論　文

Taxus cuspidata 葉の抽出成分とイチイ属樹木のケモタキソノミー

黒瀬　勝彦*・伊藤　和貴*・橘　　燦郎*・入江　和也*・沖　　妙*

Extractives of *Taxus cuspidata* Sieb. *et* Zucc. Leaves and Chemotaxonomy of Trees of Genus *Taxus*

Katsuhiko KUROSE*, Kazutaka ITOH*, Sanro TACHIBANA*, Kazuya IRIE* and Tae OKI*

Summary : A diterpenoid of the taxane type: taxinine (1), a diterpene alkaloid: taxol (2), two bisflavones: sciadopitysin (3) and ginkgetin (4), and two flavonols: isorhamnetin (5) and quercetin (6), were isolated and identified from the methanolic extractives of Ichii, *Taxus cuspidata* Sieb. *et* Zucc., leaves. Ginkgetin (4), isorhamnetin (5), and quercetin (6) were isolated for the first time from *T. cuspidata*. These bisflavones and flavonols, especially bisflavones, may be useful chemotaxonomical markers of *Taxus* species in the distinction between species of this genus.

要　旨　イチイ（*Taxus cuspidata* Sieb. *et Zucc.*）葉のメタノール抽出物から，1種のタキサン型ジテルペン taxinine (1)，1種のジテルペンアルカロイド taxol (2)，2種のビスフラボン sciadopitysin (3)と ginkgetin (4)および2種のフラボノール isorhamnetin (5)と quercetin (6)を単離同定した。イチイ葉から，ginkgetin (4)，isorhamnetin (5)および quercetin (6)を単離したのは今回が初めてである。これらビスフラボンとフラボノール，特にビスフラボンは，*Taxus* 属の化学分類学的指標として有効と考えられた。

1．INTRODUCTION

In continuation of our recent works on utilization of wood extractives[1-7] and on search of potential compounds in the extractives of trees for converting them to biologically active

＊　森林資源利用化学研究室　Laboratory of Chemistry and Biotechnology for Utilization of Forest Resources

substances[7, 8], we investigated the extractives of leaves of Ichii, *Taxus cuspidata* Sieb. *et* Zucc.

T. cuspidata with dark, linear and evergreen foliage is a needle-leaf tree in Japan. Several studies[9–15] on the leaf terpenoids of *T. cuspidata* have been conducted. However, only one study on the leaf flavonoids of *T. cuspidata* was conducted by Kariyone and Sawada[16] in 1958. They isolated sciadopitysin as a sole compound from the leaves. In addition, leaf flavonoids of Kyaraboku, *T. cuspidata var. nana* Rehder, a dwarf variety of Ichii, were recently reported by Tachibana *et al.*[7] However, detailed investigations of the leaf flavonoids of Ichii have not been reported to date. Flavonoids are generally recognized to be excellent plant taxonomic markers[17, 18]. This report deals with our investigations of the extractives in the leaves of *T. cuspidata* for the discovery of potential compounds for converting them to biologically active substances as well as investigation of the leaf flavonoids of *T. cuspidata* leaves from the point of view of chemotaxonomy of the genus *Taxus*.

2. RESULTS AND DISCUSSION

2. 1 Extractives from the leaves of *T. cuspidata*

Fresh leaves of *T. cuspidata* were extracted with methanol and gave extractives in the yield of 15.3% of the fresh leaves. The extractives were suspended in water and were successively extracted with *n*-hexane, chloroform, ethyl acetate, and *n*-butanol as shown in Fig. 1. Each fraction was checked on a TLC (thin-layer chromatography) plate. The chromatogram of TLC of each fraction is shown in Fig. 2. *n*-Hexane-soluble, chloroform-soluble, and ethyl acetate-soluble

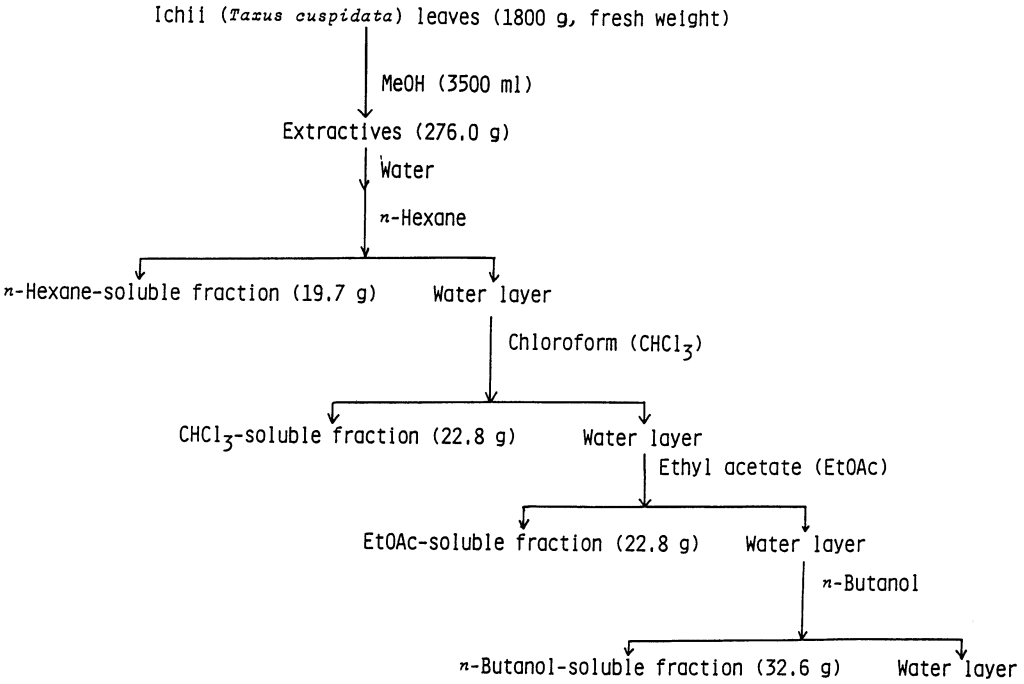


Fig. 1 Extraction and separation scheme from the leaves of *Taxus cuspidata* Sieb. *et* Zucc. (Ichii, Japanese yew).

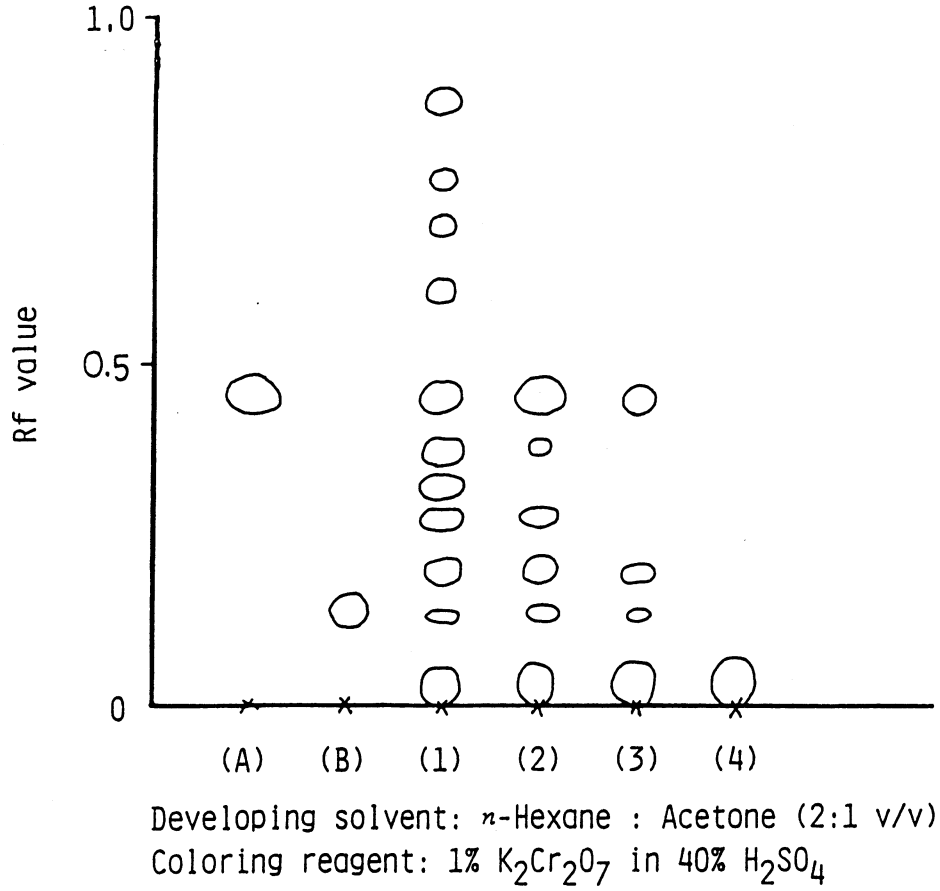


Fig. 2 TLC-chromatogram of each fraction obtained from the methanolic extractives by solvent extraction.

Note : (1): *n*-Hexane-soluble fraction; (2): $CHCl_3$-soluble fraction; (3): Ethyl acetate-soluble fraction; (4): *n*-Butanol-soluble fraction; (A): Taxinine; (B): Taxol

fractions gave positive color reactions of diterpenoids of the taxne type and diterpene alkaloids on a TLC plate. Chloroform-soluble and ethyl acetate-soluble fractions gave positive color reactions of flavonoids with magnesium and hydrochloric acid (Mg-HCl). Therefore, the three fractions were combined and were named Fraction A. The Fraction A was separated into two fractions, terpenoid and flavonoid fractions, by a silica gel column chromatography as shown in Fig. 3.

From the Fraction A, six compounds, namely, taxinine (1), taxol (2), sciadopitysin (3), ginkgetin (4), isorhamnetin (5), and quercetin (6), were isolated. Compounds (4)—(6) were isolated for the first time from the leaves of *T. cuspidata*. Structures of compounds (1)—(6) are shown in Fig. 4. Their structures were determined by UV (ultraviolet), ^{1}H-NMR (proton nuclear magnetic resonance), ^{13}C-NMR (carbon thirteen nuclear magnetic resonance), and MS (mass) spectroscopies. The content of each compound (1)—(6) isolated from *T. cuspidata* leaves is shown in Table 1.

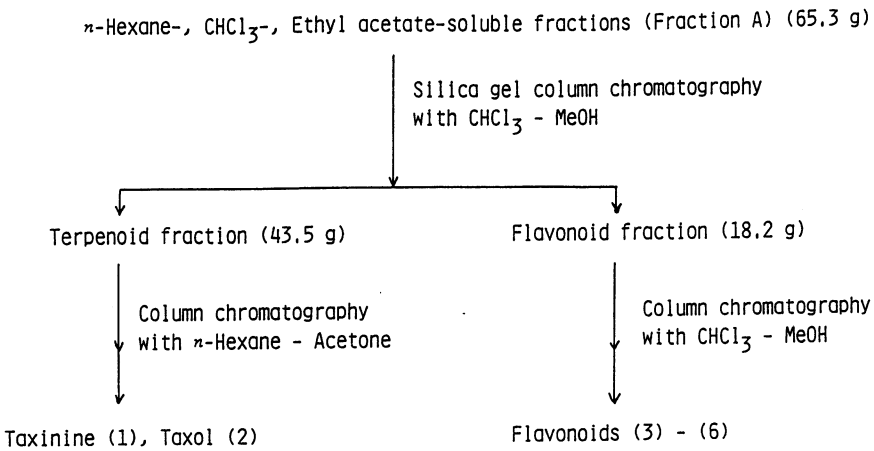
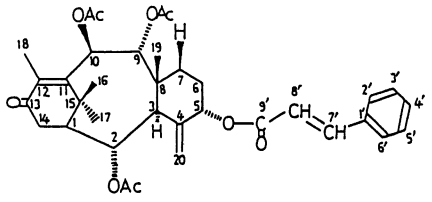
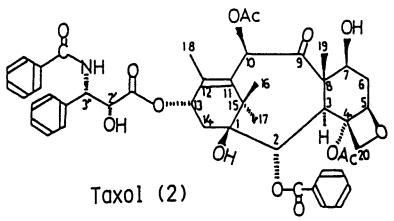


Fig. 3 Isolation scheme of taxinine (1), taxol (2), and flavonoids (3) to (6) from the Fraction A in the extractives of *Taxus csupidata* Sieb. *et* Zucc. leaves.

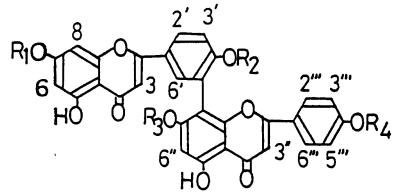
Note : Structures of compounds (1) to (6) refer to Fig. 4.



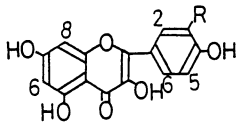
Taxinine (1)



Taxol (2)



Sciadopitysin (3) $R_1 = R_2 = R_4 = Me$, $R_3 = H$
Ginkgetin (4) $R_1 = R_2 = Me$, $R_3 = R_4 = H$
Sotetsuflavone (7) $R_3 = Me$, $R_1 = R_2 = R_4 = H$
Sequiaflavone (8) $R_1 = Me$, $R_2 = R_3 = R_4 = H$
Amentoflavone (9) $R_1 = R_2 = R_3 = R_4 = H$



Isorhamnetin (5) R = OMe
Quercetin (6) R = OH
Kaempferol (10) R = H

Fig. 4 Compounds (1) to (6) isolated from the leaves of *Taxus cuspidata Sieb. et* Zucc. and structures of the bisflavones (7) to (9) and flavonol (10).

Table 1 The content of compounds (1) to (6) isolated from *Taxus cuspidata* Sieb. *et* Zucc. leaves.

Compound	Content(%, fresh weight)
Taxinine (1)	0.055
Taxol (2)	0.0003
Sciadopitysin (3)	0.006
Ginkgetin (4)	0.003
Isorhamnetin (5)	0.004
Quercetin (6)	0.028

Note : Chemical structures of compounds (1) to (6) refer to Fig. 4.

2．1．1 Diterpenoids of the taxane type and diterpene alkaloids in the leaves of *T. cuspidata*

As shown in Fig. 3, compounds (1) and (2) were isolated from the methanolic extractives of the leaves of *T. cuspidata*.

Compound (1), $C_{35}H_{42}O_9$ (M^+ (molecular ion peak)=606), mp 266−267℃, was made up of colorless needles. The UV specrurm (λ_{max}^{MeOH} : 218 and 278nm) of compound (1) showed a characteristic UV absorption of the taxane type diterpenoids[19] and was suggested to be taxinine. MS and ^{1}H-NMR spectra of compound (1) were in good agreement with those of authentic sample of taxinine isolated from *T. cuspidata var. nana* leaves[7], ^{13}C-NMR spectrum of compound (1) shown in Fig. 5 explained well the structure of taxinine. Therefore, compound (1) was identified as taxinine. Compound (1) was obtained in the yield of 0.055% of the fresh leaves.

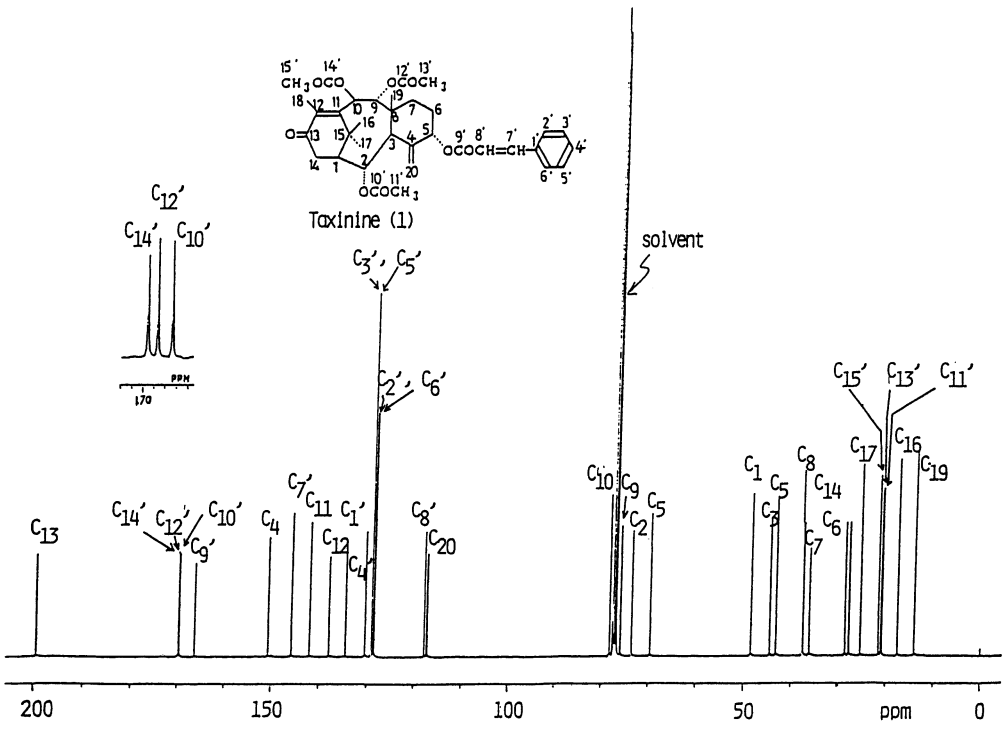


Fig. 5 carbon tirteen nuclear magnetic resonance (^{13}C-NMR) spectrum of compound (1).

Taxinine (1), a major constituent of the taxane type diterpenoids, and taxinines B, E, and J, three minor constituents of the diterpenoids, were isolated from *T. cuspidata* leaves by Woods *et al.*[20] Taxinine (1) and taxinine B were also isolated from *T. cuspidata var. nana* leaves[7]. But, in this research taxinines B, E, and J were not isolated. Generally, contents of constituents in trees vary from trees to trees and in growing areas and conditions. A seasonal variation of taxinine content in *T. cuspidata* leaves by HPLC analysis was conducted by Yoshizaki *et al.*[13] They reported that the maximum content was obtained in January (0.31% of fresh leaves), whereas the minimum content was obtained in June (0.18% of fresh leaves). No isolation of the three taxinines may be due to either variation of their contents in *T. cuspidata* leaves by differences in

growing areas and conditions or seasonal variation of their contents in the leaves. However, further investigation clarifying whether the three taxinines are existed or not in the extractives from *T. cuspidata* leaves will be needed by HPLC (high-performance liquid chromatography) or GLC (gas liquid chromatography) analysis.

Compound (2), $C_{47}H_{51}NO_{14}$ (M^+=853), mp 213−215℃, was in the form of colorless crystals. UV spectrum of compound (2) showed the characteristic UV absorption of taxol[21]. FD−MS spectrum of compound (2) is shown in Fig. 6. It was in good agreement with that of authentic taxol isolated from *T. brevifolia* bark[21]. A mixed-melting-point test of the compound (2) with an authentic sample was undepressed. Therefore, compound (2) was identified as taxol. The content of taxol was 0.0013% of the fresh leaves by HPLC analysis.

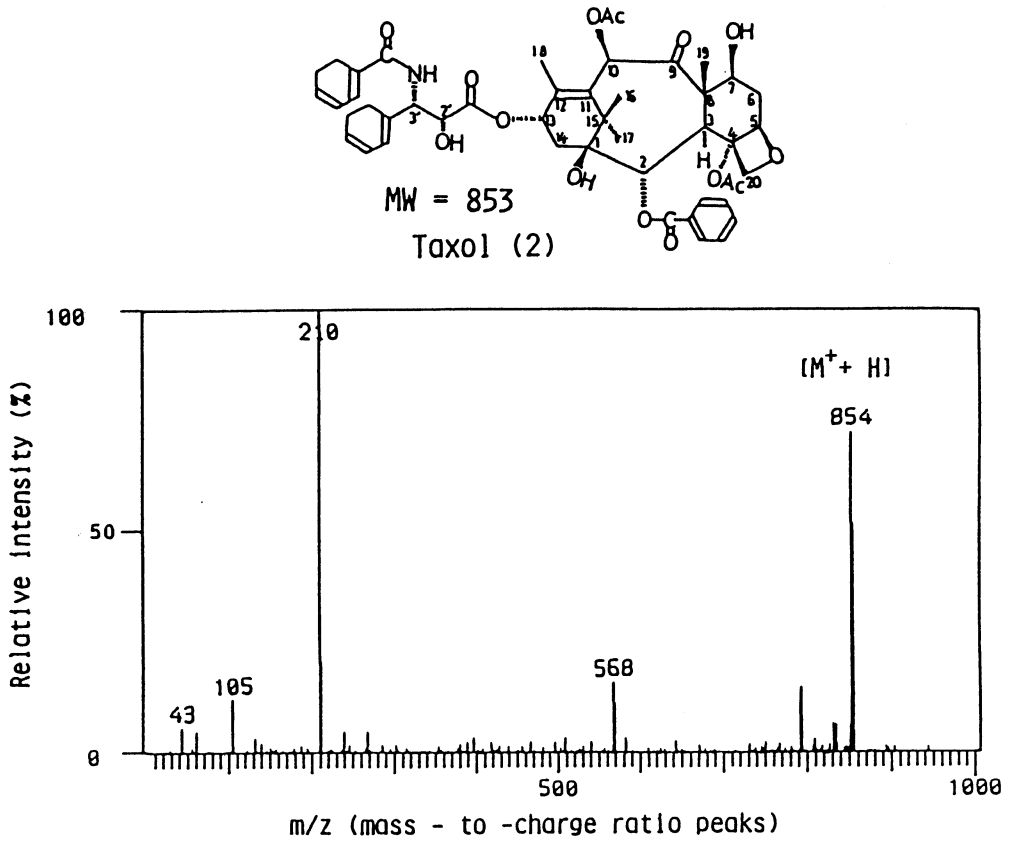


Fig. 6 Field desorption (FD) mass spectrum of compound (2).

Several studies have indicated that taxol is contained in the leaves of *Taxus* species plants[21-23]. Taxol has strong anti-leukemic and tumor-inhibiting activities[21, 24, 25], Fett Neto and Dicosmo[22] have reported that taxol is contained in the leaves, stems and bark of *T. cuspidata* grown in Canada. In this report, we also found that taxol was contained in the leaves of *T. cuspidata* grown in Japan. The content of taxol in the leaves of the latter was a little less than that in the leaves of the former[26].

Taxinine (1) is considered to be converted to taxol mimics having anti-leukemic and tumor-inhibiting activities by microbial and enzymatical conversions with phenylisoserine. Taxol (2)

has strong anti-leukemic and tumor-inhibiting activities[21, 24, 45], although its content in *Taxus* species plants is very small. However, the content of taxol is considered to be increased by tissue culture of *T. cuspidata* and other *Taxus* species.

From the above consideration, taxinine (1) was found to be a potential compound for the biotechnological and chemical production of biologically active substances. Furthermore, we also found that calli cultures of the leaves and stems of *T. cuspidata var. nana* produced taxol in amounts almost equivalent to the leaves and stems of the intact plant[27]. Trials for the production of taxol and taxol mimics are now in progress.

2．1．2 Flavonoids in the leaves of *T. cuspidata*

The following four flavonoids (3)－(6) were isolated from the flavonoid fraction of the Fraction A as shown in Fig. 3.: sciadopitysin (3), ginkgetin (4), isorhamnetin (5), and quercetin (6). Their structures are shown in Fig. 4. Whereas compound (3) had been known in *T. cuspidata*[17], *T. cuspidata var. nana*[7], *T. brevifolia*[8] and *T. baccata*[28-32], the compounds (4) to (6) were isolated for the first time from the leaves of *T. cuspidata*.

Compound (3), $C_{33}H_{24}O_{10}$ ($M^+=580$), was yellowish crystals whose UV spectrum has absorption bands at λ_{max}^{MeOH} : 272 and 327nm, suggesting it to be sciadopitysin[33], a kind of bisflavone. MS spectrum of compound (3) was in agreement with that of sciadopitysin[33]. It formed a crystalline triacetate, mp 270－272℃. ^{1}H-NMR spectrum of the acetate is shown in Fig. 7. Aromatic proton signals of 1‴, 4‴-disubstituted ring E, 4', 5'-disubstituted ring B, and 5", 7", 8"-trisubsti-tuted ring D were assigned. Furthermore, signals of three methoxy groups (3.86ppm, 3.81ppm, and 3.76

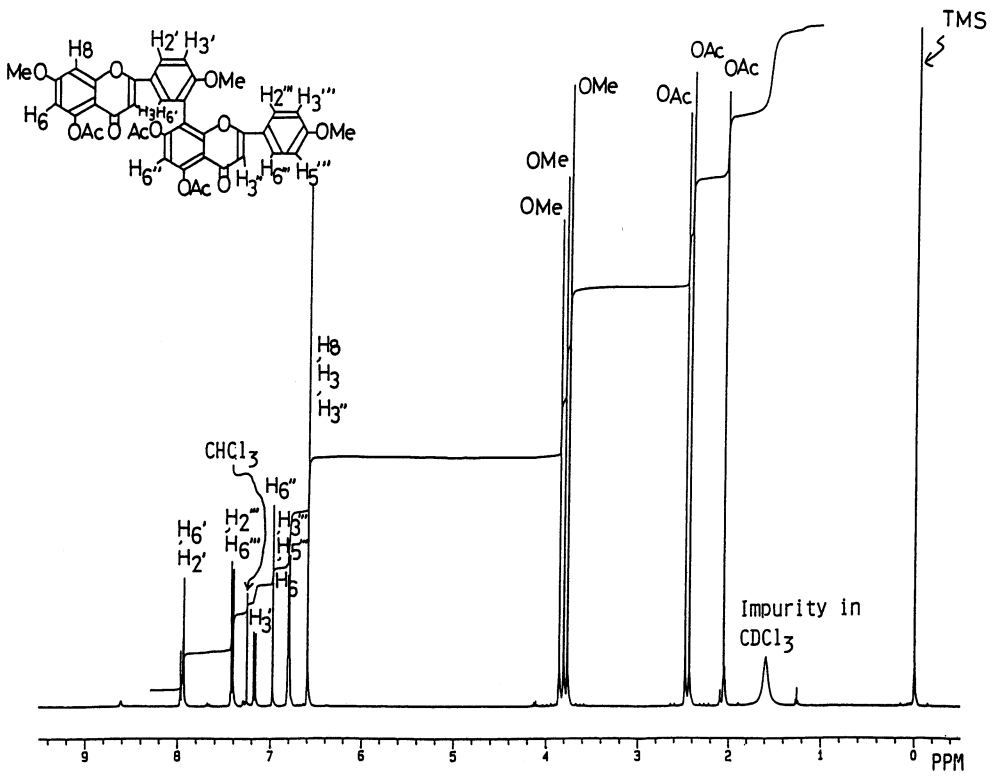


Fig. 7 Proton nuclear magnetic resonance (^{1}H-NMR) spectrum of the acetate of compound (3).

ppm) substituted at A−7, B−4', and E−4''', respectively, were observed. These data were in good agreement with those of an authentic sample isolated from the leaves of Icho (*Ginkgo bilova L.*)[33].

Compound (4), $C_{32}H_{22}O_{10}$ (M^+=566) was yellowish crystals. Its UV spectrum (λ_{max}^{MeOH} : 269 and 335nm) showed a characteristic UV absorption of bisflavonoid[33], MS spectrum of compound (4) was in agreement with that of ginkgetin[34]. It formed a crystalline tetra-acetate, mp 264−267℃. In the ^{1}H-NMR spectrum of the acetate, the ring substituent pattern was the same as in that of the acetate of compound (3). Furthermore, signals of two methoxy groups at 3.87ppm and 3.81ppm observed in the spectrum were attributed to the substituents at A−7 and B−4', respectively. These data were in good agreement with those of authentic ginkgetin isolated from *G. biloba* leaves[33]. Therefore, compound (4) was identified as ginkgetin. Compounds (3) and (4) were obtained in the yields of 0.006% and 0.003%, respectively, of the fresh leaves.

Compound (5), $C_{16}H_{12}O_7$ (M^+=316) was yellowish needles. Its UV spectrum λ_{max}^{MeOH} : 253 and 382nm) showed a characteristic UV absorption of flavonol[35]. MS spectrum of compound (5) was in agreement with that of isorhamnetin[34]. It formed a crystalline tetra-acetate, mp 203−205℃. In the ^{1}H-NMR spectrum of the acetate, aromatic proton signals of 5, 7-disubstituted ring A and 1', 3', 4'-trisubstituted ring B were assigned. Furthermore, a signal of methoxy group at 3.89ppm was attributed to the substituent at B−3'. These data were in good agreement with those of authentic isorhamnetin[36]. Therefore, compound (5) was identified as isorhamnetin.

Compound (6), $C_{15}H_{10}O_7$ (M^+=302) was yellowish needles. UV spectrum (λ_{max}^{MeOH} : 256 and 382nm) of compound (6) showed a characteristic UV absorption of flavonol[35]. MS spectrum of compound (6) was in agreement with that of quercetin[34]. It formed a crystalline penta-acetate, mp 192−194℃. In the ^{1}H-NMR spectrum of the acetate, the ring substituent pattern was the same as in that of the acetate of compound (5). Furthermore, no signal of methoxy group was observed in the spectrum. These data were in good agreement with those of authentic quercetin[37]. Therefore, compound (6) was idetified as quercetin. Compounds (5) and (6) were obtained in the yields of 0.004% and 0.028%, respectively, of the fresh leaves.

It is said that flavonoids have some usefully biological activities[38], *e. g.* anti-fungal activity, hypotensive activity, and so on. Kurose[39] found that four flavonoids (3)−(6) isolated from the leaves of *T. cuspidata* had anti-fungal activity to a plant pathogenic fungus, *Cochliobolus miyabeanus*. The details will be published later. This shows that the flavonoids may be used as naturally-occurring fungicides instead of chemically synthesized ones.

2. 2 Chemotaxonomy of trees of genus *Taxus* by use of flavonoids as chemical markers

Sciadopitysin (3) was the only bisflavonoid isolated from the leaves of *T. cuspidata* leaves[16]. However, beside sciadopitysin (3), ginkgetin (4) and two flavonols, isorhamnetin (5) and quercetin (6) were isolated for the first time from the leaves of *T. cuspidata*.The flavonoids (4)−(6) were also isolated from the leaves of *T. cuspidata var. nana*[7]. Sciadopitysin (3) as the sole bisflavone and three flavonols, isorhamnetin (5), quercetin (6), and kaempferol (10) were isolated and identified

from *T. brevifolia* leaves[8]. *T. wallichiana*[40, 41] contains three bisflavonoids: namely, sciadopitysin (3), sotetsuflavone (7), and amentoflavone (9). Compounds (3), (4), and sequiaflavone (8) were isolated and identified from *T. baccata* leaves by Khan *et al.*[28] On the other hand, DiModica *et al.*[29-32] isolated a crystalline mixture of two bisflavonoids from *T. baccata* leaves and one of them was identified as sciadopitysin (3). They estimated the other bisflavonoid as sotetsuflavone (7) by chemical analysis. Chemical structures of bisflavones (3), (4) and (7) to (9) and flavonols (5), (6), and (10) are shown in Fig. 4. These results concerning chemotaxonomical interest are summarized in Table 2. This table shows that *Taxus* species shown in the table are possible to distinguish between the species by use of flavonoids as chemical markers. Khan *et al.*[42] have reported that bisflavonyls as chemical markers are useful for chemotaxonomical distinction between *Araucariales* species. Therefore, the bisflavones and flavonols, especially bisflavones of *Taus* species, seemed to be useful chemical markers in the distinction between species in this genus. However, eight *Taxus* species grow in the world, further investigations of the leaf flavonoids of another four *Taxus* species other than four *Taxus* species listed in Table 2 will need to clarify chemotaxonomical distinction between the genus *Taxus*.

Table 2 Distribution of bisflavones and flavonols in *Taxus* species.

Taxus species	Bisflavones					Flavonols			References*A
	(3)	(4)	(7)	(8)	(9)	(5)	(6)	(10)	
Taxus baccata (European yew)	+	+	−	+	−	NR	NR	NR	[28]
T. wallichiana (Indian yew)	+	−	+	−	+	NR	NR	NR	[40, 41]
T. cuspidata (Japanese yew)	+	+	−	−	−	+	+	−	this investigation
T. cuspidata var. nana (Japanese dwarf yew)	+	+	−	−	−	+	+	−	[7]
T. brevifolia (Pacific yew)	+	−	−	−	−	+	+	+	[8]

Notes : Compounds (3)−(10) refer to Fig. 4. *A : Numbers in the bracket correspond to that of the references in the paper. NR: not reported.

3. EXPERIMENT

3. 1 Plant material

Fresh leaves of *T. cuspidata* were collected in April, 1994, in the suburbs of Saijyo City, Ehime Prefecture.

3. 2 Instrumentation and chromatography materials

Melting points were measured by a Yanagimoto model MP-S_0 micro melting point apparatus. All melting points were uncorrected. A HITACHI model 200−20 double beam spectrometer was used for UV spectra. ^{1}H-NMR and ^{13}C-NMR spectra were taken by the use of JNM−GSX270 and

JNM−EX400 spectrometers with TMS (tetramethylsilane) as an internal standard. Chemical shifts and coupling constants were given in δ value and Hz, respectively. MS spectra were taken by the use of a HITACHI M−80B mass spectrometer. HPLC (high-perform-ance liquid chromatography) was performed on a HITACHI 655 liquid chromatograph equipped with a L−4000 UV detector.

All solvents and chemicals were reagent or HPLC grade unless otherwise stated. Analytical and preparative TLC utilized silica gel (Kieselgel 60 F_{254}) plates. CC (column chromatography) employed silica gel (Kishida Chemicals, 100−200 mesh).

3．3 Extraction from the leaves of *T. cuspidata*

The extraction and separation scheme from the leaves of *T. cuspidata* is shown in Fig. 1. The fresh leaves of *T. cuspidata* (1800g) were extracted twice with methanol for seven days at room temperature. The methanolic extractives (276.0g) were suspended in water and successively extracted with *n*-hexane, chloroform, ethyl acetate, and *n*-butanol. The yield of each soluble fraction is shown in Fig. 1. The TLC chromatogram of each soluble fraction is shown in Fig. 2. The *n*-hexane-soluble, chloroform-soluble, and ethyl acetate-soluble fractions gave positive color reactions of deterpenoids of the taxane type with 1% potassium dichromate in 40% sulfuric acid on a TLC plate. Chloroform-soluble and ethyl acetate-soluble fractions gave positive color reactions of flavonoids and bisflavonoids with Mg-HCl. The *n*-hexane-soluble, chloroform-sol-uble, and ethyl acetate-soluble fractions were found to contain the taxane type diterpenoids, flavonoids and bisflavonoids by the color reactions. So the three fractions were combined and named Fraction A.

3．4 Isolation of taxinine (1) and taxol (2) from the Fraction A

The Fraction A (65.3g) was separated into terpenoids and flavonoids fractions, by a silica gel column chromatography with chloroform-methanol as shown in Fig. 3. The terpenoids fraction (43.5g) was chromatographed on a silica gel column using a *n*-hexane-acetone solvent system. The compound (1) and taxol (2) were eluted out in the order of numbers 1−2.

3．4．1 Taxinine (1)

The waxy solid obtained from the initial eluate was recrystallized from methylene chloride and ethyl acetate and yielded colorless needles (1) (981.6mg), mp (melting point) 266−267℃. (lit mp 266−268℃)[7], UV λ_{max}^{MeOH} nm (log ε): 218 (3.64), 278 (4.21). FAB−MS (fast atom bombardment MS) m/z (mass-to-charge ratio peaks): 607 (M^++H), 606 (M^+), 547, 459, 307, 289, 154 (100%), 131, 107. ^{1}H-NMR (270 MHz, $CDCl_3$) δ (chemical shift): 0.94 ((3H (protons), s (singlet), 19−Me)), 1.16 (3H, s, 17−Me), 1.74 ((2H, m (multiplet), 7−H)), 1.77 (3H, s, 16−Me), 2.00 (2H, m, 6−H), 2.06 (3H, s, 2−OAc), 2.07 (3H, s, 9−OAc), 2.08 (3H, s, 10−OAc), 2.23 ((1H, dd (double doublet), J (coupling constant) =6.5, 19.8Hz, 14−H)), 2.29 (3H, s, 18−Me), 2.43 (1H, d (doublet), J=19.8Hz, 14−H), 2.84 (1H, dd, J=6.5, 19.8Hz, 14−H), 3.41 ((1H, br (broad) d, J=6.5Hz, 3−H)), 4.85 (1H, m, 20−H), 5.36 (2H, m, 20−H and 5−H), 5.56 (1H, dd, J=2.0, 6.5Hz,

2−H), 5.90 (1H, d, J=10.0 Hz, 9−H), 6.05 (1H, d, J=10 Hz, 10−H), 6.44 (1H, d, J=16Hz, 22−H), 7.35−7.80 (5H, m, aromatic protons), 7.65 (1H, d, J=16.0 Hz, 23−H). ^{13}C-NMR (67.5MHz, $CDCl_3$) δ : 48.54 (C_1), 77.05 (C_2), 44.47 (C_3), 150.62 (C_4), 69.63 (C_5), 28.37 (C_6), 36.05 (C_7), 37.64 (C_8), 77.25 (C_9), 77.37 (C_{10}), 141.94 (C_{11}), 137.98 (C_{12}), 199.46 (C_{13}), 37.39 (C_{14}), 43.17 (C_{15}), 17.49 (C_{16}), 25.19 (C_{17}), 27.52 (C_{18}), 14.00 (C_{19}), 117.26 (C_{20}), 169.48 (CO in 2−OAc), 20.74 (Me in 2−OAc), 169.74 (CO in 9−OAc), 20.92 (Me in 9−OAc), 169.92 (CO in 10−OAc), 21.43 (Me in 10−OAc), 134.52 ($C_{1'}$), 128.50 ($C_{2'}$ and $C_{6'}$), 128.96 ($C_{3'}$ and $C_{5'}$), 130.39 ($C_{4'}$), 145.76 ($C_{7'}$), 117.83 ($C_{8'}$), 166.31 ($C_{9'}$). The ^{13}C-NMR spectrum of compound (1) is shown in Fig. 5. UV and NMR spectra of compound (1) were identical with those of authentic taxinine isolated from *T. cuspidata var. nana*[7]. The mixed-melting-point test of compound (1) with an authentic sample was undepressed.

3．4．2 Taxol (2)

The fraction containing taxol (95.2㎎) was dissolved in 5ml of chloroform and 1 ml of the solution was subjected to a preparative TLC and rechromatographed with chloroform-methanol (10 : 1 v/v). This yielded taxol (3.7㎎) as colorless crystals after recrystallization from methanol and water, mp 213−215℃. (lit mp 213−216℃)[21]. UV λ_{max}^{MeOH} nm (log ε): 227 (4.47), 273 (3.23). FD−MS (field desorption MS) m/z : 854 (M^++H), 853 (M^+), 568, 210 (100%), 105, 43. The FD−MS spectrum of compound (2) is shown in Fig. 6. UV spectrum of compound (2) was identical with that of authentic taxol isolated from *T. brevifolia*[20]. The mixed-melting-point test of the compound (2) with an authentic sample was undepressed.

Analysis of taxol in the fraction by HPLC was performed on a reverse-phase column (Hitachi Gel #3056) in a Hitachi 655 liquid chromatograph equipped with a L−4000 UV detector (wave length: 227nm) by isocratic elution with a miture of methanol-water-acetonitrile (20 : 43 : 37 v/v) as a mobile phase. The flow rate was 1 ml/min, and all chromatograms were plotted at the absorption maximum of taxol, 227nm. A 4μl of the chloroform solution of the fraction (5ml) was injected to the column. A calibration curve was obtained using authentic taxol. The content of taxol was 0.0013% of the fresh leaves. Identification of taxol was also conducted by coincidence of the retention time with that of authentic taxol isolated from *T. brevifolia* and by adding authentic taxol.

3．5 Isolation of bisflavones (3) and (4), and flavonols (5) and (6) from the Fraction A

The flavonoids fraction (18.2g) was eluated with chloroform-methanol by solvent gradient as shown in Fig. 3. Four compounds (3) to (6) were eluted out in the order of numbers 3−6.

3．5．1 Sciadopitysin (3)

The solid obtained from the initial eluate was purified by recrystallization from methanol and pyridine to afford yellowish crystals (3) (107.2㎎), mp 293−296℃. (lit mp 295−297℃)[17]. UV λ_{max}^{MeOH} nm (log ε): 272 (4, 34), 327 (4, 15). Ms m/z : 580 (M^+) (100%), 579, 565, 550, 283, 166, 132, 117. Compound (3) (10㎎) was acetylated with acetic anhydride in pyridine, and the following treatment was conducted in the usual manner to afford tri-acetate (11㎎), mp 270−272℃. (lit mp 271−272℃)[33]. ^{1}H-NMR (400MHz, $CDCl_3$) δ : 2.06 (3H, s, OAc), 2.44 (3H, s, OAc), 2.48 (3H, s,

OAc), 3.76 (3H, s, OMe), 3.81 (3H, s, OMe), 3.86 (3H, s, OMe), 6.59 (3H, s, 8−H, 3−H, and 3''−H), 6.80 (2H, d, J=8.7Hz, 3'''−H and 5'''−H), 6.82 (1H, d, J=3.9Hz, 6−H), 6.98 (1H, s, 6''−H), 7.17 (1H, d, J=8.3Hz, 3'−H), 7.42 (2H, d, J=8.7Hz,2'''−H and 6'''−H), 7.95 (1H, dd, J=10.6, 2.4Hz, 2'−H), 7.97 (1H, d, J=2.4Hz, 6'−H). ^{1}H-NMR spectrum of the acetate of compound (3) is shown in Fig. 7. Compound (3) and the tri-acetate were identical with authentic sciadopitysin isolated from *Ginkgo biloba* leaves[21] and its acetate[21], respectively.

3．5．2 Ginkgetin (4)

The solid obtained from the second eluate was purified by recrystallization from methanol and pyridine to afford yellowish crystals (4) (53.6mg), mp>300℃. (lit mp 336℃)[43]. UV λ_{max}^{MeOH} nm (log ε): 269 (4.56), 335 (4.50). MS m/z：566 (M^+) (100%), 565, 536, 283, 269, 166, 121, 118. The tetra-acetate prepared by the usual method was obtained as colorless needles from ethanol, mp 264−267℃. (lit mp 264−266℃)[33]. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.06 (3H, s, OAc), 2.28 (3H, s, OAc), 2.44 (3H, s, OAc), 2.49 (3H, s, OAc), 3.81 (3H, s, OMe), 3.87 (3H, s, OMe), 6.59 (1H, d, J=2.4Hz, 6−H), 6.60 (1H, d, J=2.4Hz, 8−H), 6.66 (1H, s, 6''−H), 6.82 (1H, s, 3−H), 7.01 (1H, s, 3''−H),7.07 (2H, d, J=9.0Hz, 3'''−H and 5'''−H), 7.17 (1H, d, J=9.0Hz, 3'−H), 7.50 (2H, d, J=9.0Hz, 2'''−H and 6'''−H), 7.93 (1H, d, J=2.2Hz, 6'−H), 7.95 (1H, dd, J=9.0, 2.2Hz, 2'−H). Compound (4) and the tetra-acetate were identical with authentic ginkgetin isolated from *G. biloba* leaves[33] and its acetate[33], respectively.

3．5．3 Isorhamnetin (5)

The crystals obtained from the third eluate were purified by recrystallization from methanol and pyridine to afford yellowish needles (5) (72.2mg), mp 303−305℃. (lit mp 304−305℃)[36], UV λ_{max}^{MeOH} nm (log ε): 253 (4.03), 382 (3.93). MS m/z：316 (M^+) (100%), 315, 301, 288, 273, 153, 151, 124, 123. The tetra-acetate prepared by the usual method was obtained in colorless needles from ethanol, mp 203−205℃. (lit mp 205−207℃)[36], ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 2.32 (3H, s, OAc), 2.35 (6H, s, 2×OAc), 2.44 (3H, s, OAc), 3.89 (3H, s, OMe), 6.87 (1H, d, J=2.2Hz, 6−H), 7.17 (1H, d, J=8.2Hz, 5'−H), 7.34 (1H, d, J=2.2Hz, 8−H), 7.39 (1H, d, J=2.2Hz, 2'−H), 7.41 (1H, dd, J=8.2, 2.2Hz, 6'−H). Compound (5) and the tetra-acetate were identical with authentic isorhamnetin and its acetate, respectively.

3．5．4 Quercetin (6)

The crystals obtained from the fourth eluate were purified by recrystallization from methanol and pyridine to afford yellowish needles (6) (502.0mg), mp 312−314℃. (lit mp 313−314℃)[37]. UV λ_{max}^{MeOH} nm (log ε): 256 (4.07), 382 (4.02). MS m/z：302 (M^+) (100%), 301, 274, 153, 137, 124, 108. The penta-acetate prepared by the usual method was obtained in colorless needles from ethanol, mp 192−194℃. (lit mp 192−194℃)[37]. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 2.34 (12H, s, 4×OAc), 2.44 (3H, s, OAc), 6.88 (1H, d, J=2.4Hz, 6−H), 7.33 (1H, d, J=2.4Hz, 8−H), 7.35 (1H, d, J=8.4Hz, 5'−H), 7.69 (1H, d, J=2.2Hz, 2'−H), 7.72 (1H, dd, J=8.4, 2.2Hz, 6'−H), Compound (6) and the penta-acetate were identical with authentic quercetin and its acetate, respectively.

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