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FLAVONOIDS FROM *METASEQUOIA GLYPTOSTROBOIDES*

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Abstract: From the autumnal leaves of *Metasequoia glyptostroboides* were isolated: 3'–O–glucoside tricetin and ginkgetin, bilobetin, 2,3–dihydroisoginkgetin – new compounds in this plant.

Keywords: *Metasequoia glyptostroboides*; flavone O–glycoside; biflavones, aglycones

Until the year 1941, when the natural habitant of *M. glyptostroboides* (Taxodiaceae) was discovered in China, this genus was recognized as the extinct one (1). Since that time, the chemical composition of the essential oil (2) and flavonoids in the leaves of *M. glyptostroboides* has been recognized (3–6). In contrast to O–glycosides, biflavones, because of their significance as chemotaxonomic markers, were intensively analysed in most of species of the family Taxodiaceae (3,4,7). In *M. glyptostroboides*, flavonoids including biflavones, flavone and flavonol O–glycosides were investigated by Beckmann et al. (3), Gadek and Quinn (4), Geiger and Groot–Pfleiderer (5), Katon and Homma (6). Some of the structures of biflavones were re–examined and corrected by Geiger (4), for example: 7–O–methylamentoflavone instead of previously isolated 7''–O–methylamentoflavone, isoginkgetin instead of 7'',4'''–O–dimethylamentoflavone. The results of research on biflavonoids in autumnal and green leaves of *M. glyptostroboides* were similar, the one exception being the occurrence in this species of 2,3–dihydrosciadopitysin (4,5). Its presence in *M. glyptostroboides* has not been confirmed by Gadek and Quinn (4) on the basis of TLC analysis.

The present study on *M. glyptostroboides*, growing in Poland, was undertaken with the hope it might explain and confirm some differences in a flavonoid complex described in the literature (4,5).

EXPERIMENTAL

Plant material

The autumnal leaves of *Metasequoia glyptostroboides* Miki ex Hu et Cheng (Taxodiaceae) were collected from the Botanical Garden in Gdańsk–Oliwa (Poland) in October 1997 and the voucher specimen No. 97–017 was deposited in the Herbarium of the Medicinal Plants Garden of the Medical University of Gdańsk (Poland).

Extraction and isolation

Dried and pulverized leaves of *M. glyptostroboides* (3 kg) were preliminary extracted with petroleum ether in a Soxhlet apparatus. Next, the purified material was extracted with chloroform (Soxhlet apparatus), and after drying it with methanol (3 × 8 l) (temp. 60°C). The concentrated chloroform extract was subjected to column chromatography over a polyamide column (50 g, 40 × 2 cm) eluted with $CHCl_3$–MeOEt (4:3). The obtained fractions 18–20 were subjected to preparative TLC on the polyamide with $CHCl_3$–MeOEt (4:1) and next to chromatography over sephadex LH–20 (5 g, 8 × 1 cm) with MeOH giving compound [**II**] (5 mg) The combined and concentrated methanol extracts (60 ml) were placed into a refrigerator (24 h). The precipitate of impurities was filtered. The filtrate was subjected to column chromatography over the polyamide column (150 g, 54 × 4 cm) and successively eluted with MeOH–H_2O at increasing concentration of MeOH: 30%, 60%, 80% and MeOH. The fractions 70–73 and 74–76 eluted with MeOH was separately subjected to column chromatography over sephadex LH–20 (20 g, 40 × 1.5 cm) with MeOH yielding compound [**I**] (20 mg). From the obtained eluates 7–10 and 8–10 compounds [**V**] (2 mg) were separated by preparative TLC on the polyamide with $CHCl_3$–MeOH–MeOEt (4:8:6) and compound [**VII**] (2 mg), [**VIII**] (2 mg) on silica gel RP–18 with MeOH–H_2O–HCOOH (70:30:6).

From the further fractions 77–81 eluted with MeOH after column chromatography over sephadex LH–20, compounds [**III**] (6 mg) and [**IV**] (4 mg)

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were separated by preparative TLC on the polyamide with $CHCl_3$–MeOEt–MeOH (40:30:5) and $CHCl_3$–MeOEt–MeOH (4:8:6), respectively and the next by re–chromatography over sephadex LH–20.

Reagents

TLC analysis was performed on glass plates covered with cellulose (Merck, Germany) and a polyamide (Merck, Germany) with mobile phases respecitvely: CH_3COOH–H_2O (30:70) (I), BuOH–CH_3COOH–H_2O (4:1:5) upper phase (II) and $CHCl_3$–MeCOEt–MeOH (4:2:3) (III), (4:8:6) (IV). Acid hydrolysis was done according to the literature data (8). NMR spectra were recorded on a Bruker MSL 300 instrument at 500 MHz (for ^1H) and 75.5 MHz (for ^{13}C) in DMSO–d_3 using TMS as an internal standard. LSI–ME (+) (NBA, Cs^+, 6 keV) mass spectral data were obtained using an AMD–Intectra spectrometer.

An HPLC system from Knauer (Berlin, Germany) was used. HPLC analysis was carried out on a Spherisorb ODS II (250 × 4 mm, 5 μm) (Knauer) with the program of gradient elution described earlier (8).

Identification

3'–O–glucoside tricetin (3'–O–β–D–glucopyranoside–5,7,3',4',5'–pentahydroxyflavone) **[I]**. Amorphous powder. TLC cellulose R_f(II)–0.30: R_f(I)–0.12; polyamide R_f(III)–0.03: R_f(IV)–0.17.HPLC t_R: 31.5 min. UV λ_{max} (MeOH) nm: 254sh, 272, 301sh, 374; +$AlCl_3$: 261, 271sh, 316sh, 445; +$AlCl_3$/HCl: 266, 275sh, 360sh, 428; +CH_3ONa: 258sh, 269, 322sh, 423; +CH_3COONa: 269, 410; + CH_3COONa/H_3BO_3: 259, 322sh, 421, LSI–MS (+) *m/z* (% rel. int.): 465 $[M+H]^+$ (58), 303 [A+H]+ (25). ^1H NMR (500 MHz, DMSO–d_6) δ: 6.18 (1H, d, J=1.8 Hz, H–6), 6.47 (1H, d, J=1.8 Hz, H–8), 6.69, (1H, s, H–3), 7.18 (1H, d, J=1.8 Hz, H–6'), 7.36 (1H, d, J=1.8 Hz, H–2'), 4.81 (1H, d, J=7.4 Hz, H–1''–O–glucose), 5.21 (1H, brs, OH–2''), 5.08 (1H, d, J=4.3 Hz, OH–4''), 5.02 (1H, d, J=5.5 Hz, OH–3''), 4.62 (1H, t, OH–6''), 3.80 (1H, dd, J=5.8/10.7 H–6''a/e), 3.55 (2H, m, Hz, H–5'', 6''a/e), 3.4–3.3 (m, H–2''–3''), 3.15 (1H, m, H–4''), 9.03 (1H, s, C–OH–3'). 9.40, (1H, s, C–OH–4'). 12.95 (1H, s, C–OH–5), 10.80, (1H, s, C–OH–7). ^{13}C NMR (DMSO–d_6) δ: 182.0 (C–4), 164.8 (C–2), 164.2 (C–7), 162.1 (C–5), 157.9 (C–9), 146.9, 146.8 (C–3', 5'), 139.7 (C–4'), 121.2 (C–1'), 102.9 (C–2'), 107.0 (C–6'), 104.3 (C–10), 103.9 (C–3), 102.9 (C–1''), 99.5 (C–6), 94.6 (C–8), 78.0 (C–5''), 76.5 (C–3''), 74.0 (C–2''), 70.7 (C–4''), 61.5 (C–6'').

2,3–DIHYDROISOGINKGETIN (5,5',7,7''–TETRAHYDROXY–4',4'''–DIMETHOXY–(3'→8'')–FLAVANONE–FLAVONE) **[II]**

Amorphous powder. TLC polyamide: R_f(III)–0.84. HPLC t_R: 64.2 min. UV (12), LSI–MS (+) *m/z* (% rel. int.): 569 $[M+H]^+$ (75). ^1H, ^{13}C NMR (12).

GINKGETIN (4''',5,'',7''–TETRAHYDROXY–4',7–DIMETHOXY–(3'→8'')–BIFLAVONE) **[III]**

Amorphous powder. TLC polyamide R_f(III)–0.77: R_f(IV)–0.80. HPLC t_R: 55.2 min. UV (11). LSI–MS (+) *m/z* (% rel.int.): 567 $[M+H]^+$ (100), 535 $[M+H–32]^+$ (12). ^1H, ^{13}C NMR (10, 11).

BILOBETIN (4''',5,5'',7,7''–PENTEHYDROXY–4'–METHOXY–(3'→8'')–BIFLAVONE) **[IV]**

Amorphous powder. TLC polyamide R_f(III)–0.43: R_f(IV)–0.56. HPLC t_R: 48.6 min. UV (11). LSI–MS (+) *m/z* (% rel.int.): 553 $[M+H]^+$ (100). ^1H, ^{13}C NMR (10, 11).

KAEMPFEROL **[V]**

Amorphous powder. TLC (8). HPLC t_R: 42.6 min. UV (9). LSI–MS (+) *m/z* (% rel.int.): 287 $[M+H]^+$ (100).

QUERCETIN **[VI]**

Amorphous powder. TLC (8). HPLC t_R: 41.7 min. UV (9). LSI–MS (+) *m/z* (% rel. int.): 303 $[M+H]^+$ (100).

APIGENIN **[VII]**

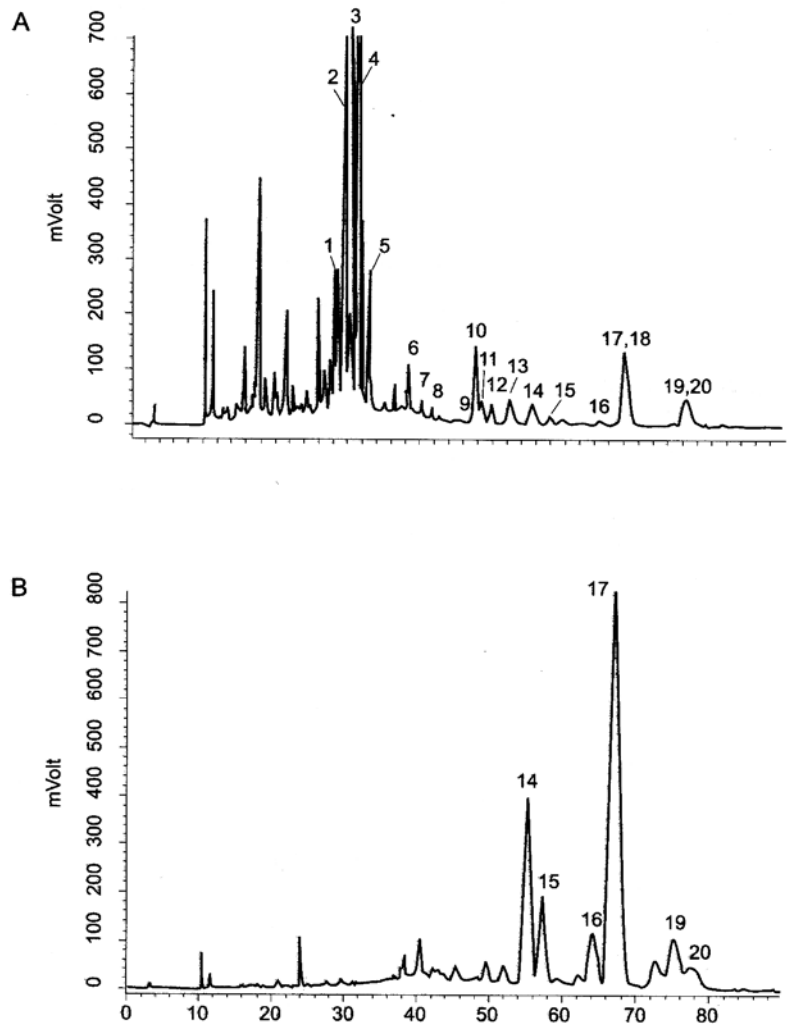
Amorphous powder. TLC cellulose R_f(II)–0.51; polyamide R_f(III)–0.45: R_f(IV)–0.55. HPLC t_R: 40.3 min. UV (9). LSI–MS (+) *m/z* (% rel. int.): 271 $[M+H]^+$ (80).

LUTEOLIN **[VIII]**

Amorphous powder. TLC cellulose R_f(II)–0.79; polyamide R_f(III)–0.22: R_f(IV)–0.33. HPLC t_R: 38.4 min. UV (9). LSI–MS (+) *m/z* (% rel. int.): 287 $[M+H]^+$ (100).

RESULTS AND DISCUSSION

As a result of the preparative column chromatography and preparative TLC of the methanol and chloroform extracts from the leaves of *M. glyptostroboides*, a number of flavonoids not described earlier in this species were isolated, i.e.: compound **[I]**, 2,3–dihydroisoginkgetin **[II]**, ginkgetin **[III]**, bilobetin **[IV]**, quercetin **[V]**, kaempferol **[VI]**, apigenin **[VII]**, luteolin **[VIII]** among with those already identified: 3–O–rhamnoside quercetin, 3–O–rhamnoside kaempferol, 7–O–glucoside apigenin, 7–O–glucoside luteolin, amentoflavone, 4'''–O–methylamentoflavone, 7–O–methylamentoflavone, isoginkgetin, sciadopitysin, hinokiflavone, 2,3–dihydrohinokiflavone, 2,3–dihydrosciadopitysin (3–6). Their structures were established on the



: 1. HPLC chromatograms of the methanol (A) and chloroform (B) extracts from *Metasequoia glyptostroboides*: 1– 7–O–glucoside n; 2– 7–O–glucoside apigenin; 3– 3'–O–glucoside tricetin; 4– 3–O–rhamnoside quercetin; 5– 3–O–rhamnoside kaempferol; 6– n; 7– apigenin; 8– quercetin; 9– kaempferol; 10– amentoflavone; 11– bilobetin; 12– 4'''–O–methylamentoflavone; 13– nethylamentoflavone; 14– ginkgetin; 15– isoginkgetin; 16– 2,3–dihydroisoginkgetin; 17– sciadopitysin; 18– hinokiflavone; 19– hydrosciadopitysin; 20– 2,3–dihydrohinokiflavone.

of co–chromatography with standards (TLC, C) (Figure 1) and UV, MS spectra – aglycones lso ^1H, ^{13}C NMR spectra – biflavones (9–12). now, one of these – 2,3–dihydroisoginkgetin identified only in the yellow leaves of *Ginkgo a* (12). Regarding the literature data, it seems ble that 2,3–dihydroisoginkgetin was earlier ted from *M. glyptostroboides* as 2,3–dihy-7'',4'''–O–dimethylamentoflavone (3).

Upon the acid hydrolysis, compound [**I**] was ded to an aglycone with R_f value (R_f–0.42;) lower than in other standard flavones (api-, luteolin) and sugar–glucose. In the LSI–MS spectrum, the molecular ion $[M+H]^+$ at 465 *m/z* suggests a molecular structure of compound [**I**] as $C_{20}H_{18}O_{12}$. Moreover, the fragmental ion $[A+H]^+$ at 303 *m/z* indicates that aglycones is pentahydroxy-flavone. The ^1H NMR spectrum of compound [**I**] reveals the presence of four singlets at δ 9.03 (C–OH–5'), 9.40 (C–OH–4'), 12.95 (C–OH–5), 10.80, (C–OH–7) assigned to the phenolic hydroxyl groups, two equivalents protons of the B ring (δ_H: 7.36, 7.18) as dublets with J=1.8 Hz (H–2' and H–6'), the singlet at δ_H 6.69 characteristic of H–3 of flavone and also the signals of two *meta*–coupled protons of the A ring (δ_H 6.47 and 6.18, J=1.8

Hz, H–6 and H–8) (12). Moreover, in the HSQC spectrum of compound [**I**], the signal of an anomeric proton of sugar occurring at δ_H 4.81 (J=7.4 Hz), is correlated with the anomeric carbon at δ_C 102.9, what confirms the presence of O–glycosidic linkage between one –OH– group of the side phenyl and sugar moiety. The values of chemical shifts of glucose protons were established from data of HMBC and HSQC spectra. In the ^{13}C NMR spectrum of compound [**I**], in comparison with spectrum of tricetin 3–O–glucoside (9), the carbon signals C–3' and C–5' as well as the carbons C–2' and C–6' were resolved and shifted about $\Delta\delta$=0.14 ppm and $\Delta\delta$=2 ppm, respectively. The values of δ carbon signals in the range 78–61 ppm were characteristic of a sugar – glucose (9). The position of the attachment of a sugar moiety was established from the HMBC spectrum of compound [**I**] and the correlation at δ_H 7.4/δ_C 146.9 (146.8) between H–1'' of glucose and C–3'(5') of aglycone. From the results, therefore, the structure of compound [**1**] was elucidated as 3'–O–β–D–glucopiranoside 5,7, 3',4',5'–penthahydroxyflavone (tricetin). It is worthy of notice, that the occurrence of other tricetin glycosides, namely 7–O–glycoside tricetin has been earlier revealed in *M. glyptostroboides* (5). From this point of view, *M. glyptostroboides* could be classified to the small group of plants containing this flavonoid (14). Tricetin is a rare flavone in the plant kingdom and among its glycosides are described 7–, 3'–mono– and diglucosides, besides 3',5'–diglucosides (14). Lamer (15) identified 3'–O–glucoside tricetin in leaves of *Thuja occidentalis* (Cupressaceae). A number of derivatives of 4'–methyl ether tricetin were separated from some species of the families Boraginaceae (16) and Sapindaceae (17).

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