筑波実験植物園研報 11: 23-34, 1992.
Ann. Tsukuba Bot. Gard.

The Flavonoid Glycosides in the Leaves of *Cornus* Species II. The Flavonoids of *C. canadensis* and *C. suesica*

By

Tsukasa IWASHINA* and Hiroaki HATTA*

岩科 司*・八田洋章*：ミズキ属植物の葉に含まれるフラボノイド配糖体
II. ゴゼンタチバナおよびエゾゴゼンタチバナのフラボノイド

Cornus canadensis L. and *C. suecica* L. (Cornaceae), small evergreen perennial herbs, are widespreadly distributed in circumpolar zone of Northern Hemisphere. In Japan, the former species is found in Hokkaido, Chûbu and Tôhoku areas of Honshu, and rarely in Nara and Ehime Prefectures, and the latter is found in eastern Hokkaido only in Japan (Kitagawa 1982). The two species were classified to section *Arctocrania* (Bentham and Hooker 1867), to sub-genus *Arctocrania* (Wangerin 1910, Ferguson 1966 a, b), or were given independence from *Cornus* as the genus *Chamaepericlymenum* Hill. (Hutchinson 1942, 1967, Kitagawa 1982).

In our chemotaxonomic study which clarify the flavonoid profiles of *Cornus* species, we have found quercetin 3-*O*-glucoside (isoquercitrin) from *C. controversa* Hemsl., *C. brachypoda* Wall., *C. darvasica* (Pojark.) Pilip. and *C. drummondii* C. A. Mey., and quercetin 3-*O*-rhamnoside (quercitrin) in addition to isoquercitrin from the former two species (Iwashina and Hatta 1990). Moreover, partially characterized another quercetin glycoside from *C. brachypoda* and kaempferol 3-*O*-glycoside from *C. darvasica* (Iwashina and Hatta 1990).

In this paper, we report the isolation and identification of flavonoids in the leaves of *C. canadensis* and *C. suecica.*

Materials and Methods

Plant materials

C. canadensis was collected in Mt. Akaishi, Shizuoka Pref., Japan and *C. suecica* in Shibetsu, Hokkaido, Japan.

Isolation of flavonoids

The fresh leaves (143 g) of *C. canadensis* were extracted with methanol, filtrated and evaporated to dryness. After monitored the flavonoid composition by two-dimensional paper-chromatography (2D-PC), crops were dissolved with water, shaken with petroleum ether and then ethyl acetate (EtOAc). EtOAc Layer which contained almost flavonoid compounds was concentrated to dryness, dissolved with 70% methanol,

* Tsukuba Botanical Garden, National Science Museum, Tsukuba, 305. 国立科学博物館 筑波実験植物園.

subjected in polyamide column (Polyamide C–200, Wako Pure Chemicals, i.d. 30 mm × 400 mm), and eluted with 70% methanol. The fractions which contained flavonoid **4** were combined, evaporated, applied to sephadex LH–20 column (Pharmacia, i.d. 10 mm × 200 mm) and eluted with 70% methanol. After cooling for 1 week, yellow needles were crystalized from methanol. Yield ca. 20 mg. Other fractions having same flavonoid composition were also combined, applied to preparative paper-chromatography (PPC) using BAW and then 15%AcOH (see below). Eight flavonoids (**1–3, 5–9**) were obtained as methanol solutions, and finally purified by sephadex LH–20 column (70% methanol).

Paper-chromatography

Solvent systems using to paper-chromatography (including PPC and 2D–PC) are follows: BAW (n–BuOH–AcOH–H_2O=4 : 1 : 5, upper phase), BEW (n–BuOH–EtOH–H_2O=4 : 1 : 2.2), Forestal (AcOH–conc.HCl–H_2O=30 : 3 : 10), 15%AcOH (AcOH–H_2O=15 : 85) and 5%AcOH (AcOH–H_2O=5 : 95) for flavonoid glycosides or aglycones; BBPW (n–BuOH–Benzene–Pyridine–H_2O=5 : 1 : 3 : 3) and BTPW (n–BuOH–Toluene–Pyridine–H_2O=5 : 1 : 3 : 3) for glycosidic sugars.

High-performance liquid chromatography (HPLC)

HPLC Separations were performed with JASCO HPLC systems including a 880–PU pump, 880–51 2–line degasser and Syringe loading sample injector 25 model 7125 (Rheodyne Inc.). Multi channel UV–visible detector Multi–330 coupled with a computer was used recording chromatograms and UV spectra. A Finepak SIL $C_{18}S$ column 5 μm (i.d. 4.6 mm × 150 mm) was used. Crude methanol extracts or authentic flavonoid solutions were filtrated through Toyopak ODS M (Tosoh) and then Maisyoridisc, 0.45 μm (Tosoh), and eluted with acetonitrile–H_2O–H_3PO_4 (22 : 78 : 0.2). Detection was at 340 nm and flow-rate was 1.0 ml/min.

UV spectra

UV Spectra of flavonoid glycosides and their aglycones were measured in methanol solutions before and after addition of sodium methylate (NaOMe), $AlCl_3$, $AlCl_3$+HCl, sodium acetate (NaOAc) or NaOAc+H_3BO_3 according to Mabry *et al.* (1970).

Acid hydrolysis

Flavonoid glycosides were hydrolyzed with 6% aq.HCl for 30 min. on a boiling water bath. After cooling, the solution was shaken with ether, whereby aglycones in ether phase and sugars in mother liquor were separated from each other.

Fast atom bombardment mass-spectra (FAB–MS)

FAB–MS was measured using nitrobenzyl alcohol (NBA).

Results and Discussion

Flavonoid identification from C. canadensis.

Nine flavonoids were isolated from the leaves of *C. canadensis.*

Flavonoid **1** (quercetin 3-*O*-xylosylgalactoside).

UV Spectra of this flavonoid on addition of $AlCl_3$, $AlCl_3$+HCl or NaOAc+H_3BO_3 showed the presence of free hydroxyls at the 5-, 3′- and 4′-positions (Table 2) (Mabry *et al.* 1970). The presence of free 7-hydroxyl and a substituted 3-hydroxyl group were proved by UV spectra on addition of NaOAc and NaOMe, respectively. An aglycone and two sugars which were obtained by acid hydrolysis were identified as quercetin, galactose and xylose by direct comparisons with authentic specimens (Table

Table 1. Chromatographic and HPLC data of flavonoids from *C. canadensis*

Flavonoids	Rf-values				Colors		HPLC Rt*
	BAW	BEW	15%AcOH	5%AcOH	UV	UV/NH_3	
1	0. 33	0. 46	0. 61	0. 43	dark purple	yellow	6. 18
2	0. 43	0. 57	0. 60	0. 41	dark purple	dark greenish yellow	8. 56
3	0. 61	0. 74	0. 46	0. 24	dark purple	dark greenish yellow	10. 89
4	0. 48	0. 59	0. 40	0. 17	dark purple	yellow	8. 01
5	0. 61	0. 74	0. 46	0. 24	dark purple	dark greenish yellow	12. 62
6	0. 43	0. 37	0. 28	0. 14	dark purple	dark yellow	8. 18
7	0. 43	0. 57	0. 60	0. 41	dark purple	dark greenish yellow	11. 41
8	0. 37	0. 52	0. 37	0. 19	dark purple	dark yellow	7. 37
9	0. 82	0. 85	—	—	yellow	greenish yellow	—
Authentic specimens:							
kaempferol 3-rutinoside	0. 43	0. 57	0. 59	0. 39	dark purple	dark greenish yellow	8. 56
quercetin 3-rutinoside	0. 30	0. 44	0. 54	0. 36	dark purple	yellow	7. 57
quercetin 3-glucoside	0. 50	0. 61	0. 41	0. 20	dark purple	yellow	8. 93
quercetin 3-galactoside	0. 48	0. 59	0. 39	0. 17	dark purple	yellow	8. 01
kaempferol 3-galactoside	0. 61	0. 74	0. 46	0. 24	dark purple	dark greenish yellow	10. 89
quercetin 3-rhamnoside	0. 65	0. 75	0. 52	0. 31	dark purple	dark yellow	13. 57

BAW=n-BuOH-AcOH-H_2O (4 : 1 : 5, upper phase), BEW=n-BuOH-EtOH-H_2O (4 : 1 : 2.2), 15% AcOH=AcOH-H_2O (15 : 85) and 5%AcOH=AcOH-H_2O (5 : 95).

* Eluent: Acetonitrile-H_2O-H_3PO_4 (22 : 78 : 0.2), Flow-rate: 1.0ml/min, Injection: 10 μl.

1=quercetin 3-xylosylgalactoside, **2**=kaempferol 3-rutinoside, **3**=kaempferol 3-galactoside, **4**=quercetin 3-galactoside, **5**=kaempferol 3-glucoside, **6**=quercetin 3-glycoside, **7**=kaempferol 3-xylosylgalactoside, **8**=quercetin 3-glycosylgalactoside (probably 3-apiosylgalactoside) and **9**=kaempferol (free).

Table 2. UV spectral properties of flavonoids from *C. canadensis*

Flavonoids	λmax (nm)					
	in MeOH	+NaOMe	+$AlCl_3$	+$AlCl_3$+HCl	+NaOAc	+NaOAc+H_3BO_3
1	256	272	275	269	274	262
	264sh	329	435	297sh	323	377
	357	406*		359	387	
				402		
2 and 7	266	275	274	275	275	267
	296sh	326	305	303	307	354
	350	400*	353	348	383	
			398	395		
3	266	275	273	275	274	267
	296sh	324	305	302	309	353
	351	399*	352	347	385	
			398	398		
4	256	272	275	269	275	262
	266sh	328	439	300	322	379
	360	409*		364	388	
				405		
5	266	275	274	275	274	267
	298sh	325	304	302	309	353
	351	399*	352	346	386	
			398	397		
6	259	274	275	270	274	262
	263sh	324sh	435	298sh	321	378
	360	410*		365	385	
				400		
8	259	275	276	271	274	263
	264sh	325	438	298sh	321	299
	360	408*		363sh	385	378
				403		
9	266	281	269	269	275	267
	291sh	422	305	304	308	368
	366	(dec)	352	350	388	
			422	422	(dec)	

sh=shoulder, dec=decomposition.

* Remarkable increase in intensity relative to the peak of methanolic solution.

3 and 4). Their data showed the attachment of xylose and galactose to 3-hydroxyl of quercetin. Moreover, FAB-MS indicated $[M-H]^-$ at m/z 595, calcd for $C_{26}H_{28}O_{16}$, which showed that quercetin, galactose and xylose were each 1 mol, and [M-xylogalactosyl-H]$^-$ at m/z 301 (aglycone). Accordingly, flavonoid **1** was identified as quercetin 3-*O*-xylosylgalactoside (Fig. 2) which have been found in the leaves of *Armoracia rusticana* G., M. and Sch. (Cruciferae) as quercetin 3-*O*-(2″-*O*-*β*-D-xylopyranosyl)-*β*-D-galactopyranoside (Larsen *et al.* 1982).

Flavonoids **2** (kaempferol 3-*O*-rutinoside) and **7** (kaempferol 3-*O*-xylosylgalactoside).

Their flavonoids could not be isolated by PPC, since they have extremely similar Rf values (Table 1), so that the flavonoids were obtained as a mixture solution. Acid hydrolysis of their glycosides gave kaempferol and four sugars, i.e. glucose, galactose, rhamnose and xylose which were identified by direct comparisons with authentic specimens (Table 3 and 4). UV Spectra of the mixture on addition of various reagents showed the presence of free 5, 7, 4′-trihydroxyls and a substituted 3-hydoxyl which exhibited that both flavonoids were kaempferol 3-*O*-glycosides. In HPLC survey, the mixture appeared as two peaks (Retention times **2**: 8.56 and **7**: 11.41) and Rt of flavonoid **2** coincided with that of authentic kaempferol 3-*O*-rutinoside. Accordingly, of their glycosides, flavonoid **2** was regarded as kaempferol 3-*O*-*α*-L-rhamnosyl (1→6) glucoside, and another flavonoid **7** must be kaempferol 3-*O*-xylosylgalactoside. Moreover, FAB-MS exhibited $[M-H]^-$ at m/z 593, calcd for $C_{27}H_{30}O_{15}$ corresponding to kaempferol 3-*O*-rhamnosylglucoside, $[M-H]^-$ at m/z 579, calcd for $C_{26}H_{28}O_{15}$ corresponding to kaempferol 3-*O*-xylosylgalactoside, [M-rhamnosyl-H]$^-$ and [M-xylosyl-H]$^-$ at m/z 447 showing direct attachment of glucose or galactose to kaempferol, and [M-rhamnoglucosyl-H]$^-$ and [M-xylogalactosyl-H]$^-$ at m/z 285 (aglycone).

From the results described above, flavonoid **2** and **7** were identified as kaempferol 3-*O*-rutinoside (nicotiflorin) and 3-*O*-xylosylgalactoside, respectively (Fig. 2). Nicotiflorin have been found in many plant species, e.g. *Nicotiana sylvestris* Speg. et Comes.

Table 3. Chromatographic data of flavonoid aglycones obtained by acid hydrolysis

Aglycones	Rf-values			Colors	
	BAW	BEW	Forestal	UV	UV/NH_3
1	0. 65	0. 71	0. 43	yellow	bright yellow
2	0. 82	0. 85	0. 59	yellow	greenish yellow
3	0. 82	0. 85	0. 59	yellow	greenish yellow
4	0. 65	0. 71	0. 43	yellow	bright yellow
5	0. 82	0. 85	0. 59	yellow	greenish yellow
6	0. 65	0. 71	0. 43	yellow	bright yellow
7	0. 82	0. 85	0. 59	yellow	greenish yellow
8	0. 65	0. 71	0. 43	yellow	bright yellow
Authentic specimens:					
quercetin	0. 65	0. 71	0. 43	yellow	bright yellow
kaempferol	0. 82	0. 85	0. 59	yellow	greenish yellow

Forestal=AcOH-conc.HCl-H_2O (30 : 3 : 10).

Table 4. Chromatographic data of glycosidic sugars obtained by acid hydrolysis

Sugars	Rf-values		Colors	Identity
	BBPW	BTPW	Aniline hydrochloride	
1	0. 24	0. 17	brown	galactose
	0. 40	0. 33	red-brown	xylose
2+**7**	0. 25	0. 17	brown	galactose
	0. 28	0. 17	brown	glucose
	0. 53	0. 50	yellow-brown	rhamnose
	0. 42	0. 35	red-brown	xylose
3	0. 25	0. 17	brown	galactose
4	0. 24	0. 17	brown	galactose
5	0. 28	0. 17	brown	glucose
8	0. 24	0. 17	brown	galactose
	0. 63	0. 58	yellow-brown	unknown (apiose?)
Authentic specimens :				
galactose	0. 25	0. 17	brown	
glucose	0. 28	0. 18	brown	
allose	0. 30	0. 25	brown	
mannose	0. 35	0. 30	brown	
rhamnose	0. 53	0. 50	yellow-brown	
xylose	0. 42	0. 35	red-brown	
arabinose	0. 35	0. 29	red-brown	

BBPW=n-BuOH-Benzene-Pyridine-H_2O (5 : 1 : 3 : 3),
BTPW=n-BuOH-Toluene-Pyridine-H_2O (5 : 1 : 3 : 3).

(Solanaceae) (Wada 1952), *Hyptis capitata* Jacq. (Labiatae) (Kobayashi 1952), *Cerbera manghas* L. (Apocynaceae) (Sakushima *et al.* 1976), *Echinopsis huotii* Lab. (Cactaceae) (Iwashina *et al.* 1986). On the other hand, kaempferol 3-*O*-xylosylgalactoside have been reported from *Armoracia rusticana* with quercetin 3-*O*-xylosylgalactoside (**1**) described above as kaempferol 3-*O*-(2″-*O*-*β*-D-xylopyranosyl)-*β*-D-galactopyranoside (Larsen *et al.* 1982).

Flavonoid **3** (kaempferol 3-*O*-galactoside).

Kaempferol and galactose which were identified by direct comparisons with authentic specimens (Table 3 and 4) were given by acid hydrolysis of this flavonoid **3**. UV Spectral survey of the original component showed the presence of free hydroxyl groups at the 5-, 7- and 4′-positions and a substituted hydroxyl group at the 3-position (Table 2).

Finally, flavonoid **3** was determined as kaempferol 3-*O*-galactoside (trifolin, Fig. 2) by careful comparison of PC and HPLC behavior with authentic samples (Table 1). Trifolin has also been found in many plants, e.g. *Panax ginseng* C. A. Meyer (Araliaceae) (Komatsu *et al.* 1969), *Cirsium arvense* (L.) Scopili (Compositae) (Wallace 1974), *Rhododendron* spp. (Ericaceae) (King 1977) etc.

Flavonoid **4** (quercetin 3–*O*–galactoside).

Acid hydrolysis of flavonoid **4** which was obtained as yellow needles gave quercetin and galactose which were identified by direct comparisons with authentic samples (Table 3 and 4). The attachment of galactose moiety to 3–hydroxyl group on quercetin was showed by UV spectral analysis (Table 2).

Finally, PC and HPLC data of this compound were completely agreed with those of authentic quercetin 3–*O*–galactoside (hyperin, Fig. 2) from the tepals of *Notocactus ottonis* (Lehm.) Berg. (Iwashina *et al.* 1982). Hyperin has been reported from the barks of *Cornus stolonifera* Michx. in Cornaceae (Nair and Rudloff 1960) and many species in other families.

Flavonoid **5** (kaempferol 3–*O*–glucoside).

Flavonoid **5** having very similar Rf-values with those of kaempferol 3–*O*–galactoside (**3**) (Table 1) gave kaempferol and glucose by acid hydrolysis (Table 3 and 4). UV Spectral analysis of the original glycoside exhibited the presence of free hydroxyls at the 5–, 7– and 4′–positions and a substituted 3–hydroxyl group showing attachment of glucose to 3–position on kaempferol.

From the results described above, flavonoid **5** was identified as kaempferol 3–*O*–glucoside (astragalin, Fig. 2). It has been showed that astragalin was distributed among many plants, e.g. ferns, *Cyrtomium falcatum* Presl. (Kishimoto 1956), *Pteridium aquilinum* (L.) Kuhn (Nakabayashi 1955) and angiosperm, *Begonia* spp. (Harborne and Hall 1964), *Rhododendron* spp. (King 1977), *Anodendron affine* Durce (Shima *et al.* 1972) etc.

Flavonoid **6** (quercetin 3–*O*–glycoside).

The presence of the free 5, 7, 3′, 4′–tetrahydroxyls and a substituted 3–hydroxyl of the flavonoid **6** was showed by UV spectral analysis (Table 2). Though the aglycone which was obtained by acid hydrolysis was identified as quercetin, glycosidic sugar could not be determined on account of the minimal amount of the original glycoside.

Flavonoid **8** (quercetin 3–*O*–apiosylgalactoside?).

Flavonoid **8** was also showed to have free hydroxyl groups at the 5–, 7–, 3′– and 4′–positions and a substituted hydroxyl at the 3–position by UV spectral analysis (Table 2). Two glycosidic sugars and an aglycone which was identified as quercetin by direct chromatographic comparison with authentic specimen (Table 3). Of their sugars, chromatographic properties of one were coincident with those of the authentic galactose. The spots of another sugar showed higher mobilities than those of seven authentic specimens using BBPW and BTPW on the chromatograms (Table 4).

Until now, seven monosaccharides, i.e. glucose, galactose, allose, arabinose, rhamnose, xylose and apiose have been found as glycosidic sugars of flavones and flavonols (Harborne and Williams 1988) except glucuronic acid and galacuturonic acid which were more strongly resistant to common acid hydrolysis (Harborne 1965). Of their

monosaccharides, apiose, which could not be use as authentic specimen, showed most similar chromatographic properties with those of unknown sugar from flavonoid **8** (Harborne 1984). Accordingly, flavonoid **8** was presumed as quercetin 3–*O*–apiosyl-galactoside which have been found in *Securidaca diversifolia* S. F. Blake (Polygalaceae) (Hamburger *et al.* 1985).

Flavonoid **9** (kaempferol).

Flavonoid **9** was soluble in ether and could not be hydrolyzed showing to be the flavonoid aglycone. UV Spectral analysis of this compound exhibited the presence of 3, 5, 7, 4′–tetrahydroxyls (Table 2).

Finally, flavonoid **9** was identified as free kaempferol (Fig. 2) by direct PC comparison with authentic specimen. It has been found that kaempferol was present as free state in some plant species (Wollenweber and Dietz 1981). However, it did not known that whether kaempferol was natural product or artifact from glycosides in isolation or purification.

HPLC Analysis of flavonoids in C. suecica

Five flavonoids were found in the leaves of *C. suecica* by three dimensional HPLC analysis (Fig. 1, see Materials and Methods). Four of 5 flavonoids were identified as

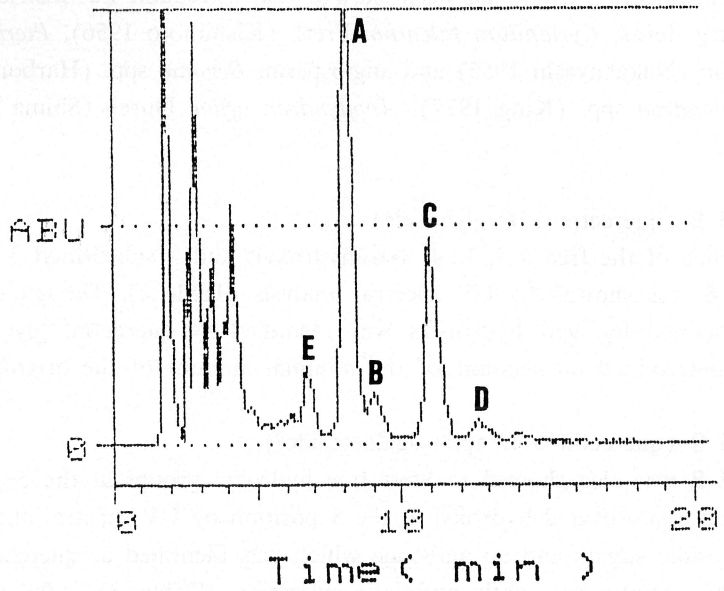
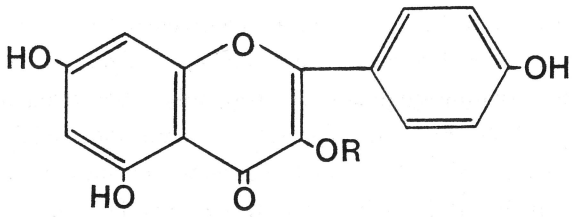
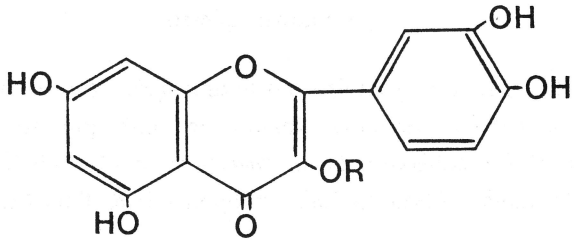


Fig. 1. Separation of flavonoid glycosides in the leaves of *C. suecica* by HPLC.
Eluent: Acetonitrile–H_2O–H_3PO_4 (22 : 78 : 0.2), Flow-rate: 1.0ml/min, Injection: 10 μl, Detection: 340 nm.
A=quercetin 3–galactoside, B=quercetin 3–glucoside, C=kaempferol 3–galactoside, D=kaempferol 3–glucoside and E=unknown flavonoid.
It was proved by UV spectra that all other peaks were not flavonoid.



R=rhamnoglucosyl : kaempferol 3-*O*-rutinoside (nicotiflorin, **2**)
R=galactosyl : kaempferol 3-*O*-galactoside (trifolin, **3**)
R=glucosyl : kaempferol 3-*O*-glucoside (astragalin, **5**)
R=xylogalactosyl : kaempferol 3-*O*-xylosylgalactoside (**7**)
R=H : kaempferol (**9**)



R=xylogalactosyl : quercetin 3-*O*-xylosylgalactoside (**1**)
R=galactosyl : quercetin 3-*O*-galactoside (hyperin, **4**)
R=glycosyl : quercetin 3-*O*-glycoside (**6**)
R=glycogalactosyl : quercetin 3-*O*-glycosylgalactoside (probabry 3-*O*-apiosylgalactoside, **8**)
R=glucosyl : quercetin 3-*O*-glucoside (isoquercitrin)

Fig. 2. The structures of flavonoids from *C. canadensis* and *C. suecica.*

kaempferol 3-*O*-glucoside and 3-*O*-galactoside, and quercetin 3-*O*-glucoside and 3-*O*-galactoside, by comparisons of retention times with authentic specimens. Rt of remained one did not agreed with those of authentic samples which could use as references in this experiment.

Until now, the flavonoids which has been found in *Cornus* species were mainly various quercetin 3-*O*-glycosides, i.e. 3-*O*-glucoside from the leaves of *C. controversa, C. darvasica, C. drummondii,* and *C. brachypoda* (Iwashina and Hatta 1990, Nakaoki and Morita 1958) and the flowers of *C. mas* L. (Egger und Keil 1969), 3-*O*-rhamnoside from the leaves of *C. controversa* and *C. brachypoda* (Iwashina and Hatta 1990), 3-*O*-galactoside from the barks of *C. stolonifera* (Nair and Rudloff 1960), 3-*O*-glucuronide and 3-*O*-rutinoside from the flowers of *C. mas* (Delaveau et Paris 1961, Egger und Keil 1969), and a kaempferol glycoside was reported as minor component in

C. darvasica (Iwashina and Hatta 1990). On the other hand, it was proved in this experiment that five of nine flavonoids in *C. canadensis* were kaempferol and its 3–*O*–glycosides. Moreover, their flavonoids were variously glycosylated with galactose, glucose, xylosylgalactose, rhamnosylglucose or probably apiosylgalactose. *C. suecica* also had two kaempferol 3–*O*–glycosides (galactoside and glucoside) in addition to two quercetin 3–*O*–glycosides (Fig. 1). Such flavonoid profiles of *C. canadensis* and *C. suecica* which were included in sub-genus *Arctocrania* (Ferguson 1966a) were clearly different from other *Cornus* species. While almost *Cornus* species represented comparatively simple flavonoid compositions, *C. canadensis* expressed the complicated glycosylated pattern. If a hypothesis that evolution proceeds were in general to a loss in the ability to synthesize some flavonoids (Hiraoka 1978) is applied, *C. canadensis* is apparently primitive than other *Cornus* species, e. g. *C. controversa, C. darvasica, C. drummondii* and *C. brachypoda.*

Acknowledgement

The authors thank to Dr. Kunijiro Yoshitama (Fac. Sci. of Kumamoto Univ.) for supplying authentic trifolin. Authors' thanks are also due to Mr. Yutaka Tamura (Shibetsu, Hokkaido) for collection of *C. suecica,* and Mr. Yukio Nishizawa and Miss Masami Nishida (Tsukuba Research Lab., Nippon Oil & Fats Co., Ltd.) for FAB–MS measurement.

Summary

Nine flavonoid components in the leaves of *Cornus canadensis* were isolated by column and paper chromatographic manner and identified as quercetin 3–*O*–xylosylgalactoside (**1**), kaempferol 3–*O*–rutinoside (**2**), kaempferol 3–*O*–galactoside (**3**), quercetin 3–*O*–galactoside (**4**), kaempferol 3–*O*–glucoside (**5**), partially characterized quercetin 3–*O*–glycoside (**6**), kaempferol 3–*O*–xylosylgalactoside (**7**), quercetin 3–*O*–glycosylgalactoside (probably 3–*O*–apiosylgalactoside) (**8**) and free kaempferol (**9**). Among their flavonoids, eight were found in *Cornus* species for the first time except quercetin 3–*O*–galactoside. The flavonoid glycosides in *C. suecica* were analyzed by HPLC manner, and quercetin 3–*O*–galactoside and 3–*O*–glucoside, and kaempferol 3–*O*–galactoside and 3–*O*–glucoside were identified by comparisons with authentic specimens. If a hypothesis that evolution proceeds were to a loss in the ability to synthesize some flavonoids is applied, *C. canadensis* is more primitive than other *Cornus* species, e. g. *C. controversa, C. darvasica, C. drummondii* and *C. brachypoda,* since *C. canadensis* showed a comparatively complicated flavonoid pattern than other *Cornus* species which have been surveyed.

摘　　要

ゴゼンタチバナ (*Cornus canadensis*) の葉に含まれる9種類のフラボノイドがカラムおよび

ペーパークロマト法などによって分離され，酸加水分解，UV 吸収スペクトルの測定，基準標品とのペーパークロマトあるいは HPLC による比較，さらには質量スペクトル分析などによって以下のように同定された。すなわち，quercetin 3-*O*-xylosylgalactoside (**1**), kaempferol 3-*O*-rutinoside(nicotiflorin, **2**), kaempferol 3-*O*-galactoside (trifolin, **3**), quercetin 3-*O*-galactoside (hyperin, **4**), kaempferol 3-*O*-glucoside (astragalin, 5), kaempferol 3-*O*-glycoside (6), kaempferol 3-*O*-xylosylgalactoside (**7**), quercetin 3-*O*-glycosylgalactoside (3-*O*-apiosylgalactoside と推定される，**8**) および遊離の kaempferol (**9**)。これらのフラボノイドのうち，以前に *C. stolonifera* の樹皮から報告されている hyperin を除くと，すべてが今までミズキ属で発見されたことのないものである。エゾゴゼンタチバナ (*Cornus suecica*) の葉からは quercetin の 3-*O*-galactoside と 3-*O*-glucoside, kaempferol の 3-*O*-galactoside と 3-*O*-glucoside，および未同定のフラボノイドが HPLC 分析によって検出された。ゴゼンタチバナとエゾゴゼンタチバナは *Arctocrania* 亜属に類別されるが，quercetin の配糖体に加えて kaempferol の配糖体を主要成分として含んでおり，これらの点で従来分析されたミズキ (*C. controversa*)，クマノミズキ (*C. brachypoda*)，*C. darvasica* あるいは，*C. drummondii* とは区別される。また，植物の進化はいくつかのフラボノイドを合成する能力の欠失への方向とする従来の仮説を適用すると，ゴゼンタチバナ (複雑なフラボノイドパターンをもつ) は明らかに今まで分析された他のミズキ属植物 (簡単なフラボノイドパターンをもつ) と比較して原始的であると推論された。

References

Bentham, G. and J. D. Hokker, 1876. Genera plantarum **1** : 947–953.

Delaveau, P. et R. Paris, 1961. Sua la présence de rutoside et de dérivés gallique dans les fleurs de *Cornus mas* L. Recherches préliminaires chez le *C. sanguinea* L. Bull. Soc. Chim. Biol. **43** : 661–666.

Egger, K. und M. Keil, 1969. Flavonolglykoside in Blüten von *Cornus mas* L. Z. Pflanzenphysiol. **61** : 346–347.

Ferguson, I. K., 1966a. Notes on the nomenclature of *Cornus*. J. Arnold Arb. **47** : 100–105.

———, 1966b. The Cornaceae in the southeastern United States. J. Arnold Arb. **47** : 106–116.

Hamburger, M., M. Gupta and K. Hostettmann, 1985. Flavonol glycosides from *Scuridaca diversifolia*. Phytochemistry **24** : 2689–2692.

Harborne, J. B. and E. Hall, 1964. Plant polyphenols—XIII. The systematic distribution and origin of anthocyanins containing branched trisaccharides. Phytochemistry **3** : 453–463.

———, 1965. Plant polyphenols—XIV. Characterization of flavonoid glycosides by acidic and enzymic hydrolyses. Phytochemistry **4** : 107–120.

———, 1984. Phytochemical methods. A guide to modern techniques of plant analysis. Second Ed. Chapman and Hall, London. p. 222–242.

——— and C. A. Williams, 1988. Flavone and flavonol glycosides. *In* Harborne, J. B., The flavonoids : Advances in research since 1980. Chapman and Hall, London. p. 304–328.

Hiraoka, A., 1978. Flavonoid patterns in Athyriaceae and Dryopteridaceae. Biochem. Syst. Ecol. **6** : 171–175.

Huchinson, J., 1942. Neglected generic characters in the family Cornaceae. Ann. Bot. N.S. **6** : 84–93.

———, 1967. The genera of flowering plants. Vol. II. Clarendon Press, Oxford.

Iwashina, T., S. Ootani, T. Gotoh and N. Kondo, 1982. Distribution of flavonol glycosides in the sub-family Cereoideae and some chemotaxonomic consideration thereof. Sci. Rep. Res. Inst. Evolut. Biol. **1** : 83–102 (in Japanese).

———, ——— and K. Hayashi, 1986. Determination of minor flavonol-glycosides and sugar-free flavonols in the tepals of several species of Cereoideae (Cactaceae). Bot. Mag. Tokyo **99** : 53–62.

——— and H. Hatta, 1990. The flavonoid glycosides in the leaves of *Cornus* species I. The flavonoids of *C. controversa, C. brachypoda, C. darvasica* and *C. drummondii*. Ann. Tsukuba Bot. Gard. **9** : 41–47 (in Japanese).

King, B.L., 1977. The flavonoids of the deciduous *Rhododendron* of North America (Ericaceae). Amer. J. Bot. **64** : 350–360.

Kishimoto, Y., 1956. Pharmaceutical studies on the ferns. X. Flavonoids of *Cyrtomium* species (2). On the flavonoid glucosides. Yakugaku Zasshi **76** : 250–253 (in Japanese).

Kitagawa, M., 1982. Cornaceae. *In* Satake, Y., J. Ohwi, S. Kitamura, S. Watari and T. Tominari, Wild flowers of Japan II. Herbaceous plants—Choripetalae. Heibonsha, Tokyo (in Japanese). p. 274.

Kobayashi, K., 1952. Studies on the components of the leaves of *Hyptis capitata* Jacq. Yakugaku Zasshi **72**: 1–3 (in Japanese).

Komatsu, M., T. Tomimori and Y. Makiguchi, 1969. Studies on the constituents of the herbs of *Panax ginseng* C. A. Meyer. II. On the flavonoid constituents. Yakugaku Zasshi **89**: 122–126 (in Japanese).

Larsen, L. M., J. K. Nielsen and H. Sφrensen, 1982. Identification of 3-*O*-[2-*O*-(β-D-xylopyranosyl)-β-D-galactopyranosyl] flavonoids in horseradish leaves acting as feeding stimulants for a flea beetle. Phytochemistry **21**: 1029–1033.

Mabry, T. J., K. R. Markham and M. B. Thomas, 1970. The systematic identification of flavonoids. Springer-Verlag, Berlin. p. 35–164.

Nair, G. V. and E. von Rudloff, 1960. Isolation of hyperin from red-osier dogwood (*Cornus stolonifera* Michx.). Can. J. Chem. **38**: 2531–2533.

Nakabayashi, T., 1955. Studies on thiamine decomposing activity of flavonoid pigments of *Pteridium aquilinum*. Vitamin **8**: 410–414 (in Japanese).

Nakaoki, T. and N. Morita, 1958. Studies on the medicinal resources XII. Components of the leaves of *Cornus controversa* Hemsl., *Ailanthus altissima* Swingl, and *Ricinus communis* L. Yakugaku Zasshi **78**: 558–559 (in Japanese).

Sakushima, A., S. Nishibe, S. Hisada, Y. Noro and Y. Hisada, 1976. Studies on the constituents of Apocynaceae plants. Isolation of flavonol glycosides and some other components from the leaves of *Cerbera manghas* L.(1). Yakugaku Zasshi **96**: 1046–1048 (in Japanese).

Shima, K., S. Hisada and I. Inagaki, 1972. Studies on the constituents of *Anodendron affine* Durce. IV. Isolation of kaempferol, astragalin and dambonitol from leaves. Yakugaku Zasshi **92**: 507–509 (in Japanese).

Wada, E., 1952. Chemical constituents of tobacco. Part 3. Isolation of a kaempferol-3-rhamnoglucoside from the flowers of *N. sylvestris* Speg. et Comes. Nippon Nôgeikagaku Kaishi **26**: 159–162 (in Japanese).

Wallace, J. W., 1974. Tricin 5-*O*-glucoside and other flavonoids of *Cirsium arvense*. Phytochemistry **13**: 2320–2321.

Wangerin, W., 1910. Cornaceae. *In* Engler, A., Das Pflanzenreich. 41. (IV 229). Engelmann, Berlin. p. 1–110.

Wollenweber, E. and V. H. Dietz, 1981. Occurrence and distribution of free flavonoid aglycones in plants. Phytochemistry **20**: 869–932.