

Notes

Toshikazu Harada and Yasuhisa Saiki: Pharmaceutical Studies on Ferns. VIII.^{1, 2)} Distribution of Flavonoids in Ferns.³⁾ (2)

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The previous paper³⁾ reported existence of flavonoid compounds in many ferns, and showed that they were almost all flavonoid glycosides, but the known flavonoid compounds number nearly 260, and too many to be determined by paper chromatography that they must be examined from their hydrolysed products. The fern flavonoid aglycones were explored by paper chromatography, and obtained new information. Though, there are nearly 130 kinds of flavonoid aglycones in flowering plants, most of them are included in a few particular plants and the widely distributed flavonoid aglycones are merely naringenin, apigenin, luteolin, kaempferol, quercetin, isorhamnetin, and myricetin. The writers thought that common flavonoids might exist in ferns and compared them by one dimensional paper chromatography. Solvents used are AcOH·H₂O (volume ratio 3:2(1), and 3:17(2)) and PhOH saturated with H₂O (3).

According to the writers' study and to the report of Gage, *et al.*,⁴⁾ (1) gives good R_f values for almost all flavonoid compounds and the values become higher in the order of flavonol, flavone, and flavanone, the smaller the number of hydroxyl groups, the higher the R_f values become. (2) gives good R_f values only for flavanone and flavonoid glycosides, and flavone and flavonol aglycones stop near the starting point. (3) gives R_f for almost all flavonoids, but are occasionally interfered by other pigments.

In the present experiments, spotty ferns in previous report were compared with flavonoid samples, and distribution of luteolin, apigenin, kaempferol, and quercetin in many ferns, and absence of morin, myricetin, isorhamnetin, hesperetin, liquiritigenin, naringenin, acacetin, baicalein, and wogonin were known. Distribution of matteucinol⁵⁾ and desmethoxymatteucinol,⁶⁾ contained only in *Pentarhizidium japonicum* Hayata (= *Matteuccia orientalis* Trautv.), were unknown as obscure coloring in spite of existence of resembling spots in some ferns.

Therefore, most flavonoids of ferns are indistinct except luteolin, apigenin, quercetin and kaempferol, and seem new materials or rare flavonoids. Majority of these ferns are hardly gathered in a large amount and contain less than 0.1% of dry matter. The writers, therefore, intend to study ferns which can be collected easily.

TABLE I. Paper Chromatography of Flavonoid Aglycones

Compounds	m.p.* (°C)	R _f × 100			Color of Spots				
		AcOH·H ₂ O		H ₂ O-satd. PhOH					
		3:2	3:17		(1)	(2)	(3)	(4)	(5)
Flavones									
Apigenin	>330	78	04	95	—	Y	FY	Y	FYG

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1) Paper presented at the Annual Meeting of the Pharmaceutical Society of Japan (1955).

2) Part VII: Y. Kishimoto: J. Pharm. Soc. Japan, **75**, 1437(1955).

3) Part VI: *Ibid.*, **75**, 267(1955).

4) Gage, *et al.*: Anal. Chem., **23**, 1582(1951); Casteel, *et al.*: *Ibid.*, **25**, 508(1953).

5) T. Munesada: J. Pharm. Soc. Japan, **44**, 187(1924).

6) S. Fujise: J. Chem. Soc. Japan, **50**, 111(1929); *ibid.*, **55**, 1024(1934).

Luteolin	316	55	04	90	Y	Gy	Gy	Y	Gy
Acacetin	251	79	00	93	—	—	—	Y	Gy
Baicalein	260	72	12	76	Y	Gy	GyBr	Y→Gy	DBr
Wogonin	198	84	00	87	Y	Gy	Gy	Y	PBr
Flavonols									
Kaempferol	267	50	04	74	Y	Y	Y	Y	DY
Morin	290	70	15	65	Y	YG	YG	Y	DY
Quercetin	305	38	04	45	Y	Y	Y	Br	DBr
Isorhamnetin	300	45	04	88	Y	Y	Y	Y	DY
Myricetin	350	27	03	22	Y	YBr	YBr	Y→G→V	Br
Flavanones									
Hesperetin	233	87	40	87	—	YG	WBl	YBr	—
Naringenin	248	85	48	95	—	YG	Bl	YBr	LY
Liquiritigenin	203	87	43	83	—	—	FBl	YBr	Cr
Matteucinol	174	86	00	85	—	Gy	Gy	PY	Gy

* All melting points are uncorrected.

One-dimensional ascending method: Toyo Roshi No. 50.

(1) Under ordinary light in untreated case; (2) under ultraviolet light in untreated case; (3) under ultraviolet light with use of MgAc_2 -EtOH solution; (4) under ordinary light with use of 1% aq. Na_2CO_3 ; (5) under ultraviolet light with use of 1% aq. Na_2CO_3 .

Abbreviations: Bl: Blue Br: Brown Cr: Cream D: Dark F: Faint G: Green Gy: Gray L: Light P: Pale V: Violet Y: Yellow —: Colorless, →: Color changes

TABLE II. Detection of Flavonoid in Ferns by Paper Chromatography

Name of Fern ^{a)}	K	Q	A	L	Other Spots	Name of Fern ^{a)}	K	Q	A	L	Other Spots
Psilotaceae						<i>Mecodium badium</i> Cop.					1
<i>Psilotum nudum</i> Griesb.					2	<i>M. polyanthos</i> Cop.	+				2
Lycopodiaceae						<i>M. flexile</i> Cop.					1
<i>Lycopodium Fordii</i> Bak.					1	<i>Nesopteris thysanostoma</i> Cop.	+				
<i>L. Sieboldii</i> Miq.					1	Pteridaceae					
<i>L. nikoense</i> Fr. et Sav.					1	<i>Aleuritopteris argentea</i> Fée					2
<i>L. serratum</i> Thunb.			+			<i>A. Kuhni</i> Ching var.					1
<i>L. obscurum</i> L.					1	<i>Brandt</i> Tagawa					
<i>L. Phlegmaria</i> L.			+			<i>Cheilanthes mysurensis</i> Wall.			+		
<i>L. clavatum</i> L.			+			<i>Cibotium Barometz</i> J. Sm.	+				
<i>L. cernuum</i> L.			+			<i>Coniogramme intermedia</i>					1
Selaginellaceae						Hieron.					
<i>Selaginella tamariscina</i> Sp.			+			<i>Coptidopteris Wilfordii</i> Nakai					1
<i>S. pachystachys</i> Koids.			+			et Momose			+		
Equisetaceae						<i>Dennstaedtia hirsuta</i> Mett.					1
<i>Equisetum ramoissimum</i> Desf.			+	+		<i>D. scabra</i> Moore					1
Marattiaceae						<i>Monachosorum flagellare</i>			+	+	
<i>Angiopteris lygodiiifolia</i> Ros.				+		Hayata					
Ophioglossaceae						<i>Onychium japonicum</i> Kuntze					3
<i>Botrychium japonicum</i> Und.				+	1	<i>Pteridium aquilinum</i> Kuhn ^{b)}	+	+	+		1
<i>B. ternatum</i> Sw.				+		<i>Pteris multifida</i> Poir.		+			2
<i>B. strictum</i> Und.			+		1	<i>P. cretica</i> L.				+	
<i>B. virginianum</i> Sw.					1	var. <i>albolineata</i> Hook.				+	1
<i>Ophioderma pendulum</i> Pr.			+		1	<i>P. dispar</i> Kuntze		+		+	1
Osmundaceae						<i>P. kiuschiuensis</i> Hieron.					1
<i>Osmunda lancea</i> Thunb.			+		1	<i>P. oshimensis</i> Hieron.					2
<i>O. bromeliifolia</i> Cop.			+			<i>P. Wallichiana</i> Ag.			+	+	1
<i>O. cinnamomea</i> L.					4	<i>Ptilopteris Maximowiczii</i>					1
Gleicheniaceae						Hance					
<i>Dicranopteris dichotoma</i>			+	+	+	<i>Stenoloma chusanum</i> Ching				+	3
Bernh.					1	Parkeriaceae					
Schizaeaceae						<i>Ceratopteris siliquosa</i> Cop.				+	
<i>Lygodium japonicum</i> Sw.					1	Plagiogyriaceae					
Hymenophyllaceae						<i>Plagiogyria japonica</i> Nakai					1
<i>Gonocormus minutus</i> Bosch.					1	<i>P. euphlebia</i> Mett.					2
<i>Hymenophyllum barbatum</i>			+			<i>P. Matsumuraeana</i> Makino					5
Bak.											

Name of Fern ^{a)}	K	Q	A	L	Other Spots	Name of Fern ^{a)}	K	Q	A	L	Other Spots
Cyateaceae						<i>Polystichum polyblepharum</i> Pr. var.					1
<i>Cyatea boninshimensis</i> Cop.					1	<i>fibrilospaleaceum</i> Tagawa					1
Aspidiaceae						<i>P. pseudo-Makinoi</i> Tagawa					3
<i>Acystopteris japonica</i>	+					var. <i>ambiguum</i> Tagawa					1
Nakai						<i>P. Makinoi</i> Tagawa					2
<i>Athyrium mesosorum</i> Makino				+	1	<i>P. Kurokawae</i> Tagawa					1
<i>A. unifurcatum</i> Chr.			+			<i>P. retrosopaleaceum</i> Tagawa					2
<i>A. iseanum</i> Ros.			+			var. <i>ovatopaleaceum</i> Tagawa					3
<i>A. melanolepis</i> Chr.					1	<i>P. microchlamys</i> Kodama					1
<i>A. nipponicum</i> Hance		+		+		<i>P. craspedosorum</i> Diels.				+	1
<i>A. otophorum</i> Koids.					1	<i>Pseudathyrium alpestre</i>				+	
<i>A. Vidalii</i> Nakai					1	Newm.					
<i>A. yokoscense</i> Chr.					1	<i>Rumohra Standishii</i> Ching	+				1
<i>A. Wardii</i> Makino				+		<i>R. mutica</i> Ching			+		1
<i>Bolbitis Nakaii</i> H. Ito					2	<i>R. pseudo-aristata</i> Tagawa					1
<i>Ctenitis shikokiana</i> H. Ito					2	<i>R. aristata</i> Ching					1
<i>Cyclosorus goggilodus</i> Farw.	+	+			3	<i>R. simplicior</i> Ching					2
var. <i>glaber</i> H. Ito						var. <i>major</i> H. Ito					1
<i>Cyrtomium Fortunei</i> J. Sm.					3	<i>R. amabilis</i> Ching					1
var. <i>clivicola</i> Tagawa					3	<i>R. nipponica</i> Ching					1
<i>C. Yamamotoi</i> Tagawa					1	<i>Sagenia pteropus</i> Moore					1
<i>C. falcatum</i> Pr.					3	<i>Thelypteris palustris</i> Schott.	+				
<i>Dictyocline Griffithii</i> Moore					1	<i>T. oligophlebia</i> Ching		+	+		2
<i>Diplaziopsis Cavaleriana</i> Chr.					1	var. <i>elegans</i> Ching					
<i>Dryopteris crassirhizoma</i>					2	var. <i>subtripinnata</i> H. Ito	+				
Nakai						<i>T. laxa</i> Ching					2
<i>D. polylepis</i> Chr.				+		<i>T. japonica</i> Ching					1
<i>D. tokyoensis</i> Chr.	+					<i>T. glanduligera</i> Ching				+	1
<i>D. cycadina</i> Chr.					1	<i>T. quelpaertensis</i> Ching	+	+		+	1
<i>D. monticola</i> Chr.	+				1	Blechnaceae					
<i>D. sparsa</i> Kuntze	+					<i>Struthiopteris amabilis</i> Tagawa					1
<i>D. Sabaei</i> Chr.					2	<i>S. nipponica</i> Nakai					2
<i>D. erythrosora</i> Kuntze					1	<i>Woodwardia orientalis</i> Sw.					2
<i>D. cystolepidota</i> Chr.					1	<i>W. unigemmata</i> Nakai					2
<i>D. hondoensis</i> Koids.					1	Aspleniaceae					
<i>D. decipiens</i> Kuntze	+					<i>Asplenium normale</i> Don.					1
<i>D. Championi</i> Ching					1	<i>A. Trichomanes</i> L.	+		+		
<i>Gifubenishida</i> (<i>D. kinkiensis</i>					1	<i>A. anceps</i> L.	+				
Tagawa)						<i>A. prolongatum</i> Hook.					1
<i>D. sacrosancta</i> Koids.					1	<i>A. Sarelii</i> Hook.				+	
<i>D. varia</i> Kuntze					2	var. <i>pekinense</i> Chr.					
<i>D. gymnophylla</i> Chr.					1	<i>A. ritoense</i> Hayata				+	
<i>D. chinensis</i> Koids.					1	<i>Phyllitis scolopendrium</i> Newm.				+	1
<i>D. formosana</i> Hayata				+	1	Polypodiaceae					
<i>D. austriaca</i> Woy. Schinz					1	<i>Artemesia Lehmanni</i> Ching					1
et Thell.						<i>Cheiropleuria bicuspis</i> Pr.					1
<i>D. amurensis</i> Chr.					2	var. <i>integrifolia</i> Eaton					
<i>Glaphyopteris falciloba</i> H. Ito					3	<i>Colysis elliptica</i> Ching					1
<i>Gymnocarpium Dryopteris</i>					1	<i>C. pothifolia</i> H. Ito					1
Newm.						<i>Crypsinus hastatus</i> Thunb.					2
<i>Leptorumohra Miqueliana</i>					1	<i>C. Engleri</i> Cop.	+				
H. Ito						<i>Drynaria coronans</i> J. Sm.					1
<i>Matteuccia Struthiopteris</i>	+	+			2	<i>Lemmaphyllum</i>					2
Todaro						<i>microphyllum</i> Pr.					
<i>Onoclea sensibilis</i> L.	+	+			2	<i>Lepisorus</i>					4
var. <i>interrupta</i> Maxim						<i>Thunbergianus</i> Ching					1
<i>Pentarhizidium japonicum</i> ^{c)}					3	<i>L. annuifrons</i> Ching					2
Hayata						<i>L. ussuriensis</i> Ching					
<i>Phegopteris subaurita</i> Tagawa					2	<i>Loxogramme</i>				+	1
<i>P. polypodioides</i> Fée	+				1	<i>Saziran</i> Tagawa					1
<i>Physematum manchuriense</i>	+					<i>L. salicifolia</i> Makino					1
Nakai											

Name of Fern ^{a)}	K	Q	A	L	Other Spots	Name of Fern ^{a)}	K	Q	A	L	Other Spots
<i>Microsorium</i>					2	<i>Pyrrosia lingua</i> Farwl.					1
<i>Buergerianum</i> Ching						<i>P. hastata</i> Ching					2
<i>Prosaptia urceolaris</i> Cop.	+				1						

a) Classification of the family according to Copeland: "Genera Filicum" (1947).

b) Under study by Y. Ohshima (Kyushu University).

c) Except matteucinol and desmethoxymatteucinol.

Abbreviations: K: Kaempferol, Q: Quercetin, A: Apigenin, L: Luteolin,

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Experimental

Method of Paper Chromatography—Toyo Roshi No. 50 (2×40 cm.) was used in one-dimensional ascending method.

Testing Solution of Fern Flavonoids—Dry cut leaves of fern (5~10 g.) were extracted with cold MeOH during 24 hrs. After evaporation of MeOH, its residue was hydrolysed with 5% aq. H₂SO₄ in boiling water bath for 5~6 hrs. The hydrolysed solution was extracted with EtOAc, shaking vigorously to extract the precipitate, and the EtOAc extract was concentrated.

Samples of Flavonoid Aglycones—Apigenin was extracted from flowers of *Daphne Genkwa* Sieb. et Zucc.⁸⁾ Luteolin was given by Dr. T. Nakaoki. Acacetin was from the University of Kyoto. Wogonin and baicalein were extracted from the root of *Scutellaria baicalensis* Georgi.⁹⁾ Quercetin and myricetin were obtained by hydrolysis of rutin and myricitrin. Morin (Merck). Kaempferol was extracted from *Dicranopteris dichotoma* Bernh.²⁾ by Kishimoto. Isorhamnetin was from the pollen of *Typha angustata* Bory et Chaub.¹⁰⁾ Hesperetin was from the pericarps of *Citrus Unshu* Marcov.¹¹⁾ Naringenin was from the pericarps of *Citrus Natsudaidai* Hayata.¹²⁾ Liquiritigenin was from the root of *Glycyrrhiza* spp.¹³⁾ Matteucinol was from leaves of *Pentarrhizidium japonicum* Hayata.⁶⁾

Summary

Flavonoids were explored in 290 kinds of ferns by paper chromatography, developed with AcOH·H₂O (3:2 and 3:17) and PhOH saturated with water, by one-dimensional ascending chromatography. As a result, luteolin, apigenin, quercetin, and kaempferol were found in some ferns, but many unknown spots were found which were assumed to be new materials or rare flavonoids.

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