

## Flavonoids from the Genus *Taxus*

Mirosława Krauze-Baranowska

Department of Pharmacognosy, Medical University of Gdańsk, Gen. J. Hallera 107 str., 80-416 Gdańsk, Poland. Fax: +48583493206. E-mail: krauze@farmacja.amg.gda.pl

Z. Naturforsch. **59c**, 43–47 (2004); received November 11, 2002/May 26, 2003

From the needles of *Taxus baccata* the following flavonoids were isolated: 3-O-rutinosides quercetin, myricetin and kaempferol, 7-O-glucosides kaempferol and quercetin, kaempferol, quercetin, myricetin. The composition of flavonols and biflavones in some of the species of the genus *Taxus*, namely *T. celebica*, *T. cuspidata*, *T. media* and cultivar varieties *T. baccata* 'Aurea', *T. baccata* 'Aurea decora', *T. baccata* 'Elegantissima', *T. baccata* 'Fastigiata', *T. baccata* 'Pyramidalis', *T. media* 'Hatfieldii' were compared by HPLC separation.

**Key words:** Flavonols, Biflavones, HPLC, *Taxus*

### Introduction

The genus *Taxus* – a natural source of paclitaxel – was intensively investigated for the content of taxoids, that could be used in a semi-synthesis of this diterpene (Li *et al.*, 2001). At the same time, several reports concerning other chemical constituents occurring in this genus, belonging to a group of biflavones (Di Modica *et al.*, 1962; Khan *et al.*, 1976; Das *et al.*, 1994, 1995; Konda *et al.*, 1995; Reddy and Krupadanam, 1996; Parveen *et al.*, 1985; Singh *et al.*, 1997; Wollenweber *et al.*, 1998; Krauze-Baranowska and Wiwart, 2002) and lignans (Das *et al.*, 1995; Singh *et al.*, 1997) were published.

The literature data confirm, that flavonoids present in species of the genus *Taxus* are apigenin C-8''/C-3' dimers (Di Modica *et al.*, 1962; Khan *et al.*, 1976; Das *et al.*, 1994, 1995; Konda *et al.*, 1995; Reddy and Krupadanam, 1996; Parveen *et al.*, 1985; Singh *et al.*, 1997; Krauze-Baranowska and Wiwart, 2002). It is worth to notice, that other groups of flavonoids in the genus *Taxus* have not yet been investigated in detail (Niemann, 1988). The presence of the following biflavones was revealed: sciadopitysin, ginkgetin in needles and stem barks of *T. baccata* (Khan *et al.*, 1976; Das *et al.*, 1995; Reddy and Krupadanam, 1996), in *T. wallichiana* (Parveen *et al.*, 1985; Singh *et al.*, 1997) and in *T. cuspidata* (Konda *et al.*, 1995), kavaflavone, amentoflavone in needles and stem barks of *T. baccata* (Das *et al.*, 1994, 1995), and *T. wallichiana* (Parveen *et al.*, 1985; Singh *et al.*, 1997), 7-O-methylamentoflavone in *T. baccata* (Khan *et al.*, 1976), 7''-O-methylamentoflavone in *T. baccata* (Di Modica *et al.*, 1962), bilobetin and 4''-O-

methylamentoflavone in needles of *T. baccata* (Krauze-Baranowska and Wiwart, 2002). Moreover, Wollenweber *et al.* (1998) reported the presence of sciadopitysin, ginkgetin, amentoflavone and bilobetin as external biflavonoids accumulated on the surface of needles of *T. baccata*.

The objective of this work was to isolate and identify flavonoids other than biflavones, present in the needles of *Taxus baccata* as well as the chromatographic analysis (HPLC) of the flavonoid complexes occurring in needles of several species and cultivar varieties of the genus *Taxus*.

### Material and Methods

#### Plant material

The needles of *Taxus baccata* L. were collected from the Medicinal Plants Garden of Medical University of Gdańsk (Poland) in January 1997. The needles of cultivar varieties of *T. baccata* namely, *T. baccata* 'Aurea decora', *T. baccata* 'Aurea', *T. baccata* 'Elegantissima', *T. baccata* 'Fastigiata', *T. baccata* 'Pyramidalis' and two other species of the genus *Taxus*, *T. celebica* Li. and *T. media* Rehd., were obtained from the Botanical Garden of the University of Wrocław (Poland) in February 1997. The needles of *Taxus cuspidata* Sieb. et Zucc., and *Taxus media* 'Hatfieldii' were collected from the Arboretum of the Botanical Garden in Wirty (Poland) in September 1997. The above plants are deposited at the Herbarium of the Department of Pharmacognosy of the Medical University of Gdańsk (Poland) with the following numbers of voucher specimens: 97-001 (*Taxus baccata*), 97-002 (*T. baccata* 'Aurea decora'), 97-003 (*T. baccata*

'Aurea'), 97-004 (*T. baccata* 'Fastigiata'), 97-005 (*T. baccata* 'Elegantissima'), 97-006 (*T. baccata* 'Pyramidalis'), 97-007 (*T. celebica*), 97-008 (*T. media*), 97-009 (*T. media* 'Hatfieldii'), 97-010 (*T. cuspidata*).

#### Extraction and isolation

Dried and pulverized needles of *T. baccata* (0.5 kg) were extracted in a Soxhlet apparatus with: petroleum ether (b. p. 61 °C), chloroform and methanol. The methanol extract was concentrated (50 ml) and chromatographed over a polyamide column (100 g, 45 cm × 3 cm, 15 ml each eluate) using methanol/water mixtures with increasing concentration of MeOH (v/v): 30% (eluates 1–22), 60% (eluates 23–44), 80% (eluates 45–51). Compound **1** was separated from eluates 5–19 over a polyamide column (10 g, 9 cm × 1.5 cm, eluates 1–28, 5 ml each eluate) with a mobile phase F and obtained from eluates 8–20 in crystalline form (25 mg). Compounds **2** (10 mg) and **3** (10 mg) were isolated from the filtrate of eluates 8–20 by preparative TLC on cellulose with the mobile phase C and next purified over Sephadex LH-20 column (5 g, 8 cm × 1 cm, 1 ml each eluate). From the eluates 25–34 a mixture of compound **4** and **5** was precipitated as pale yellow powder (25 mg). Both compounds, **4** (6 mg) and **5** (6 mg), were isolated from a precipitate by preparative TLC on cellulose with the mobile phase D and subsequently purified over Sephadex G-10 column (5 g, 8 cm × 1 cm, 1 ml each eluate) with MeOH. Eluates 45–51 were chromatographed over Sephadex LH-20 column (10 g, 18 cm × 1.5 cm, 1 ml each eluate) with MeOH and from the obtained eluates 9, and 10–12, respectively, compounds **8** (1.0 mg), **7** (4.0 mg) and **6** (1.5 mg) were purified by preparative TLC on polyamide with the mobile phase A.

NMR spectra were recorded on a Bruker MSL 300 instrument at 500 MHz (for <sup>1</sup>H) and 75,5 MHz (for <sup>13</sup>C) in DMSO-d<sub>6</sub> using TMS as an internal standard. FAB-MS (+) and LSI-MS (+) (NBA, Cs<sup>+</sup>, 6 keV) mass spectral data were obtained using an AMD-Intectra spectrometer.

Analytical and preparative TLC were carried out on precoated plates with polyamide 11 F<sub>254</sub> (Merck, 20 cm × 20 cm, 0,25 mm thickness) and cellulose F<sub>254</sub> using mobile phases: CHCl<sub>3</sub>-MeC-OEt-MeOH (4:8:6 v/v/v) (A), IsoPrOH-HCOOH-H<sub>2</sub>O (2:5:5 v/v/v) (B), BuOH-H<sub>2</sub>O-CH<sub>3</sub>COOH (4:1:5 v/v/v) (C), CH<sub>3</sub>COOH-H<sub>2</sub>O (30:70 v/v) (D),

(15:75 v/v) (E), BuOH-MeOH-H<sub>2</sub>O (40:5:5 v/v/v) (F). Column chromatography was performed with polyamide (Roth) and Sephadex LH-20 (Pharmacia). Total hydrolysis was done by heating 1 mg of compound with 1 N HCl (100 °C, 30 min). Partial hydrolysis was made by heating 1 mg of compound with 1% HCl (100 °C, 15 min). Enzymatic hydrolysis was performed by incubation a solution of compound (1 mg) with β-glucosidase (2 mg) at 34 °C for two days. Sugar analysis was carried out on aluminium sheets precoated with Si gel 60 F<sub>254</sub> (Merck, 0,2 mm thickness) using mobile phase AcCN:H<sub>2</sub>O (15:85 v/v). The chromatograms were visualized by spraying with aniline phthalate, followed by heating at 105 °C.

*3-O-Rutinoside quercetin* (**1**): TLC cellulose: R<sub>f</sub>(C) = 0.38, R<sub>f</sub>(E) = 0.34. – HPLC: t<sub>R</sub> = 22.5 min. – LSI-MS (+): m/z (rel. int.) = 611 [M+H]<sup>+</sup> (85), 466 [M+H-rhamnose]<sup>+</sup> (10), 303 [A+H]<sup>+</sup> (39). – UV, <sup>1</sup>H and <sup>13</sup>C NMR data are in agreement with literature data (Krauze-Baranowska and Cisowski, 1995).

*3-O-Rutinoside kaempferol* (**2**): TLC cellulose: R<sub>f</sub>(C) = 0.45, R<sub>f</sub>(E) = 0.37. – HPLC: t<sub>R</sub> = 19.5 min. – LSI-MS (+): m/z (rel. int.) = 595 [M+H]<sup>+</sup> (72), 449 [M+H-rhamnose]<sup>+</sup> (15), 287 [A+H]<sup>+</sup> (28). – UV, <sup>1</sup>H and <sup>13</sup>C NMR data are in agreement with literature data (Chaurasia and Wichtl, 1987).

*3-O-Rutinoside myricetin* (**3**): TLC cellulose: R<sub>f</sub>(C) = 0.26, R<sub>f</sub>(E) = 0.30. – HPLC: t<sub>R</sub> = 24.7 min. – LSI-MS (+): m/z (rel. int.) = 627 [M+H]<sup>+</sup> (65), 482 [M+H-rhamnose]<sup>+</sup> (12), 319 [A+H]<sup>+</sup> (15). – UV, <sup>1</sup>H and <sup>13</sup>C NMR data are in agreement with the literature data (Bennini and Chulia, 1994).

*7-O-Glucoside kaempferol* (**4**): TLC polyamide: R<sub>f</sub>(A) = 0.53, cellulose: R<sub>f</sub>(C) = 0.47, R<sub>f</sub>(D) = 0.71. – HPLC: t<sub>R</sub> = 30.5 min. – UV data as described in the literature (Markham, 1982). – FAB-MS: m/z (rel. int.) = 449 [M+H]<sup>+</sup> (100), 287 [A+H]<sup>+</sup> (24).

*7-O-Glucoside quercetin* (**5**): TLC polyamide: R<sub>f</sub>(A) = 0.41, cellulose: R<sub>f</sub>(C) = 0.29, R<sub>f</sub>(D) = 0.62. – HPLC: t<sub>R</sub> = 27.8 min. – UV data as described in the literature (Markham, 1982). – FAB-MS (+): m/z (rel. int.) = 465 [M+H]<sup>+</sup> (100), 303 [A+H]<sup>+</sup> (30).

*Kaempferol* (**6**): TLC polyamide: R<sub>f</sub>(A) = 0.40, cellulose: R<sub>f</sub>(B) = 0.45, R<sub>f</sub>(C) = 0.87. – HPLC: t<sub>R</sub> = 42.8 min. – UV data as described in the litera-

ture (Markham, 1982). – FAB-MS (+):  $m/z$  (rel. int.) = 287  $[M+H]^+$  (80).

*Quercetin* (7): TLC polyamide:  $R_f(A)$  = 0.31, cellulose:  $R_f(B)$  = 0.23,  $R_f(C)$  = 0.76. – HPLC:  $t_R$  = 40.5 min. – UV data as described in the literature (Markham, 1982). – FAB-MS (+):  $m/z$  (rel. int.) = 303  $[M+H]^+$  (95).

*Myricetin* (8): TLC polyamide:  $R_f(A)$  = 0.20,  $R_f(B)$  = 0.07,  $R_f(C)$  = 0.68. – HPLC:  $t_R$  = 37.8 min. – UV data as described in the literature (Markham, 1982). – FAB-MS (+):  $m/z$  (rel. int.) = 319  $[M+H]^+$  (58).

### HPLC analysis

An HPLC system from Knauer (Berlin, Germany) was used. HPLC analysis was carried out on a Lichrospher RP-18 column (250 mm × 4 mm, 5  $\mu$ m; Merck, Darmstadt, Germany) with the following program of gradient elution: THF (A),  $H_3PO_4:H_2O$  (1:99; B), from 0 min to 35 min linear gradient at increasing concentration of A from 10% to 40% in a mixture A + B, from 35 min isocratic elution at concentration A 40% in a mixture A + B, with a reequilibration period of 10 min between individual runs, flow rate 1.0 ml/min, UV detection for biflavones at 330 nm and for standard diterpenes at 228 nm. The needles of species of *Taxus* (10.0 g) were preliminary purified with petroleum ether and chloroform in a Soxhlet apparatus. Flavonoids were extracted from the plant material with methanol (100 ml). After evaporation of the solvent (20 ml) the extracts were injected. On the basis of the obtained HPLC data the content (%) of each compound in the flavonoid mixture was calculated as follows:

$$\% \text{ content of flavonoid} = \frac{\text{peak area of flavonoid}}{\text{sum of peak areas of all flavonoids}} \times 100$$

The standard biflavones were isolated from the needles of *Taxus baccata* according to the procedure described earlier (Krauze-Baranowska and Wiwart, 2002).

### Results and Discussion

For the first time the following flavonoids were isolated from the methanol extract from the needles of *Taxus baccata*: 3-O-rutinoside quercetin (1), 3-O-rutinoside kaempferol (2), 3-O-rutinoside myricetin (3), 7-O-glucoside kaempferol (4), 7-

O-glucoside quercetin (5), kaempferol (6), quercetin (7) and myricetin (8). The structures of the compounds were established by classical methods – acidic and enzymatic hydrolysis, co-chromatography with standards and spectroscopic methods –, UV, MS (1–8), NMR (1–8) (Bennini and Chulia, 1994; Chaurasia and Wichtl, 1987; Markham, 1982). The results confirm earlier report by Niemann (1988) on occurrence of flavonols in the genus *Taxus*.

Under optimized conditions of RP-HPLC analysis – gradient elution for mixture of solvents: tetrahydrofuran (organic modifier) and water-formic acid (99:1) – a good separation of all flavonoids, flavonols and biflavones present in the plant material was achieved. Moreover, the use of the above conditions, but with UV detection at 228 nm, makes it also possible to analyse the diterpenes, paclitaxel and baccatin, with the values of  $t_R$  30.1 min and 60.5 min, respectively. The dominant compounds in all investigated genera were flavonols, with 3-O-rutinoside quercetin together with 3-O-rutinoside myricetin as the major ones (Table I, Fig. 1). Other flavonoids such as 3-O-rutinoside kaempferol (*Taxus baccata*, *T. baccata* 'Aurea decora', *Taxus media*, *Taxus cuspidata*), 7-O-glucoside kaempferol (*T. baccata*, *T. cuspidata*) and 7-O-glucoside quercetin (*T. baccata*, *T. baccata* 'Aurea', *T. baccata* 'Pyramidalis', *T. cuspidata*) were present either as the main compounds (the above mentioned genera) or as minor constituents (all genera except the above mentioned) depending on the species (Table I). Besides O-glycosides, flavonol aglycones, myricetin, quercetin, kaempferol were also shown to be present in minor quantities, with the exception of *T. baccata* 'Elegantissima'. This cultivar variety differed from others by the presence of quercetin as one of the main compounds (Table I, Fig. 1). Biflavones in the *Taxus* species were mainly represented by sciadopitysin, ginkgetin, amentoflavone, 7-O-methylamentoflavone while bilobetin, 4'''-O-methylamentoflavone occurred in small amounts (*T. baccata* 'Fastigiata', *T. celebica*, *T. baccata*, *T. baccata* 'Aurea') or were absent (*T. baccata* 'Aurea decora', *T. media* 'Hatfieldii') (Table I, Fig. 1). Sciadopitysin and amentoflavone are dominant compounds in a group of biflavones from *T. baccata*, *T. media*, *T. celebica* and in cultivar varieties *T. baccata* 'Aurea', *T. baccata* 'Aurea decora', *T. baccata* 'Elegantissima', *T. baccata* 'Pyramidalis'. Ginkgetin accompanied the above mentioned biflavones as the main compo-

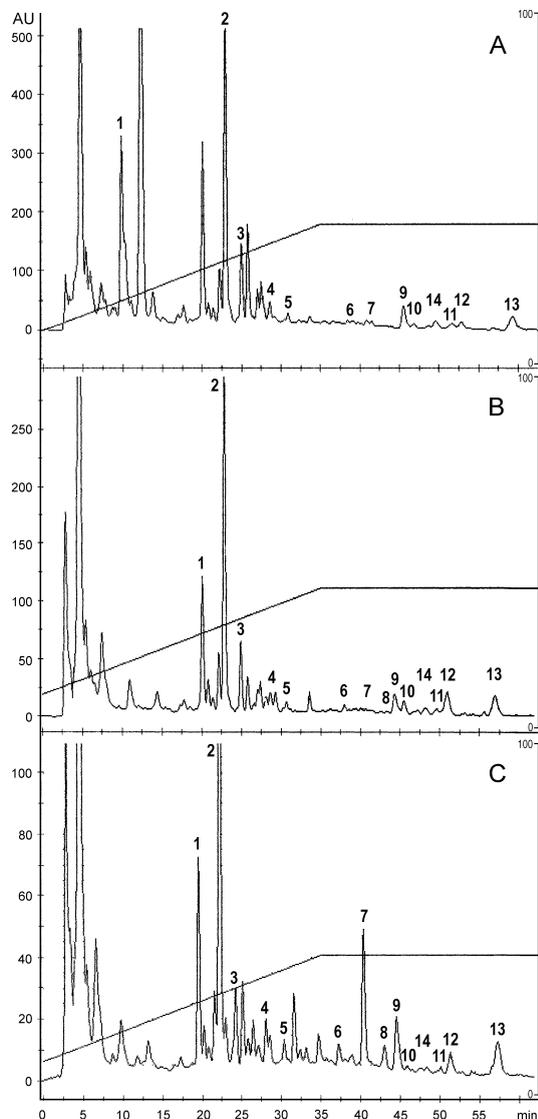


Fig. 1. HPLC chromatograms from methanol extracts from A) *Taxus media*, B) *Taxus baccata* 'Fastigiata', C) *Taxus baccata* 'Elegantissima'. **1:** 3-O-rutinoside myricetin, **2:** 3-O-rutinoside quercetin, **3:** 3-O-rutinoside kaempferol, **4:** 7-O-glucoside quercetin, **5:** 7-O-glucoside kaempferol, **6:** myricetin, **7:** quercetin, **8:** kaempferol, **9:** amentoflavone, **10:** bilobetin, **11:** 7-O-methylamentoflavone, **12:** ginkgetin, **13:** sciadopitysin, **14:** 4'''-O-methylamentoflavone.

ment in *T. baccata* 'Aurea', *T. baccata* 'Pyramidalis', *T. baccata* 'Fastigiata', *T. media* 'Hatfieldii'. Three species are different from the others, regarding composition of biflavones: in *T. cuspidata* amentoflavone significantly dominates, 4'''-O-methylamentoflavone and 7-O-methylamentoflavone occurred among main biflavones in *T. media*, and in *T. media* 'Hatfieldii', respectively. The analysis of the HPLC peak areas allows the conclusion, that flavonoid dimers constitute only *c.* 2.8% (*Taxus baccata* 'Aurea decora') to 19.9% (*T. baccata* 'Elegantissima') (Table I) of all flavonoids, which are biosynthesized in needles of several species of the genus *Taxus*. Furthermore, these results lead to the conclusion, that biosynthesis of flavonoids in plant is strictly controlled: if non-dimeric flavonoids appear as dominant compounds, biflavones are present in small amount, and opposite – if the plant is rich in biflavonoids, the amount of other flavonoid compounds is significantly lower and they even exist as traces only. This latter relationship was observed for flavonoids in *Microbiota decussata* (Krauze-Baranowska *et al.*, 2002) and *Cupressocyparis leylandii* (Cupressaceae) (Krauze-Baranowska *et al.*, 1999). Lebreton (1962) analysed the UV spectra of methanol extracts from the family Cupressaceae and also demonstrated that in some species flavonols dominated whereas in other dimeric flavones were dominant. The similar dependence was shown for bioflavonoids from an ethanol extract from the leaves of *Ginkgo biloba* (Sticher, 1993), in which biflavones dominated but several forms of flavonoid O-glycosides were present in comparatively lower amounts.

#### Acknowledgements

This research was supported by KBN grant No 4P05F00918. The author kindly thanks Prof. Dr. habil. Kazimierz Głowniak, from the Department of Pharmacognosy of the Medical University of Lublin (Poland) for an authentic standard of baccatin and Prof. Dr. habil. Małgorzata Sznitowska from the Department of Pharmaceutical Technology of the Medical University of Gdańsk (Poland) for a standard of paclitaxel.

Table I. The composition of flavonoids\* in some species of the genus *Taxus*.

Compound	Species									
	<i>Taxus baccata</i>	<i>T. baccata</i> 'Aurea'	<i>T. baccata</i> 'Aurea decora'	<i>T. baccata</i> 'Elegantissima'	<i>T. baccata</i> 'Fastigiata'	<i>T. baccata</i> 'Pyramidalis'	<i>Taxus media</i>	<i>T. media</i> 'Hatfieldii'	<i>Taxus celebica</i>	<i>Taxus cuspidata</i>
3-O-Rutinoside myricetin	15.3	19.9	14.5	15.1	18.3	10.2	24.4	21.7	15.1	20.5
3-O-Rutinoside quercetin	41.2	50.5	65.7	47.3	46.5	52.8	47.0	52.5	57.3	48.0
3-O-Rutinoside kaempferol	11.4	6.3	10.9	6.4	10.1	6.9	10.2	5.1	7.1	4.3
7-O-Glucoside quercetin	14.4	7.0	4.2	2.2	2.9	6.0	2.4	5.5	3.2	7.2
7-O-Glucoside kaempferol	11.7	5.1	0.8	1.3	0.8	6.1	1.2	4.2	0.7	10.2
Myricetin	–**	0.7	0.6	1.6	0.7	1.3	0.3	0.5	0.5	0.2
Quercetin	0.7	0.4	0.5	11.2	0.2	0.4	0.5	0.5	0.4	0.5
Kaempferol	0.2	0.2	–	1.7	0.3	0.2	–	0.2	–	0.4
Flavonols	94.9	89.9	97.2	86.8	79.8	83.9	86.0	90.3	84.3	91.3
Amentoflavone	1.5	2.1	0.9	5.3	4.1	3.0	4.5	1.5	4.0	4.4
Bilobetin	0.2	0.7	–	0.3	2.2	0.9	0.8	0.7	0.8	0.6
4"-O-Methylamentoflavone	0.3	1.2	–	0.3	1.4	1.2	1.8	–	0.6	0.5
7-O-Methylamentoflavone	0.4	0.7	0.4	0.2	0.7	0.7	1.2	2.8	2.1	0.9
Ginkgetin	0.4	2.8	0.4	1.9	5.8	4.5	1.5	2.2	2.6	0.9
Sciadopitysin	2.3	2.4	1.1	5.2	6.0	5.8	4.1	2.6	5.6	1.4
Flavones	5.1	9.1	2.8	13.2	20.2	16.1	14.0	9.7	15.7	8.7

\* % Content of compound in flavonoid complex.

\*\* Compound chromatographically detected as trace.

- Bennini B. and Chulia A. (1994), Flavonol glycosides from *Erica cinerea*. J. Nat. Prod. **57**, 178–180.
- Chaurasia N. and Wichtl M. (1987), Flavonol glycosides from *Urtica dioica*. Planta Med. **53**, 432–434.
- Das B., Rao S. P., Srinivas K. V. N. S., and Yadav J. S. (1994), Biflavones of *Taxus baccata*. Fitoterapia **65**, 189.
- Das B., Rao S. P., Srinivas K. V. N. S., and Yadav J. S. (1995), Lignans, biflavones and taxoids from Himalayan *Taxus baccata*. Phytochemistry **38**, 715–717.
- Di Modica G., Rossi P. F., and Rivero A. M. (1962), Flavones isolated from *Taxus baccata*. Atti. Acad. Nazl. Lincei. Rend. Classe Sci. Fis. Mat. e Nat. **32**, 87–90 [Chem. Abstr. **58**, 4502c (1963)].
- Khan M. S. Y., Kumar I., Prasad J. S., Nagarajan G. R., Partasarathy M. R., and Krishnamurthy H. G. (1976), Phenolic constituents of *Taxus baccata* leaves. Planta Med. **42**, 82–85.
- Konda Y., Sasaki T., Kagawa H., Takayanagi H., Hari-gaya Y., Sun X. L., Li X., and Onda M. (1995), Conformational analysis of C3'-C8 connected biflavones. J. Heterocycl. Chem. **32**, 1531–1535.
- Krauze-Baranowska M. and Cisowski W. (1995), Flavonoids from *Ecballium elaterium* herb. Herba Pol. **41**, 5–10.
- Krauze-Baranowska M., Cisowski W., Wiwart M., and Madziar B. (1999), Antifungal biflavones from *Cupressocyparis leylandii*. Planta Med. **65**, 572–574.
- Krauze-Baranowska M. and Wiwart M. (2002), Antifungal activity of biflavones from *Taxus baccata* and *Ginkgo biloba*. Z. Naturforsch. **58c**, 65–69.
- Li S., Zhang H., Yao P., Sun H., and Fong H. H. S. (2001), Taxane diterpenoids from the bark of *Taxus yunnanensis*. Phytochemistry **58**, 369–374.
- Lebreton P. (1982), Les Cupressales: une definition chimiosystematique. Candollea **37**, 243–256.
- Markham K. R. (1982), Techniques of Flavonoids Identification. Academic Press, London, pp. 36–49 and pp. 72–93.
- Niemann G. J. (1988), Distribution and evolution of the flavonoids in gymnosperms. In: The Flavonoids (J. B. Harborne, ed.). Chapman and Hall, London, p. 475.
- Reddy B. P. and Krupadanam G. L. D. (1996), Chemical constituents of the leaves of Himalayan *Taxus baccata*: use of DQF-COSY in the structure elucidation of biflavones. Indian J. Chem. Sect. B. Org. Chem. Ind. Med. Chem. **35B**, 283–285.
- Parveen N., Taufeeq H. M., and Khan N. U. D. (1985), Biflavones from the leaves of Himalayan yew: *Taxus wallichiana*. J. Nat. Prod. **48**, 994.
- Singh B., Gujral R. K., Sood R. P., and Duddeck H. (1997), Constituents from *Taxus* species. Planta Med. **63**, 191–192.
- Sticher O. (1993), Quality of *Ginkgo* preparations. Planta Med. **59**, 2–11.
- Wollenweber E., Kraut L., and Mues R. (1998), External accumulation of biflavonoids on gymnosperm leaves. Z. Naturforsch. **53c**, 946–950.