Flavonoids from the Genus Taxus

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

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), and T. wallichiana (Parveen et al., 1985; Singh et al., 1997) and in T. cuspidata (Konda et al., 1995), kayaflavone, 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 Wroclaw (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 decora’), 97-004 (T. baccata ‘Elegantissima’), 97-005 (T. baccata ‘Fastigiata’).
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 compounds 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 (5 g, 8 cm × 1 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 1H) and 75.5 MHz (for 13C) in DMSO-d6 using TMS as an internal standard. FAB-MS (+) and LSI-MS (+) (NBA, Cs+, 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 F254 (Merck, 20 cm × 20 cm, 0.25 mm thickness) and cellulose F254 using mobile phases: CHCl3/MeC-OEt-MeOH (4:8:6 v/v/v) (A), IsoPrOH-HCOOH-H2O (2:5:5 v/v/v) (B), BuOH-H2O-CH3COOH (4:1:5 v/v/v) (C), CH3COOH-H2O (30:70 v/v) (D), (15:75 v/v) (E), BuOH-MeOH-H2O (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 F254 (Merck, 0.2 mm thickness) using mobile phase AcCN:H2O (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: Rf(C) = 0.38, Rf(E) = 0.34, – HPLC: tR = 22.5 min. – LSI-MS (+): m/z (rel. int.) = 611 [M+H]+ (85), 466 [M+H-rhamnose]+ (10), 303 [A+H]+ (39), – UV, 1H and 13C NMR data are in agreement with literature data (Krauze-Baranowska and Cisowski, 1995).

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

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

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

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

Kaedempferol (6): TLC polyamide: Rf(A) = 0.40, cellulose: Rf(B) = 0.45, Rf(C) = 0.87. – HPLC: tR = 42.8 min. – UV data as described in the literature.
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 µm; Merck, Darmstadt, Germany) with the following program of gradient elution: THF (A), H3PO4:H2O (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 stan-
dard diterpenes at 228 nm. The needles of species was achieved. Moreover, the use of the above conditions, but with UV detection at 228 nm, makes it also possible to analyse the diter-
penes, paclitaxel and baccatin, with the values of tR 30.1 min and 60.5 min, respectively. The domi-
nant compounds in all investigated genera were flavonoids, 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-rutin-
toside 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, kaempfe-
ror were also shown to be present in minor quanti-
ties, with the exception of T. baccata ‘Elegantis-
sima’. 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-methylamentofla-
vone while bilobetin, 4‴-O-methylmentoflavone occurred in small amounts (T. baccata ‘Fastigiata’, T. celebica, T. baccata, T. baccata ‘Aurea’) or were absent (T. baccata ‘Aurea decora’, T. media ‘Hat-
fieldii’) (Table I, Fig. 1). Sciadopitysin and amento-
flavone 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-

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), querce-
tin (7) and myricetin (8). The structures of the compounds were established by classical meth-
ods – acidic and enzymatic hydrolysis, co-chroma-
tography with standards and spectroscopic meth-
ods –, UV, MS (1–8), NMR (1–3) (Bennini and Chulia, 1994; Chaurasia and Wichtl, 1987; Mark-
ham, 1982). The results confirm earlier report by Niemann (1988) on occurrence of flavonols in the gen-
us 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 flavo-
noids, 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 diter-
penes, paclitaxel and baccatin, with the values of tR 30.1 min and 60.5 min, respectively. The domi-
nant compounds in all investigated genera were flavonoids, 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-rutin-
toside 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, kaempfe-
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flavone 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-
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.*

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* % Content of compound in flavonoid complex.

** Compound chromatographically detected as trace.


[Chem. Abstr. 58, 4502c (1963)].


