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The Flavonoid Glycosides in the Leaves of *Cornus* Species III. The Flavonoids of Three Himalayan *Cornus* Species

By

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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
Ⅲ．3種のヒマラヤ産ミズキ属植物のフラボノイド

As parts of studies which clarify the flavonoid compositions in *Cornus* species, we have observed six species, i.e., *C. controversa* Hemsl., *C. brachypoda* Wall., *C. darvasica* (Pojark.) Pilip., *C. drummondii* C. A. Mey., *C. canadensis* L. and *C. suecica* L., and isolated and identified some flavonol glycosides based on kaempferol and quercetin, e.g., quercetin 3-*O*-glucoside (isoquercitrin), 3-*O*-galactoside (hyperin), 3-*O*-rhamnoside (quercitrin), 3-*O*-xylosylgalactoside, and kaempferol 3-*O*-glucoside (astragalin), 3-*O*-galactoside (trifolin), 3-*O*-rutinoside (nicotiflorin), 3-*O*-xylosylgalactoside etc. (Iwashina and Hatta 1990, 1992). In this paper, we describe the flavonoid profiles of three Himalayan *Cornus* species, *C. macrophylla* Wall. and *C. oblonga* Wall. (subgenus *Thelycrania*), and *C. capitata* Wall. (subgenus *Benthamia*), and chemotaxonomically discuss their situation.

Materials and Methods

Plant materials

C. oblonga and *C. macrophylla* were collected in Saipal, western Nepal in July to August, 1991 by one of authors (H. Hatta) during the expedition to the Nepal. *C. capitata* was grown in Tsukuba Botanical Garden, National Science Museum.

Isolation and identification of flavonoids

Dry (*C. oblonga* and *C. macrophylla*) or fresh (*C. capitata*) leaves were extracted with methanol and concentrated. Crops were preparatively paper-chromatographed using BAW (n-BuOH–AcOH–H_2O = 4:1:5, upper phase), 15% AcOH (AcOH–H_2O = 15:85) and once more BAW. Isolated flavonoids were finally purified by sephadex LH-20 column (solvent system: 70% methanol) and obtained as powders or pure solutions. They were identified by comparisons of paper-chromatographic and UV spectral data of original glycosides and acid hydrolysates (aglycones and glycosidic sugars) with those of authentic specimens as described before (Iwashina and Hatta 1992). Original glycosides were also characterized by high-performance liquid chromatography (HPLC) according to Iwashina and Hatta (1992).

Acid hydrolysis

Complete acid hydrolysis of flavonal glycosides were performed with 12% HCl according to

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Iwashina and Hatta (1992) and mild hydrolysis with 1.2% HCl: MeOH (1:1), 10 min, 85°C.

Characterization of aliphatic acid

Aliphatic acid which was liberated by acid hydrolysis was characterized by PC (solvent systems: BFW and PN, see Table 3) according to Harborne (1984). After development, the paper was sprayed with bromothymol blue (0.04g in 100ml 0.01M NaOH) as color reagent.

UV spectra

UV spectral survey was performed in methanol according to Mabry *et al.* (1970).

Results and Discussion

Cornus capitata Wall.

Among eight flavonols were isolated from the leaves of *C. capitata*, common four were identified as quercetin 3-*O*-glucoside (**2**), quercetin 3-*O*-galactoside (**3**), kaempferol 3-*O*-rhamnoside (**9**) and free quercetin (**1**) by PC and HPLC comparisons with authentic specimens, acid hydrolysis and UV spectra (Table 1 and 2). Quercetin 3-*O*-glucoside have been found in leaves of *C. controversa, C. darvasica* and *C. drummondii* (Nakaoki and Morita 1958, Iwashina and Hatta 1990), quercetin 3-*O*-galactoside in barks of *C. stolonifera* Michx, and leaves of *C. canadensis* L. and *C. suecica* L. (Nair and Rudloff 1960, Iwashina and Hatta 1992). Kaempferol 3-*O*-rhamnoside was found for the first time in *Cornus* species. Free quercetin seems to be artifact, because it did not appeared on the 2D-PC of crude extract.

Unusual other four flavonols were identified or characterized as follows. UV spectral properties of flavonoid **5** indicated the presence of free 5-, 7-, 3'- and 4'-hydroxyl and a substituted 3-hydroxyl groups. By complete acid hydrolysis, the glycoside gave quercetin, xylose and galactose which were identified by direct PC comparisons with authentic samples. On the other hand, by mild acid hydrolysis, quercetin 3-*O*-galactoside was produced as an intermediate. From the results described above, flavonoid **5** was identified as quercetin 3-*O*-xylosylgalactoside (Fig. 2). It was showed by similar manners that flavonoid **10** was kaempferol 3-*O*-xylosylgalactoside. Quercetin 3-*O*-xylosylgalactoside and kaempferol 3-*O*-xylosylgalactoside have been found in *C. canadensis* (Iwashina and Hatta 1992).

Complete acid hydrolysis of flavonoid **6** liberated quercetin and rhamnose. It was showed by UV spectral survey that rhamnose was attached to 3-position of quercetin (Table 2). Their data were presumed that the flavonoid was quercetin 3-*O*-rhamnoside (quercitrin). However, retention time of flavonoid **6** was higher than that of authentic quercitrin (more hydrophobic than quercitrin, Table 1), though Rf-values were very similar with those of quercitrin. It was showed by PC characterization of aliphatic acid in hydrolysates that flavonoid **6** was quercetin 3-*O*-rhamnoside which was acylated with aliphatic acid. However, chromatographic properties of the acid did not agreed with those of four general aliphatic acids, malonic acid, succinic acid, malic acid and tartaric acid (Table 3). Flavonoid **11** was kaempferol 3-*O*-glucoside acylated with unknown aliphatic acid which was same acid with that of flavonoid **6** showed by HPLC comparison with authentic astragalin and PC characterization of hydrolysates. Acylated flavonol glycosides were reported for the first time from *Cornus* species.

All of their flavonols in this experiment were based on quercetin and kaempferol, and *O*-glycosylated with various sugar moieties, e.g., glucose, acylated glucose, galactose, rhamnose, acylated rhamnose and xylosylgalactose at 3-hydroxyl, and they may be represent the comparatively

Table 1. PC and HPLC properties of flavonols from *Cornus capitata* and *C. oblonga*

Flavonols	Rf-values				Colors		HPLC*	Origins
	BAW	BEW	15%AcOH	5%AcOH	U V	UV/NH_3	Rt(min)	
quercetin (**1**)	0.71	0.67	–	–	y	br-y	–	*C. capitata*
quercetin 3-glucoside (**2**)	0.66	0.66	0.36	0.21	dp	y	10.83	*C. capitata* *C. oblonga*
quercetin 3-galactoside (**3**)	0.64	0.64	0.36	0.19	dp	y	10.19	*C. capitata* *C. oblonga*
quercetin 3-diglucoside (**4**)	0.50	0.51	0.50	0.33	dp	y	7.12	*C. oblonga*
quercetin 3-xylosylgalactoside (**5**)	0.51	0.53	0.58	0.43	dp	y	5.22	*C. capitata*
quercetin 3-rhamnoside (acylated) (**6**)	0.80	0.80	0.48	0.33	dp	dy	19.32	*C. capitata*
kaempferol 3-glucoside (**7**)	0.78	0.77	0.46	0.23	dp	dgy	13.30	*C. oblonga*
kaempferol 3-galactoside (**8**)	0.75	0.77	0.46	0.23	dp	dgy	11.33	*C. oblonga*
kaempferol 3-rhamnoside (**9**)	0.85	0.85	0.49	0.29	dp	dgy	9.67	*C. capitata*
kaempferol 3-xylosylgalactoside (**10**)	0.61	0.65	0.60	0.40	dp	dgy	7.83	*C. capitata*
kaempferol 3-glucoside (acylated) (**11**)	0.80	0.80	0.48	0.26	dp	dgy	18.69	*C. capitata*

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).
*Column: Finepak SIL $C_{18}S$, Eluent: Acetonitrile–H_2O–H_3PO_4 (22:78:0.2), Flow-rate: 1.0ml/min and Detection: 345nm.
dp = dark purple, br-y = bright yellow, y = yellow, dy = dark yellow and dgy = dark greenish yellow.

advanced characters.

Cornus oblonga Wall.

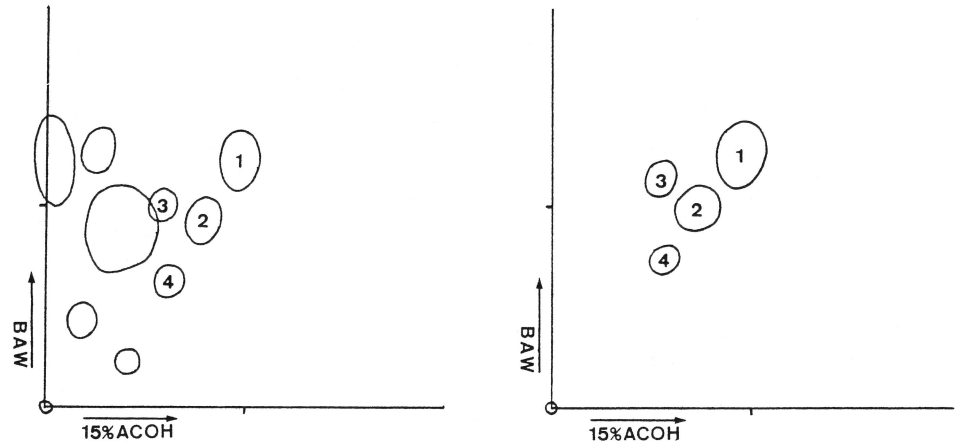
In this experiment, five flavonol glycosides were isolated as a pure solution and two mixtures from this evergreen *Cornus* species. Two mixtures were identified as quercetin 3-*O*-glucoside (**2**) and 3-*O*-galactoside (**3**), and kaempferol 3-*O*-glucoside (**7**) and 3-*O*-galactoside (**8**) by direct PC and HPLC comparisons with authentic specimens, respectively (Table 1). Another one was characterized as quercetin 3-*O*-diglucoside (**4**) by identification of hydrolysates (aglycone and sugar) and UV spectra

Table 2. UV spectral properties of flavonols from *Cornus capitata* and *C. oblonga*

Flavonols	λ max (nm)					
	in MeOH	+NaOMe	+$AlCl_3$	+$AlCl_3$/HCl	+NaOAc	+NaOAc/H_3BO_3
quercetin (**1**)	255 372	dec	272 456	264 298sh 361 426	274 322 390	259 387
quercetin 3-glucoside and 3-galactoside (**2** and **3**)	257 360	273 328 410 (inc)	274 437	269 298 366 404	273 325 390	262 379
quercetin 3-diglucoside (**4**)	258 362	274 316sh 414 (inc)	274 434	269 299 362 395	273 321 405	261 378
quercetin 3-xylosylgalactoside (**5**)	257 356	272 326 405 (inc)	275 434	270 297sh 360 401	274 326 388	262 375
quercetin 3-rhamnoside (acylated) (**6**)	256 350	272 325 398 (inc)	274 422	272 299 351 397	273 317 378	262 362
kaempferol 3-glucoside and 3-galactoside (**7** and **8**)	266 349	275 326 400 (inc)	274 305 353 395	275 302 348 392	274 310 390	267 352
kaempferol 3-rhamnoside (**9**)	264 342	273 323 389 (inc)	273 303 349 397	274 301 342 393	273 303 376	265 346
kaempferol 3-xylosylgalactoside (**10**)	267 349	275 324 397 (inc)	274 304 352 394	275 302 346 394	274 313 387	267 352

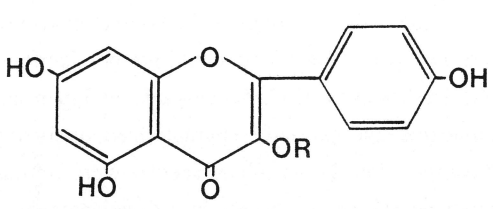
dec = Decomposition.
inc = Remarkable increase in intensity relative to the peak of methanolic solution.
sh = Shoulder.

(Table 2). Eyde (1988) has emphasized that *C. oblonga* was phylogenetically more primitive in *Cornus*, since the species retained the unique morphological characters, e.g., evergreen, 4-3 carpel, etc. We have reported that *C. canadensis* which had the more complicatedly glycosylated flavonols was apparently primitive (Iwashina and Hatta 1992). However, if *C. oblonga*, which had more simple flavonoid composition (glycosylation with only glucose and galactose) and lesser biosynthetic ability, was considered as primitive species, evolutionary proceeding of flavonoids in *Cornus* was

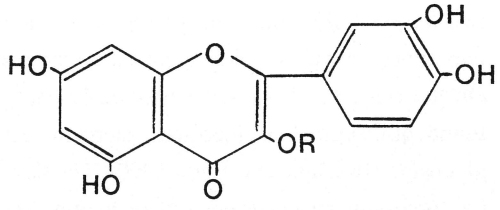


C. macrophylla (Himalaya) *C. brachypoda* (Japan)

Fig. 1. Two-dimensional chromatograms of *Cornus macrophylla* and *C. bracypoda*.
1 = quercetin 3-rhamnoside, **2** = quercetin 3-glucoside or 3-galactoside, **3** and **4** = unknown flavonol glycosides.
Other spots seem to be non-flavonoid phenolic substances.



R = glucosyl: kaempferol 3-*O*-glucoside (astragalin, **7**)
R = galactosyl: kaempferol 3-*O*-galactoside (trifolin, **8**)
R = rhamnosyl: kaempferol 3-*O*-rhamnoside (afzelin, **9**)
R = xylogalactosyl: kaempferol 3-*O*-xylosylgalactoside (**10**)
R = acylated glucosyl: acylated kaempferol 3-*O*-glucoside (**11**)



R = H: quercetin (**1**)
R = glucosyl: quercetin 3-*O*-glucoside (isoquercitrin, **2**)
R = galactosyl: quercetin 3-*O*-galactoside (hyperin, **3**)
R = rhamnosyl: quercetin 3-*O*-rhamnoside (quercitrin)
R = diglucosyl: quercetin 3-*O*-diglucoside (**4**)
R = xylogalactosyl: quercetin 3-*O*-xylogalactoside (**5**)
R = acylated rhamnosyl: acylated quercetin 3-*O*-rhamnoside (**6**)

Fig. 2. The structures of flavonols from three Himalayan *Cornus* species.

"simplicity→complication", and it seems that *C. canadensis* was more advanced than other *Cornus* species.

Cornus macrophylla Wall.

Some authors (e.g., Rehder 1916, Li 1944, Hu and Song 1983) considered that *C. macrophylla* which was distributed to Himalaya regions from China was identical with *C. brachypoda* native to Japan and Korean Peninsula. On the other hand, according to Eyde (1988), Koehne and Wangerin has concluded two species as a single species, *C. macrophylla*.

In this two-dimensional paper chromatography (2D-PC) and HPLC survey of *C. macrophylla*,

Table 3. Rf values of aliphatic acid from acylated quercetin 3-rhamnoside and kaempferol 3-glucoside in *Cornus capitata*.

Aliphatic acids	Rf values*	
	BFW	PN
6 and **11**	0.27	0.42
Authentic specimens:		
tartaric acid	0.35	0.20
malic acid	0.53	0.23
malonic acid	0.68	0.23
succinic acid	0.76	0.27

BFW = n-BuOH–Formic acid–H_2O (4:1:5, upper phase), PN = n-PrOH–1M NH_4OH (7:3).
*Bromothymol blue (0.04g in 100ml 0.01M NaOH) was used as color reagent.

two flavonol glycosides, quercetin 3-*O*-galactoside and quercetin 3-*O*-rhamnoside were found with two minor flavonols which appeared on the paper chromatogram (Fig. 1) but could not be identified. On the other hand, we have previously analyzed the fresh leaves of *C. brachypoda* in Japan and found quercetin 3-*O*-glucoside, quercetin 3-*O*-rhamnoside and partially characterized quercetin-glycoside (Iwashina and Hatta 1990). The flavonoid compositions between two species were similar, i.e., predominant occurrence of quercetin 3-*O*-rhamnoside and minor quercetin 3-*O*-galactoside or 3-*O*-glucoside with two unidentified flavonols. However, they were clearly distinguished by occurrence of other major spots of which color reaction (blue, but immediately change to dull blue or dull yellow under UV light, and bright blue under UV after exposure to fuming ammonia) seems to be not flavonoids, from *C. macrophylla*. Moreover, their unknown compounds were also found in *C. amomum* Mill., *C. arnoldiana* Rehd., *C. asperifolia* Michx., *C. grabrata* Benth., *C. hemsleyi* Schneid. & Wanger. and *C. horseyi* Rehd. which included in subgenus *Thelycrania* (Iwashina and Hatta, unpublished data). It is now in problem that either the such chemical differences should be considered as inter-specific variation between *C. macrophylla* and *C. brachypoda* or as intra-specific variation in *C. macrophylla sensu lato*.

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Summary

As series of our chemotaxonomical surveys, the flavonoid compounds of three Himalayan *Cornus*

species, *C. capitata, C. oblonga* and *C. macrophylla* were isolated and identified. The flavonoids which were found from their species were follows: *C. capitata*; free quercetin (**1**), quercetin 3-*O*-glucoside (**2**), quercetin 3-*O*-galactoside (**3**), quercetin 3-*O*-xylosylgalactoside (**5**), acylated quercetin 3-*O*-rhamnoside (**6**), kaempferol 3-*O*-rhamnoside (**9**), kaempferol 3-*O*-xylosylgalactoside (**10**) and acylated kaempferol 3-*O*-glucoside (**11**): *C. oblonga*; quercetin 3-*O*-diglucoside (**4**), kaempferol 3-*O*-glucoside (**7**), kaempferol 3-*O*-galactoside (**8**), (**2**) and (**3**): *C. macrophylla*; quercetin 3-*O*-rhamnoside and (**3**).

The flavonoid composition of *C. macrophylla* was similar with that of *C. brachypoda* which was occasionally considered to be same species with *C. macrophylla* and have been surveyed the flavonoid profile. However, 2D-PC pattern of *C. macrophylla* was different from *C. brachypoda* by occurrence of major spots of non-flavonoid phenolic compounds.

C. oblonga had simply glycosylated flavonols and was lower flavonoid synthetic ability. Though we have previously considered that *C. canadensis* which had the more complicatedly glycosylated flavonols was more primitive, if *C. oblonga* was recognized to be phylogenetically more primitive in *Cornus* as emphasized by some authors, evolutionary proceeding of flavonoids was "simplicity→complication" and it seems that *C. canadensis* was more advanced.

摘　　要

フラボノイド化合物を指標としたミズキ属植物の化学分類学的研究の一環として，ヒマラヤ地域に自生するヒマラヤヤマボウシ（*Cornus capitata*），*C. oblonga* および *C. macrophylla* の葉に含まれるフラボノイドが分離同定された。

ヒマラヤヤマボウシからは遊離の quercetin (**1**), quercetin 3-*O*-glucoside (**2**), qurecetin 3-*O*-galactoside (**3**), quercetin 3-*O*-xylosylgalactoside (**5**), アシル化された quercetin 3-*O*-rhamnoside (**6**), kaempferol 3-*O*-rhamnoside (**9**), kaempferol 3-*O*-xylosylgalactoside (**10**) およびアシル化された kaempferol 3-*O*-glucoside (**11**) が，*C. oblonga* からは quercetin 3-*O*-diglucoside (**4**), kaempferol 3-*O*-glucoside (**7**), kaempferol 3-*O*-galactoside (**8**), (**2**) および (**3**) が，また *C. macrophylla* からは quercetin 3-*O*-rhamnoside と (**3**) が検出された。

C. macrophylla は形態的には研究者によって *C. brachypoda* と同一種とみなされたり，別種とみなされたりしているが，本研究で得られた両者のフラボノイド組成は極めて類似している一方で，前種には主要成分として出現する非フラボノイドのフェノール性化合物とみられるスポットが後種にはまったく認められないことが判明した。しかしながら，これらの成分の有無が *C. macrophylla* と *C. brachypoda* とを区別する化学的特徴であるのか，広義の *C. macrophylla* の種内変異に当たるのかは判別できない。

C. oblonga は数名の研究者によって，いくつかの形態的特徴からミズキ属植物のなかでより原始的であると考えられている。この種から今回得られたフラボノイドはいずれも一般的なグルコースあるいはガラクトースを結合したフラボノール配糖体であり，しかもこの種のフラボノイド合成能力は低い。筆者らは以前にゴゼンタチバナ（*C. canadensis*）の分析を行い，グルコースやガラクトースだけでなくキシロシルガラクトース，ルチノース，アピオシルガラクトースなど様々な糖でグリコシル化された多くのフラボノールを分離し，これらのデータからゴゼンタチバナをミズキ属の中でより原始的な種とみなしたけれど，もし単純なフラボノール配糖体しか持たず，しかもその合成能力の低い *C. oblonga* が原始的とみなされるのならば，ミズキ属でのフラボノイドを指標とした進化の方向は“簡素なフラボノイド組成→複雑化”であり，ゴゼンタチバナ

はより進化していると考えられる。

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