Oct	KI, Owase	-
<i>A. japonicum</i> COPELAND var. <i>dimorphophyllum</i> OHWII		Seitakashikeshida	Aug	YS, Kibune	-	<i>A. wardii</i> MAKINO var. <i>major</i> MAKINO		Ohirohanoinuwarabi	Nov	OS, Yakushima	+
<i>A. japonicum</i> COPELAND var. <i>grammitoides</i> MILDE*		Koshikeshida	Aug	SR, Shizuoka	-	<i>A. wichurae</i> OHWII		Nokogirishida	Aug	ST, Minō	+
<i>A. maximum</i> COPELAND		Hirohanokogirishida	Sep	SR, Shizuoka	-				Oct	IG, Kōchidani	+
			Nov	OS, Yakushima	+				Oct	KI, Kozagawa	+
			Aug	SR, Shizuoka	+				Oct	KI, Owase	+
<i>A. melanolepis</i> H. CHRIST		Meshida	Nov	SR, Shizuoka	+				Oct	KI, Owase	+
<i>A. mesosorum</i> MAKINO		Nuriwarabi	Nov	OS, Yakushima	+				Oct	KI, Nachi	+
			Aug	ST, Minō	+				Oct	KI, Owase	+
			Oct	SI, Minō	+				Oct	SG, Yugawara	-
			Aug	IG, Kōchidani	+				Oct	SR, Shizuoka	-
			Aug	RC, Akka	+				Oct	TS, Ioki	-
			Sep	YS, Kibune	+	<i>A. yakushimensis</i> TAGAWA*		Yakushimataniinuwarabi	Oct	TS, Mt. Taniyama	+
			Sep	KL, Owase	+				Nov	OS, Yakushima	+
			Oct	KL, Owase	+				Nov	OS, Yakushima	+
			Oct	TS, Mt. Taniyama	+				Nov	OS, Yakushima	+
			Nov	OS, Yakushima	+				Jun	OS, Yakushima	+
			May	KI, Nachi	+				Jun	RZ, Mt. Izumi	+
<i>A. mellenianum</i> OHWII		Miyamanokogirishida	Nov	OS, Yakushima	+				Sep	RZ, Sendai	+
<i>A. mellenianum</i> OHWII var. <i>fauriei</i> OHWII		Hosobanokogirishida	Nov	OS, Yakushima	+				Sep	ST, Minō	+
<i>A. multifidum</i> ROSENSTOCK		Satoneshida	Nov	OS, Yakushima	+				Sep	ST, Minō	+
			May	RZ, Sakunani	-				Sep	RZ, Okunikaawa	+
<i>A. multifidum</i> ROSENSTOCK f. <i>acutissimum</i> KURATA		Togariibaneshida	Jun	RZ, Shirasawa	-				Sep	SR, Shizuoka	+
<i>A. naganumanum</i> OHWII		Hikagawarabi	Aug	IG, Kōchidani	+				Nov	OS, Yakushima	+
<i>A. nakanoi</i> MAKINO		Himehobishida	Sep	RZ, Sekiyama	+				Nov	OS, Yakushima	+
			Oct	SR, Shizuoka	+				Nov	OS, Yakushima	+
			Aug	SR, Shizuoka	+				Aug	SR, Shizuoka	+
			Nov	OS, Yakushima	+				Oct	KI, Owase	+
			Nov	OS, Yakushima	+				Nov	OS, Yakushima	+
			May	RZ, Sakunani	+				Aug	AW, Mt. Kiyosumi	+
<i>A. niponicum</i> HANCE		Inuwarabi	May	RZ, Sekiyama	+				Aug	KI, Katsura	+
			Sep	RZ, Sekiyama	+				Aug	YI, Murō	+
			Oct	KI, Owase	+				Oct	TS, Mt. Yokogura	+
			Nov	UZ, Higashine	+				Oct	KI, Owase	+
			Nov	RZ, Sekiyama	+				Oct	SG, Yugawara	+
			Nov	RZ, Sekiyama	+				Oct	KI, Owase	+
			Nov	RZ, Sekiyama	+				Oct	KI, Kozagawa	-

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C. sinii OHWI		Satsumashida	Sep	SR, Shizuoka	+	D. lacera O. KUNTZE		Kumawarabi	Nov	RZ, Sekiyama	+
C. subglaucescens CHING		Katsunoinode	May	KI, Katsunura	-	D. laticornis C. CHRISTENSEN*		Ivakegevarabi	Sep	RC, Akka	+
			Aug	AW, Mt. Kiyosumi	+	D. melanocarpa HAYATA*		Kurominoichishida	Nov	OS, Yakushima	+
			Aug	SR, Shizuoka	+	D. monticola C. CHRISTENSEN		Miyamabenshida	Sep	SR, Shizuoka	+
			Nov	OS, Yakushima	+	D. polita ROSENTHAL*		Taitobenshida	Nov	OS, Yakushima	+
Cybalosorus acuminatus NAKAI		Hoshida	Aug	ST, Minō	-	D. polytrepis C. CHRISTENSEN		Miyamakumawarabi	Jun	RZ, Shirasawa	+
			Oct	SR, Shizuoka	-				Oct	RZ, Sakunami	-
			Oct	TS, Ioki	-	D. pycnopteroides C. CHRISTENSEN		Wakanashida	Oct	KI, Owasef	-
C. dentatus CHING		Inukeshida	Oct	SR, Shizuoka	-	D. sabaei C. CHRISTENSEN		Miyamaichishida	Jun	RZ, Shirasawa	-
C. goggeloides LINK		Tetsuhoshida	Apr	KI, Shingū	-				Aug	KI, Mt. Odagahara	-
			Oct	SR, Shizuoka	-				Aug	RZ, Mt. Izumi	-
C. parasiticus FARWELL		Kehoshida	Nov	OS, Yakushima	-	D. sieboldii C. CHRISTENSEN		Omitsude	Sep	SR, Shizuoka	-
			Nov	SM, Kagoshima	-				Oct	KI, Owasef	-
C. subpubescens CHING*		Kushinohashida	Aug	SR, Shizuoka	-	D. sordidipes TAGAWA		Yogoreitachishida	Nov	OS, Yakushima	+
C. triphyllus TARDIEU-BLOT		Komorishida	Oct	SR, Shizuoka	+				Nov	OS, Yakushima	+
			Nov	OS, Yakushima	+	D. sparsa O. KUNTZE		Nagabanoitachishida	Sep	SR, Shizuoka	+
Cystopteris fragilis BERNHARDI		Navoshida	Nov	OS, Yakushima	+				Sep	ST, Minō	-
Diclyptera griffithii MOORE var. pinnatifida BEDDOME		Amishida	Oct	KI, Katsunura	+	D. fastigiata TAGAWA		Tsukushiokujaku	Oct	KI, Owase	+
			Oct	KI, Owase	+	D. tobayonensis C. CHRISTENSEN		Tanibego	Oct	KI, Owase	+
			Oct	KI, Kozagawa	+				Oct	KI, Owasef	+
			Nov	OS, Yakushima	+	D. variiformis MAKINO		Okumawarabi	Sep	RZ, Sekiyama	+
Diplazium ovacearum KURATA*		Owaseshida	Nov	OS, Yakushima	+				May	RZ, Mt. Banzan	+
D. taiwanense TAGAWA*		Niseshiroyamashida	Nov	KI, Owasef	+	D. varia O. KUNTZE var.		Himetachishida	Oct	RZ, Sakunami	+
D. yakumontanum TAGAWA*		Yakushimawarabi	Nov	OS, Yakushima	+	secrocarpa OHWI			Aug	SR, Shizuoka	+
Dryopteris austriaca WOYNAK		Shiranewarabi	Nov	RZ, Mt. Izumi	+				Oct	KI, Owase	+
			Nov	RZ, Sekiyama	+	D. varia O. KUNTZE var.		Ikachishida	Aug	AW, Mt. Kiyosumi	+
D. bisetiana C. CHRISTENSEN*		Yamaichishida	Oct	TS, Mt. Taniyama	+				Aug	IG, Kōchidani	+
D. championi CHING		Saikobenshida	Sep	ST, Minō	+	D. varia O. KUNTZE var.		Oitachishida	Oct	SR, Shizuoka	+
D. chientsisi KONZUMI		Misakikaguna	Aug	AW, Mt. Kiyosumi	+				Aug	SR, Shizuoka	+
D. commixta TAGAWA		Tsukushiwahago	Oct	KI, Owasef	+				Oct	SR, Shizuoka	+
D. crassirhizoma NAKAI		Oshida	May	RZ, Shirasawa	+				Aug	SR, Shizuoka	+
			Jul	SN, Togakushi	+				Oct	KI, Kozagawa	+
			Aug	RC, Mt. Kurikoma	+				Nov	OS, Yakushima	+
			Oct	RZ, Sekiyama	+				Sep	SR, Shizuoka	+
			Nov	RZ, Sekiyama	+				Oct	KI, Owase	+
			Sep	YT, Murō	+				Nov	OS, Yakushima	+
D. cycadina C. CHRISTENSEN		Iwahago	Oct	KI, Kozagawa	+				Nov	OS, Yakushima	+
			Oct	TS, Mt. Taniyama	+				Aug	AW, Mt. Kiyosumi	+
			Oct	TS, Mt. Yokogura	+				Aug	IG, Kōchidani	+
			Oct	KI, Owasef	+				Aug	YT, Yoshino	+
D. dichinisi C. CHRISTENSEN		Okujakushida	Apr	KI, Katsunura	+				Oct	MS, Kawasaki	+
D. erythrosora O. KUNTZE		Benshida	Oct	IG, Kōchidani	+				Oct	KI, Kozagawa	+
			Aug	KI, Owase	+				Oct	TS, Ioki	+
			Aug	ST, Minō	+				Aug	KA, Mt. Kitadake	+
			Aug	IG, Kōchidani	+				Oct	SG, Yagawara	+
D. erythrosora O. KUNTZE var. cystolepidota NAKAI		Tegokushida	Aug	IG, Kōchidani	+				Oct	KI, Owase	+
D. erythrosora O. KUNTZE var. hoidzumiana H. ITO*		Hokozakibenshida	Nov	OS, Yakushima	+				Nov	OS, Yakushima	+
D. formosana C. CHRISTENSEN		Takasagoshida	Nov	OS, Yakushima	+				Sep	SR, Shizuoka	+
D. fuscipes C. CHRISTENSEN		Marubabenshida	May	YT, Mt. Kasuga	+				Nov	OS, Yakushima	+
			Oct	KI, Owase	+				Nov	OS, Yakushima	+
			Apr	SR, Shizuoka	+				Nov	OS, Yakushima	+
D. gymnosora C. CHRISTENSEN		Nukataichishida	Apr	SR, Shizuoka	+				Jun	OS, Yakushima	+
D. hayatai TAGAWA		Inutamashida	Nov	OS, Yakushima	+				RZ, Mt. Talhaku	+	
D. honoensis KOIZUMI		Obenshida	Apr	KI, Katsunura	+				Aug	IG, Kōchidani	+
D. indusiata MAKINO et YAMAMOTO		Nukataichishidamo-doki	Sep	SR, Shizuoka	+				Sep	RZ, Sendai	+
			Oct	KI, Owase	+				Oct	SG, Yagawara	+
			Nov	OS, Yakushima	+				Oct	TS, Mt. Sembon	+
			Nov	OS, Yakushima	+				Aug	IG, Kōchidani	+
D. lacera O. KUNTZE		Kumawarabi	Oct	IG, Kōchidani	+				Sep	ST, Minō	+
			Oct	RZ, Sakunami	+				Oct	SR, Shizuoka	+

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L. iaxa COPELAND L. oligophlebia COPELAND		Yawarashida	Nov	OS, Yakushima	—	Polystichum	<i>craspedosorum</i> DIELS	Tsurudenda	Oct	SG, Yugawara	—
		Himewarabi	May	UZ, Higashine	##					Tachidenda	Oct
L. oligophlebia COPELAND var. lasiocarpa OHWI L. onetensis COPELAND		Aragchimewarabi	Sep	SR, Shizuoka	##	P. deltoideum	DIELS	Komochinode	Aug	KI, Owasef	+
		Mizoshidamodoki	Oct	RZ, Mt. Izumi	##					Taiwan-jumonjishida	Oct
L. phegopteris BORY L. quelpaertensis COPELAND		Miyamawarabi	Jun	KA, Mt. Kitadake	—	P. eximium	C. CHRISTENSEN var. mans TAGAWA	Katainode	Sep	KI, Owase	+
		Obashorima	Aug	RZ, Mt. Izumi	—					Karaksuinode	May
L. subochthodes TAGAWA		Ibukishida	Jul	RC, Mt. Kurikoma	+	P. macrochaetys	KODAMA	Nampinode	Aug	IG, Kōchidani	##
		Himeshida	Sep	Mt. Nakuidake	+					Inode	Oct
L. thelypteris BORY		Himeshida	Aug	OM, Mt. Ibuki	+	P. obomasui	KURATA*	Saikokuinode	Apr	RZ, Mt. Izumi	##
		Mizoshida	Oct	SR, Shizuoka†	+					Inodemodoki	Oct
L. totta OHWI		Mizoshida	Oct	KI, Kozagawa	—	P. retroso-paleaceum	TAGAWA	Sakageinode	Aug	IG, Kōchidani	—
		Mizoshida	May	OS, Yakushima	—					Iwashiroinode	Nov
L. uraiensis COPELAND* Mattheuccia orientalis TREVISEN		Mizoshida	May	RZ, Ayashi	##	P. pseudo-paleaceum	TAGAWA var. covariense TAGAWA	Tsuyunashinode	Sep	YI, Murō	—
		Mizoshida	Sep	RZ, Mt. Banzan	—					Oninode	Aug
M. strathliopteris TODARO		Taiwan-hatiganewarabi	Oct	RZ, Mt. Izumi	—	P. ovato-paleaceum	TAGAWA	Jumonjishida	Aug	IG, Kōchidani	—
		Inugansoku	Nov	RZ, Okunikawa	—					Himekanawarabi	Oct
Onoclea sensibilis LINNÉ		Kusatotetsu	Nov	OS, Yakushima	—	P. rigens	TAGAWA	Okanawarabi	May	RZ, Mt. Izumi	—
		Koyawarabi	Aug	OS, Yakushima	—					Hosobakanawarabi	Jun
Phacrophlebia falcata COPELAND		Koyawarabi	Aug	RZ, Shirasawa	##	P. tsusimensis	J. SMITH	Hosobakanawarabi	Aug	ST, Minō	—
		Koyawarabi	Aug	IG, Kōchidani	##					Otokoshida	Oct
P. fortunei COPELAND		Omiyabusotetsu	Aug	RZ, Mt. Izumi	##	R. maximowiczii	CHUNG	Nantaishida	Oct	SR, Shizuoka	+
		Omiyabusotetsu	Oct	RZ, Mt. Banzan	##					Narashida	Aug
P. fortunei COPELAND var. clinicola OHWI		Koyawarabi	May	RZ, Okura	##	R. nipponica	CHUNG	Kobanokanawarabi	Sep	RZ, Mt. Izumi	+
		Omiyabusotetsu	Sep	KI, Shingu	##					Hakataashida	Nov
P. fortunei COPELAND var. intermedia OHWI		Omiyabusotetsu	Oct	SG, Hayama	##	R. pseudo-arietata	H. ITO	Ryōmenshida	Oct	YI, Murō	—
		Omiyabusotetsu	Oct	TS, Ioki	##					Karenkōamishida	Sep
P. macrophylla OKUYAMA		Miyayabusotetsu	Oct	KI, Kozagawa	—	Tectaria	kwarensis TAGAWA* Woodia polystichoides EATON	Iwadenda	Jun	RZ, Shirasawa	—
		Miyayabusotetsu	Nov	RZ, Shirasawa	—						Nov
P. tachiroana COPELAND		Miyajimashida	Dec	RZ, Shizuoka†	—	P. vittata	COPELAND		Nov	RZ, Shirasawa	—
		Miyajimashida	Oct	UZ, Yamadera	—						Dec
Pegopteris subaurita TAGAWA*		Mimigataashida	Oct	KI, Owase	—	Pegopteris	subaurita TAGAWA*		Oct	SR, Shizuoka†	—
		Mimigataashida	Oct	KI, Katsunura	—						Oct

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Blechnaceae	<i>Blechnum amabile</i> MAKINO	Osashida	May	RZ, Okunikkawa	■	<i>A. trichomanes</i> LINNÉ	Chasenshida	Oct	SR, Shizuoka	—	
	<i>B. castaneum</i> MAKINO		Jul	RC, Mt. Kurikoma	—	<i>A. unilaterale</i> LAMARCK	Hobishida	Oct	KI, Kozagawa	—	
	<i>B. niponicum</i> MAKINO	Miyamashishigashira Shishigashira	Sep	RZ, Okunikkawa	■	<i>A. wilfordii</i> METTENUS	Aoganeshida	Oct	KI, Kozagawa	—	
			Oct	SR, Shizuoka	■			Nov	OS, Yakushima	—	
			Oct	TS, Mt. Yokogura	■			Nov	OS, Yakushima	—	
			Sep	RZ, Mt. Hakokōda	■			Nov	OS, Yakushima	—	
			May	RZ, Ayashi	■	<i>A. wrightii</i> EATON	Kurumashida	Nov	OS, Yakushima	—	
			Sep	RZ, Sekiyama	■			Aug	KI, Katsura	—	
			Sep	RZ, Okunikkawa	■	<i>A. yakumontanum</i> MASAMUNE*	Yakushimashida	Sep	SR, Shizuoka	—	
			Sep	ST, Minō	■	<i>A. yoshinagae</i> MAKINO	Tokiwashida	Nov	OS, Yakushima	—	
			Oct	TS, Mt. Sembon	■	<i>Camptosorus sibiricus</i> RUBRICH	Kunonoushida	Oct	KI, Owase	—	
			Nov	RZ, Sekiyama	■			May	OM, Mt. Ibuki	—	
			Nov	RZ, Shizuoka†	■			Sep	RC, Akka	—	
			Aug	SR, Shizuoka†	—	polypodiaceae					
			Nov	OS, Yakushima	—	<i>Chiropleuria bitrispis</i> PRESL	Sujihitotsuba	Oct	KI, Owase	—	
			Nov	OS, Yakushima	■			Nov	OS, Yakushima	—	
			Nov	OS, Yakushima	■	<i>Colysis elliptica</i> CHING	Iwahitode	Sep	ST, Minō	■	
			Nov	OS, Yakushima	■			Oct	SR, Shizuoka†	■	
			Sep	SR, Shizuoka†	■	<i>C. hemionitidea</i> PRESL	Tatwankurharan	Nov	OS, Yakushima	■	
			Nov	OS, Yakushima	■			Sep	OS, Tanegashima	■	
			Oct	YI, Shimokitayama	■			Oct	SR, Shizuoka†	■	
			Oct	SR, Shizuoka	■			Nov	OS, Yakushima	■	
			Oct	KI, Owase	■			Nov	OS, Yakushima	■	
			Oct	KI, Kozagawa	■	<i>C. potiffolia</i> PRESL	Ōwahitode	Dec	OS, Tanegashima	■	
			Oct	YI, Murō	■			Sep	SR, Shizuoka†	■	
			Nov	SM, Kagoshima	■			Oct	TS, Ashizuri	■	
			Nov	SR, Shizuoka†	■			Oct	SR, Shizuoka†	■	
			Oct	SG, Yugawara	■			Oct	SR, Shizuoka†	■	
Asplenaceae	<i>Asplenium antiquum</i> MAKINO	Taniwatari	Aug	SR, Shizuoka†	■	<i>C. shimtenensis</i> H. IRO	Hitotsubaiwahitode	Nov	OS, Yakushima	■	
			Sep	OS, Tanegashima	■	<i>C. wrightii</i> CHING	Yarinohokurharan	Oct	SR, Shizuoka†	■	
			Oct	OS, Tanegashima	■			Nov	OS, Yakushima	■	
			Oct	SR, Shizuoka†	■			Nov	OS, Yakushima	■	
			Nov	OS, Yakushima	■	<i>C. wrightii</i> CHING var. <i>henryi</i> TAGAWA*	Ōyarinohokurharan	Oct	SR, Shizuoka†	■	
			Nov	OS, Yakushima	■			Nov	OS, Yakushima	■	
			Aug	SR, Shizuoka†	■	<i>Crypsinus engelii</i> COPELAND	Takanohauraboshi	Nov	OS, Yakushima	■	
			Oct	SR, Shizuoka†	■	<i>C. hastatus</i> COPELAND	Mitsudauraboshi	Mar	ST, Minō	■	
			Nov	OS, Yakushima	■			Jul	RZ, Matsushima	■	
			Nov	OS, Yakushima	■			Oct	SR, Shizuoka	■	
			Nov	OS, Yakushima	■			Oct	KI, Owase	■	
			Jun	RZ, Mt. Taihaku	—			Oct	TS, Mt. Taniyama	■	
			Aug	RZ, Mt. Izumi	—			Nov	OS, Yakushima	■	
			Oct	UZ, Yamadera	—	<i>C. yakushimensis</i> TAGAWA	Himatakanohaura-boshi	Nov	OS, Yakushima	■	
			Aug	KA, Mt. Kitadake	—	<i>Lemmaphyllum microphyllum</i> PRESL	Mamezuta	Nov	OS, Yakushima	■	
			Sep	KI, Kozagawa	—			Mar	ST, Minō	■	
			Oct	KI, Owase	—			Apr	IY, Matsuyama	■	
			Nov	OS, Yakushima	—			Aug	KI, Nachi	■	
			Nov	OS, Yakushima	—			Sep	YS, Kibune	■	
			Apr	SR, Shizuoka	—			Oct	ST, Minō	■	
			Oct	KI, Owase	—			Oct	SG, Yugawara	■	
			Nov	OS, Yakushima	—			Oct	TS, Hayama	■	
			Aug	YI, Kamikitayama	—			Oct	TS, Mt. Ishidate	■	
			Oct	KI, Owase	—			Nov	OS, Yakushima	■	
			Sep	RC, Akka	—			Nov	OS, Yakushima	■	
			Aug	ST, Minō	—			Nov	OS, Yakushima	■	
			Oct	SR, Shizuoka	—	<i>Lepisorus montisorus</i> TAGAWA*	Chizuminoishinobu	Nov	OS, Yakushima	■	
			Oct	KI, Owase	—	<i>Lovogramme graminifolia</i>	Himesajiran	Oct	OS, Yakushima	■	
			Oct	KI, Kozagawa	—	C. CHRISTENSEN					
			May	IW, Yamazu	—	<i>L. satifolia</i> MAKINO	Iwayanagishida	Oct	KI, Kozagawa	■	
			Oct	UZ, Yamadera	—			Nov	OS, Yakushima	■	
			Nov	RZ, Sekiyama	—			Nov	OS, Yakushima	■	



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<i>L. setivirens</i> TAGAWA		Sejiran	Aug	AW, Mt. Kiyosumi	+	<i>P. formosensis</i> BAKER		Taiwan-aonekazura	Aug	SR, Shizuoka†	+
			Oct	SR, Shizuoka	+					Oct	SR, Shizuoka†
<i>Microsorium buergerianum</i> CHING			Oct	KL, Owase	+	<i>P. nipponicum</i> METTENIUS		Aonekazura	Nov	OS, Yakushima	+
			Nov	TS, Mt. Ishidate	+				Oct	SR, Shizuoka	+
			Nov	OS, Yakushima	+				Oct	KI, Negoro	+
			Oct	SR, Shizuoka†	+				Nov	OS, Yakushima	+
			Nov	OS, Yakushima	+				May	SI, Mino	+
			Nov	OS, Yakushima	+				Oct	TS, Mt. Ishidate	+
<i>M. kancocitii</i> CHING		Hokozakura boshi	Nov	OS, Yakushima	+	<i>Pyrrhosia hastata</i> CHING		Oozodenda	Nov	OS, Yakushima	+
		Kurharan	Mar	ST, Mino	+				May	SI, Mino	+
			Aug	KL, Nachi	+				Nov	OS, Yakushima	+
			Oct	KL, Owase	+				Mar	ST, Mino	+
<i>Platypeltis amurjensis</i> NAKAI		Hotetsuhida	Oct	KL, Kozagawa	+	<i>P. linearifolia</i> CHING		Birōdoshida	Mar	ST, Mino	+
			Oct	TS, Mt. Taniyama	+				Aug	KI, Nachi	+
			Sep	SR, Shizuoka†	+				Sep	RZ, Okunikawa	+
			Oct	RZ, Mt. Ōmori	+				Oct	SR, Shizuoka	+
<i>P. onoei</i> OKUYAMA		Himcnokishinobu	Oct	TS, Mt. Ōmori	+	<i>P. lingua</i> FARWELL		Hifotsuba	Oct	RZ, Mt. Ōmori	+
			Oct	TS, Mt. Ishidate	+				Oct	TS, Mt. Ishidate	+
<i>P. thunbergiana</i> KAULFUS		Nokishinobu	Mar	ST, Mino	+	<i>Vittariaceae</i>			Mar	ST, Mino	+
			May	YT, Nara	+				Aug	KI, Nachi	+
			Oct	RZ, Sendai	+				Aug	AW, Mt. Kiyosumi	+
			Oct	RZ, Mt. Ōmori	+				Oct	SR, Shizuoka	+
<i>P. assuriensis</i> REEEL et MAACK var. <i>distans</i> OKUYAMA			Oct	KI, Owase	+	<i>Vittaria flexuosa</i> FÉE		Sbshiran	Oct	SR, Shizuoka	+
			Oct	TS, Mt. Taniyama	+				Oct	KI, Owase	+
			Oct	TS, Hayama	+				Oct	TS, Mt. Yokogura	+
			Oct	ST, Mino	+				Nov	OS, Yakushima	+
<i>Polypodium fauriei</i> H. CHRIST		Miyamanokishinobu	Nov	OS, Yakushima	+	<i>V. zosterifolia</i> WILDENOW		Amamoshishiran	Nov	OS, Yakushima	+
			Jul	YT, Mt. Ōdaigahara	+				Aug	SR, Shizuoka	+
			Aug	SN, Mt. Yatsugatake	+				Oct	SR, Shizuoka	+
			Nov	OS, Yakushima	+				Oct	KI, Owase	+
		May	RZ, Mt. Taihaku	+			Nov	OS, Yakushima	+		
		Nov	RZ, Sakunami	+			Nov	OS, Yakushima	+		
		Oshakujidenda	Nov	RZ, Sakunami	+			Aug	SR, Shizuoka	+	
					+			Oct	SR, Shizuoka	+	
					+			Sep	RZ, Ayashi	+	

a) The classification principally follows the manual of Ohwi. The taxa, whose sources of nomenclature are literatures other than the above manual, are asterisked (cf. footnote 7).

b) The families are arranged in the sequence adopted by Ohwi, and genera and species are given in alphabetical order within the families.

c) abbreviations: AW=Awa, HD=Hida, HZ=Hizen, IG=Iga, IW=Iwashiro, IY=Iyo, IZ=Izu, KA=Kai, KG=Kaga, KI=Kii, MS=Musashi, MT=Mutsu, OM=Ōmi, OS=Ōsumi, RC=Rikuchū, RZ=Rikuzen, SR=Suruga, SG=Sagami, SM=Satsuma, SN=Shinano, ST=Settsu, TS=Tosa, UZ=Uzen, YS=Yamashiro, YT=Yamato, YZ=Yezo, †=cultivated

d) Areas of the indicated locations vary. Specimens from the same species collected at the same time at distinct places in a location with a large area were all screened.

e) Assays were carried out on *Sarcophaga peregrina*. A test solution containing a methanol extract from a dried plant material (10 mg) was injected into each isolated larval abdomen. The results are expressed in terms of the activity indexes which are corresponding to the following average per cent puparium formation: —=0, 0<+ ≤20, 20<+ ≤40, 40<+ ≤60, 60<+ ≤80, 80<+ ≤100%.

1-4: *Athyrium aphanoneuron* may further be classified into four taxa. See the text (p. 2301).

A number of screening methods for the insect moulting hormone activity have been developed; each has respective advantage. The screening operation used for the present bioassay was the *Sarcophaga* test,<sup>8)</sup> which is rather easily carried out and gives quantitative results.

It was reported in a previous paper that the chance of finding the hormone activity from fresh plant materials was higher than that from dried ones such as crude drugs.<sup>6)</sup> However, it was not clear whether the difference in activity between fresh and dried samples arose fortuitously or because of decomposition during drying and storing. It was found in the present work that plant materials showed activity regardless of the conditions; *i.e.*, fresh or dried, new and old. In fact, dried specimens 20 years old still gave positive responses.

The insect moulting hormone activities of the *pteridophyta* species of Japanese origin were summarized in Table I. Of the total 283 species, 39 varieties, and 1 form of ferns tested, 170 species, 22 varieties, and 1 form (48 genera and 13 families) showed activity towards insect,<sup>9)</sup> while 113 species and 17 varieties (28 genera and 7 families) exhibited negative responses. Therefore, it was confirmed that ferns in general show a high probability of containing phytoecdysones.

Among the ferns found to give positive responses, 51 species and 7 varieties exhibited remarkable activity. In particular, probability of finding the strong activity was high in ferns belonging to the families Osmundaceae, Plagiogyriaceae, Cyatheaceae, Aspidiaceae, Blechnaceae, and Polypodiaceae. On the other hand, it is of interest chemotaxonomically that 7 families found to contain no active species namely Equisetaceae, Lycopodiaceae, Selaginellaceae, Ophioglossaceae, Marattiaceae, Schizaeaceae, and Hymenophyllaceae are those whose degrees of differentiation are considered to be low from a viewpoint of taxonomy. In spite of the same species being concerned, samples of different collecting dates and/or locations sometimes showed different responses. In some extreme cases, a specimen was active while another specimen in the same species was inactive. An interesting example is *Athyrium aphanoneuron* which, according to another taxonomical view, is classified into *Diplazium crassiusculum* TAGAWA (Ibudakekinoborishida,<sup>1</sup> in Table I), *D. donianum* TARDIEU (Kinoborishida,<sup>2</sup> in Table I), *D. donianum* TARDIEU var. *aphanoneuron* TAGAWA (Atsubakinoborishida,<sup>3</sup> in Table I), and *D. lobatum* TAGAWA (Kirebakinoborishida,<sup>4</sup> in Table I). Among these taxa, only *D. donianum* exhibits the strong hormone activity and the remaining *D. crassiusculum*, *D. donianum* var. *aphanoneuron*, and *D. lobatum* show only little or no activity. Therefore, the detailed classification of this group may be significant from the viewpoint of chemotaxonomy. Further, the results of the present screening test on certain plants are contradictory to those of the previous reports.<sup>5,6)</sup> Therefore, variation of contents depending upon the seasons and locations may be significant. On the whole, it was noticed that the activity of a sample collected in autumn tends to be higher than that collected in spring. Whereupon it follows that description of the collecting dates and locations is considered to be indispensable to the record of the hormone activity of plant materials.

### Experimental

**Materials**—A total of 871 specimens were selected, most of which were wild and some of which were cultivated at herbal gardens. Samples from whole plants were subjected to the following extraction for bioassay. Sampling from a large fern was performed so that a sample represented a whole plant as complete 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 reduced pressure to give the dried residue which was dissolved in 10% aqueous EtOH (1 ml). The filtrate was used as a test solution.

**Biological Assay**—Assays were carried out on larvae of *Sarcophaga peregrina* as described by Ohtaki, *et al.*<sup>8)</sup> Thus, final instar larvae, which at 24 hr after ligation formed puparia at their anterior ends but did not pupate behind ligation, were employed as test insect.

8) T. Ohtaki, R.D. Milkman, and C.M. Williams, *Biol. Bull.*, **135**, 332 (1968).

9) Taxa each of which contains at least one active specimen are counted as positive.

An aliquot of the test solution (10  $\mu$ l) was injected into each isolated larval abdomen which was kept at 26°. Ten individuals were used for each plant 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 per cent puparium formation.<sup>8)</sup> The results in Table I are expressed in terms of the activity indexes as -, +, ++, ###, ####, and #####, these indexes being corresponding to the following average per cent puparium formation: - = 0, 0 < +  $\leq$  20, 20 < ++  $\leq$  40, 40 < ###  $\leq$  60, 60 < ####  $\leq$  80, 80 < #####  $\leq$  100%.

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