Flavonoids from Some *Frullania* Species (Hepaticae)*

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From *Frullania teneriffae* 5 known flavone aglycones and 6 flavone glycosides were isolated, among them the new 6-OH-luteolin-6-O-β-D-glucoside-7-O-β-D-glucoside-6‴-(3-hydroxy-3-methylglutaroylester). *F. cesatiana* contains 2 flavone-di-C-glucosides and the new 6-OH-luteolin-7-O-β-D-glucoside-6″-(3-hydroxy-3-methylglutaroylester)-3′-O-glucuronide. The phenolic pattern of *F. eboracensis* was compared to that of *F. muscicola*. The known 6-OH-luteolin-7-O-β-D-glucoside-6″-(3-hydroxy-3-methylglutaroylester) was identified from *F. gibbosa* and *F. arecae*.

Introduction

The large liverwort genus *Frullania* has been the object of many phytochemical studies. Sesquiterpenes, bibenzyls and flavonoids are typical secondary compounds of this genus. It could also be demonstrated that these metabolites are useful markers for chemotaxonomic purposes (Kraut *et al.*, 1993, and references cited therein).

In the present paper we want to present our results on the flavonoid chemistry of 5 *Frullania* species. From *F. teneriffae*, *F. cesatiana* and *F. eboracensis* the main flavonoids were isolated; from *F. gibbosa* and *F. arecae* a previously only partly characterized acylated flavone glycoside (Yuzawa *et al.*, 1987) was now fully identified.

F. teneriffae is a species of atlantic distribution in Western Europe and occurs mainly in Macaronesia. *F. cesatiana* and *F. eboracensis* are taxa of the so called "*F. muscicola* species complex" which is about to be revised taxonomically (Sim-Sim *et al.*, Cryptogamie, in press). *F. cesatiana* is known from some localities of the Southern European Alps, and *F. eboracensis* is distributed in North America.

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Results and Discussion

F. teneriffae (F. Web.) Nees

A total of 7 samples of *F. teneriffae* from Madeira, Porto Santo and Portugal was available for chromatographic analyses by TLC and HPLC. Their patterns of phenolic compounds proved to be identical. From one sample (275 g, see Experimental), 11 flavonoids including 5 flavone aglycones and 6 flavone di-O-glycosides were isolated from the CH_2Cl_2 and the 80% aq. MeOH extract, respectively. The structures were elucidated spectroscopically and by hydrolytic procedures.

The flavone aglycones (**1–5**) belong to the luteolin and apigenin types. Thus luteolin-7,3′,4′-trimethylether (**1**), luteolin-7,3′-dimethylether (**2** = velutin), luteolin-3′,4′-dimethylether (**3**), apigenin-7-methylether (**4** = genkwanin) and apigenin-4′-methylether (**5** = acacetin) were identified. The structures of **1** and **2** could be confirmed by UV, EI-MS, ^1H and ^{13}C NMR spectroscopy; those of **3–5** were established by UV spectroscopy and cochromatography with authentic markers. Genkwanin and acacetin were so far unknown as free aglycones for bryophytes.

Apart from **10** most of the glycosides (**6–11**) were isolated in amounts too small to allow the recording of NMR spectra.

Compound **6** was identified as luteolin-7-O-neohesperidoside by its UV spectra, FAB-MS and acid hydrolysis, followed by analysis of the hydrolytic products, especially the neohesperidose,

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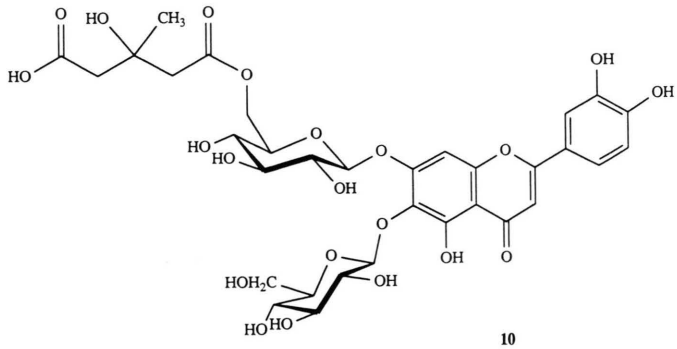

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by cochromatography with appropriate marker compounds.

Glycosides **7** and **8** are acylated luteolin 7-O-neohesperidosides, their acyl part could not be determined.

The chromatographic behaviour and the UV/VIS data characterized **9** as a luteolindiglycoside with a free ortho dihydroxy group in the B-ring, a substituted 7-OH group and an additional 6-OR substitution (Markham 1982; Voirin 1983). Acid treatment resulted in partial hydrolysis of **9**, revealing as products glucose (TLC) and 6-OH-luteolin-7-O-glucoside (**9a**; HPLC, TLC). The maximum of band II at 284 nm in the MeOH UV spectrum in contrast to 272 nm of **9** indicated the cleavage of glucose from the 6-O-position. Further hydrolysis of **9a** gave glucose again and, as aglycone, 6-OH-luteolin. Thus **9** is 6-OH-luteolin-6-O-glucoside-7-O-glucoside. As no NMR spectra were obtained, the configuration of the glucose moieties could not be confirmed, although the monoglucoside **9a** was identical to a standard sample of 6-OH-luteolin-7-O-β-D-glucopyranoside. To our knowledge **9** is a new flavone glycoside.

As the UV/VIS data of **10** were essentially the same as for **9**, the chromophore of **10** should be similar to **9**. The chromatographic behaviour on cellulose layers with H_2O (hRf = 70) and 15% HOAc (hRf = 45) and on polyamide with WEMA (hRf = 22) as solvent systems (see Experimental) was indicative for a 6-OR-luteolin-7-O-glycoside with an aliphatic dicarboxylic acid as additional substituent with a free carboxy group. The FAB-MS afforded the $[M-H]^-$-ion at *m/z* 769 and the aglycone peak at *m/z* 301, corresponding to a glycoside of 6-OH-luteolin, two hexose units and an acid residue of 162 mu. This was indicative for 3-hydroxy-3-methylglutaric acid (= HMG) because we had previously isolated flavone glycosides with HMG as acyl part from other *Frullania* species (Kraut *et al.*, 1993). The ^1H and ^{13}C NMR data (Tables I and II) validated this conclusion as all HMG-typical signals were visible (Kraut *et al.*, 1993). The sugars were identified as two glucoses with β-configuration not linked to each other. The typical shift patterns in the ^1H and ^{13}C NMR spectra proved the site of acylation of one of the glucose units to be C-6, thus characterizing **10** as

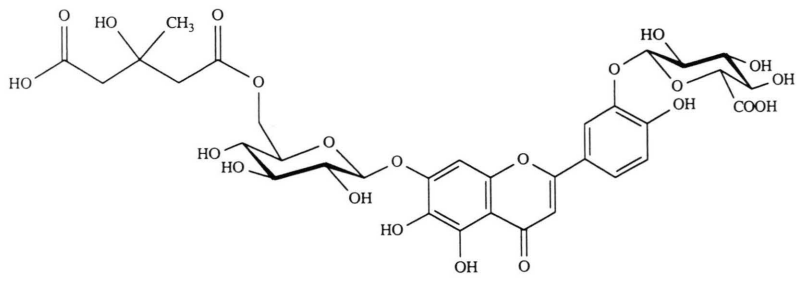

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Table I. ^{1}H NMR data of flavonoids **10** and **14** from *F. teneriffae* and *F. cesatiana* (DMSO-d_6).

H	**10**	**14**
Aglycone		
3	6.91 s*	6.98 s*
8	6.73 s*	6.84 s*
2′	7.47 s	7.84 s
5′	6.87 d (8.4)	6.97 d (7.2)
6′	7.40 d (8.4)	7.65 d (8.5)
5-OH	–	12.68 s
Glucose (at 6-OH)		
1	4.86 d (7.3)	–
Glucose (at 7-OH)		
1	5.09 d (7.2)	5.13 d (7.1)
6a	4.38 d (11.4)	4.28 d (11.8)
6b	4.06 dd (7.0/11.7)	4.13 dd (6.5/11.9)
Glucuronic acid (at 3′-OH)		
1	–	5.00 brs
HMG		
2a	2.34 s	2.38 d (14.6)
2b	2.34 s	2.31 d (14.7)
4a	2.61 d (14.1)	2.57 d (14.6)
4b	2.52 d**	2.51 d**
3-Me	1.14 s	1.10 s

* Assignments exchangeable.
** Exact coupling constant not determinable because of overlapping with DMSO-signal.

Table II. ^{13}C NMR data of the flavonoid **10** of *F. teneriffae* (DMSO-d_6).

C	**10**
Aglycone	
2	164.9
3	102.7
4	182.0
5	152.6[a]
6	129.2
7	155.8
8	94.4
9	152.3[a]
10	105.9
1′	121.3
2′	113.5
3′	146.0
4′	150.0
5′	115.8
6′	118.9
Glucose (at 6-OH)	
1	103.3
2	74.0[b]
3	76.2[c]
4	69.7[d]
5	77.0
6	60.7
Glucose (at 7-OH)	
1	100.7
2	73.2[b]
3	75.4[c]
4	68.7[d]
5	74.0
6	63.2
HMG	
1	170.4
2	45.5
3	69.8[d]
4	45.6
5	173.1
6	27.4

[a–d] Assignments exchangeable.

a 6-OH-luteolin-6-O-glucoside-7-O-glucoside with HMG esterified at one of both glucose C-6-atoms. By alkaline hydrolysis **10** was cleaved to give HMG and a diglucoside identical with **9**. According to TLC treatment with 1 N TFA resulted in 5 products: HMG, glucose, 6-OH-luteolin (**10c**), 6-OH-luteolin-7-O-glucoside (**10b**) and an intermediate product (**10a**). Because of its hRf values and the typical λ_{max} at 284 nm for band II in the MeOH UV spectrum, **10a** had to be 6-OH-luteolin-7-O-glucoside-6″-HMGester. An authentic marker from *F. muscicola* (Kraut *et al.*, 1993) confirmed this assumption. Summarizing **10** represented the new flavonoid 6-OH-luteolin-6-O-β-D-glucopyranoside-7-O-β-D-glucopyranoside-6‴-HMGester.

In contrast to **6–10** which turned yellow or yellow-orange after spraying with NA, **11** appeared as a brown-purple spot on TLC under the same conditions. According to its UV/VIS spectra with diagnostic shift reagents, **11** was a 6-OH-luteolin derivative with 6-OR, 7-OR, 3′-OR and free 4′-OH groups. The TLC data of **11** were similar to those of **10**. After acid hydrolysis with 1 N TFA we could detect the following compounds: glucose as the only sugar, nodifloretin (= 6-OH-luteolin-3′-methylether) as aglycone (**11c**) and the two intermediates **11a** and **11b**. Their chromatographic behaviour was comparable to that observed with **10a** and **10b**, suggesting for **11b** nodifloretin-7-O-glucoside and for **11a** an acylated nodifloretin-7-O-glucoside with unidentified acid residue. The

partial structure of **11** is nodifloretin-6-O-glucoside-7-O-glucoside acylated at the 7-O-glucose.

F. teneriffae is morphologically rather similar to *F. tamarisci*, both belonging to the subgenus *Frullania*. By 2D-TLC of 80% aq. MeOH extracts both species are easy to distinguish. For *F. teneriffae* the remarkable "aglycone spot" and also the position and fluorescence of the glycoside spots are typical, whereas *F. tamarisci* does not exhibit aglycone spots on its 2D-TLC's and its glycoside spots of luteolin type (Mues *et al.*, 1983) are at different positions.

F. cesatiana De Not.

From *F. cesatiana* four collections were available for comparative chromatographic studies, the flavonoid patterns of which also proved to be identical. Two of the four samples were sufficient for flavonoid analysis.

From 1 g plant material three flavone glycosides were obtained (**12–14**). The UV/VIS, FAB-MS, ^{1}H NMR and chromatographic data of **12** allowed its identification as lucenin-2 (= luteolin-6,8-di-C-β-D-glucopyranoside).

Compound **13** was characterized as lucenin-2 with an unknown rest R which was not elucidated due to unsufficient amounts of **13**.

The ^{1}H NMR spectrum of **14** afforded 5 protons in the aromatic region which were assigned to H-2′, H-5′, H-6′, H-3 and H-8 or H-6. Two anomeric protons were visible at 5.13 (d, J = 7.1 Hz) and 5.00 ppm, the latter one being a very broad singlet not showing the splitting into the expected doublet. Moreover two further protons with acyl induced downfield shifts appeared at 4.28 and 4.13 ppm. Their coupling pattern was typical for the C-6 methylene hydroxy protons of a hexose. In the higher field signals for two methylenes and a tertiary methyl group were indicative for HMG again (Table I). Its UV/VIS data characterized glycoside **14** as a 6-OH-luteolin derivative with free 6- and 4′-OH and substituted 7- and 3′-OH. Because of the very high hRf value of 94 on cellulose with H_2O as solvent, more than one carboxy group was assumed for **14**. Its FAB-MS afforded the $[M-H]^-$ at *m/z* 783, fragment peaks appeared at *m/z* 607, 505, 477, 463 and 301. The relative molecular mass of 784 mu counted for a glycoside with 6-OH-luteolin as aglycone, a hexose, a hexuronic acid and HMG. The peak at *m/z* 477 is due to the loss of a hexose-HMGester. Further cleavage of the hexuronic acid results in the aglycone peak at *m/z* 301. The peak at *m/z* 607 originates from the loss of hexuronic acid from **14** to give the acylated monohexoside **14a**. Subsequently the cleavage and fragmentation of HMG from **14a** is indicated by the peaks at *m/z* 505 and 463 resulting in the monohexoside **14b**. Finally the hexose is lost from **14b** to afford the aglycone. Thus, in addition to the ^{1}H NMR spectrum, the FAB-MS of **14** was a further proof for the linkage of HMG to the hexose.

The spectroscopic features discussed so far can be summarized into two structural possibilities for **14**: either 6-OH-luteolin-7-O-hexoside-6″-HMGester-3′-O-hexuronide or 6-OH-luteolin-7-O-hexuronide-3′-O-hexoside-6‴-HMGester. Final evidence for the structure of **14** was achieved by enzymatic hydrolysis with β-glucuronidase, resulting in the cleavage of glucuronic acid identified by TLC. The intermediate flavone glycoside **14a** now showed a yellow-orange fluorescence upon treatment with NA and a dark absorption under the influence of Benedicts Reagent (BR). Hence glucuronic acid had to be cleaved from the 3′-O-position. Moreover the chromatographic properties of **14a** were the same as for 6-OH-luteolin-7-O-glucoside-6″-HMGester from *F. muscicola* (Kraut *et al.*, 1993) as authentic sample. Acid hydrolysis of **14a** yielded HMG, glucose and 6-OH-luteolin. Thus the structure of **14** was elucidated as the new 6-OH-luteolin-7-O-β-D-glucoside-6″-HMGester-3′-O-glucuronide.

According to our results the flavonoid patterns of *F. cesatiana* and *F. muscicola*, which was considered only as a variety of *F. cesatiana* (Bisang *et al.*, 1988), are significantly different. *F. cesatiana* produces flavone-C-glycosides and a flavone-O-glucuronide whereas *F. muscicola* biosynthesizes only flavone-O-glucosides. In order to get further results on the differences in the chemistry of natural products from both taxa we performed a GC/MS from the CH_2Cl_2-extract of *F. cesatiana* and detected sesquiterpene hydrocarbons and sesquiterpene lactones. The main sesquiterpene lactone of *F. cesatiana* afforded a molecular mass at *m/z* 234 ($C_{15}H_{22}O_2$) representing a dihydrolactone, whereas the major sesquiterpene lactone of *F. muscicola* had a molecular composition of $C_{15}H_{20}O_2$ (232 mu) (Kraut *et al.*, 1994).

F. eboracensis Gott.

The phenolic pattern of this species was qualitatively almost identical to that of *F. muscicola* (Kraut *et al.*, 1993). Therefore the isolation of all compounds was not necessary.

By vacuum liquid chromatography (VLC) the 80% aq. MeOH extract was separated into two major fractions F1 and F2. F1 contained compound **15** with a blue fluorescence in UV light and was thought to be a glycerolglucosidecaffeoyl ester according to our experience with *F. muscicola*. After its purification **15** turned out to be glycerol-1-O-glucoside-4′-caffeoylester with the same chromatographic properties as the authentic marker from *F. muscicola* (Kraut *et al.*, 1993). Upon acid treatment glycerol, glucose and caffeic acid were identified. The respective 3′- and 4′-caffeoylesters of *F. muscicola* were not found in *F. eboracensis*.

Fraction F2 contained all flavonoids. Comparative chromatographic studies showed no differences from the flavonoid pattern of *F. muscicola*. The hydrolysis products gave 6-OH-luteolin, scutellarein and luteolin as aglycones, glucose as the only sugar and HMG as acid residue, i.e. the same molecular compounds as for *F. muscicola*, thus suggesting a close relationship between the two taxa.

F. gibbosa Nees and *F. arecae* (Spreng.) Gott.

In a previous chemical and morphological study (Yuzawa *et al.*, 1987) a flavonoid (**16**) of *F. gibbosa* has been characterized as 6-OH-luteolin-7-O-acylglucoside. **16** was repurified by Sephadex LH-20 and we succeeded in recording a ^{1}H NMR spectrum. From the signal pattern (see Experimental) HMG was obviously the acyl moiety esterified at C-6 of the glucose. Alkaline hydrolysis of **16** yielded HMG and 6-OH-luteolin-7-O-glucoside. The same acylglucoside was identified from *F. arecae* by acid hydrolysis and chromatographic comparisons. 6-OH-luteolin-7-O-β-D-glucopyranoside-6″-HMGester was also the main flavonoid of *F. muscicola* (Kraut *et al.*, 1993).

HMG (i.e. its CoA-ester) is a very important intermediate in the biosynthesis of terpenoid compounds. Some examples are known for this acid as acyl part of flavonoid glycosides in higher plants of very different taxa (references in Kraut *et al.*, 1993; Jung *et al.*, 1993). Among bryophytes, however, HMGesters of flavonoids are so far restricted to the genus *Frullania*. We could detect this dicarboxylic acid in the following species from different subgenera: subgenus *Frullania*: *F. teneriffae*, *F. polysticta* (Kraut *et al.*, 1993); subgenus *Trachycolea*: *F. muscicola* (Kraut *et al.*, 1993), *F. cesatiana*, *F. eboracensis* and (unpublished results) *F. dilatata*; subgenus *Chonanthelia*: *F. gibbosa*, *F. arecae*.

Experimental

Plant material

The plant material of the different *Frullania* species originated from the following localities:

F. teneriffae (= Ft): Ft1: Madeira, Juncal, Pico do Arieiro, UTM CB1823, CB1924; Ft2: Madeira, near Lombo dos Cedros, UTM BB9228; Ft3: Madeira, near Fanal, UTM CB0030; Ft4: Madeira, near Balcões, UTM CB2223, CB2323; Ft5: Portugal, Estremadura, UTM MC69; Ft6: Porto Santo, Pico do Facho, UTM CB7660, CB7661; Ft7: Porto Santo, Pico Castelo, UTM CB7560. All samples were collected in October 1990 except Ft1 (May 1989).

F. cesatiana (= Fc): Fc1: Switzerland, Tessin, Gandria, 21.6.92, Nr. 2702; Fc2: Switzerland, Tessin, Gandria, 17.9.89, Nr. 1682; Fc3: Italy, South Tirol, Meran, 23.9.59, Nr. 15348; Fc4: Italy, South Tirol, Isonzo valley, Nr. 31402.

F. eboracensis (= Fe): Fe1: USA, New York, Adirondack Mts., 1991, Nr. 156801; Fe2: see Fe1, Nr. 156802; Fe3: see Fe1, 5.1.92, Nr. 156803; Fe4: USA, New York, Rensselaer county, 11.10.92, Nr. 156804; Fe5: USA, New Jersey, Warren county, 13.9.92, Nr. 156805.

Voucher specimens of all samples are deposited in the herbarium of the Botanical Garden of Lisboa, Portugal (LISU; Ft1–7; Fe1–5), of Bern, Switzerland (BERN; Fc3–4), and of F. Rüegsegger, Switzerland (Fc1–2). For *F. gibbosa* and *F. arecae* see Yuzawa *et al.*, 1987. All samples were identified by M. Sim-Sim.

Extraction and isolation

For comparative chromatographic analyses the respective samples (30–200 mg) were preextracted with CH_2Cl_2 followed by extraction with 80% aq. MeOH. For performance of 2D-TLC of

methanolic extracts see Mues 1988. For GC/MS see Kraut *et al.*, 1994.

F. teneriffae

The ground plant material of sample Ft1 (275 g) was exhaustively extracted with CH_2Cl_2 and then with 80% aq. MeOH. The aqueous phase of the alcoholic extract was partitioned between H_2O and $CHCl_3$, afterwards between H_2O and EtOAc. The $CHCl_3$ phase was combined with the CH_2Cl_2 extract from which the aglycones **1–4** were isolated. At first the extract was chromatographed over a Sephadex LH-20 column with CH_2Cl_2–MeOH = 1:1 as solvent to remove chlorophyll. The aglycones were separated from each other and from impurities by different methods: prep. HPLC on RP18 (70, 75 or 80% MeOH in 1% H_3PO_4 as solvents), by prep. TLC on silica gel (Merck) with toluene–MeOH–HOAc = 90:6:4 and by CC on Sephadex LH-20 with 80% aq. MeOH as solvent. The EtOAc phase contained the aglycones **1**, **2** and **5** and also parts of the glycoside **6**. It was at first passed over a column with Sephadex LH-20 (70% aq. MeOH) to result in 4 major fractions. Fraction 1 contained **6** which was purified by CC on cellulose (microcristalline, for CC, Merck; solvent: 3–12% HOAc) and CC on Sephadex LH-20 (80% aq. MeOH). Fractions 2 and 3 contained pure **2** and **1** respectively. From fraction 4 we isolated **5** by prep. HPLC on RP18 (70% MeOH in 1% H_3PO_4) and CC on Sephadex LH-20 (80% aq. MeOH).

The glycosides **6–11** were isolated from the aqueous phase of the alcoholic extract by CC on cellulose (3–15% HOAc) and by repeated CC on Sephadex LH-20 (50 or 70% aq. MeOH), LPLC on RP18 (Lichroprep 40–63 µm, Merck; solvents: mixtures of MeOH with 5% HOAc) and by PC on Whatmann 3MM with BEW or AEW (see below) as solvents. The yields of the flavonoids were as follows: **1**: 17 mg; **2**: 73 mg; **3–5**: <1 mg each; **6**: 4 mg; **7–8**: <1.5 mg each; **9**: 1.5 mg; **10**: 20 mg; **11**: <1 mg.

F. cesatiana

The plant material of *F. cesatiana* for the isolation of the flavonoids (Fc1–2) was three times extracted with 80% aq. MeOH. The extract was reduced to the H_2O phase and bound on a small column with RP18 conditioned with H_2O. At first H_2O followed by 15% aq. MeOH were passed through the column by VLC to remove polar impurities. Afterwards the flavonoids **12–14** were eluted with 40% aq. MeOH. They were separated from each other by LPLC on RP18 using a gradient between 32 and 50% MeOH in 5% HOAc. Final purification was achieved with Sephadex LH-20 using 70% aq. MeOH. Yields: **12**: ca. 2 mg; **13**: <1.5 mg; **14**: 3.2 mg.

F. eboracensis

From *F. eboracensis* 2.8 g plant material of 5 combined samples (Fe1–5) were at disposal. The extraction procedure and the first isolation step were identical to those mentioned above for *F. cesatiana*. From the fraction eluted with 15% aq. MeOH we isolated **15** (<1 mg) by prep. HPLC on RP18 (25% MeOH in 1% H_3PO_4) followed by CC on Sephadex LH-20 (70% aq. MeOH). The flavonoids were enriched in the fraction which was eluted with 40% aq. MeOH. At first they were chromatographically analysed together with the flavonoids from *F. muscicola*. Afterwards the major part of them was hydrolysed with 1 N TFA for 4 hours. After removal of TFA the hydrolysis products were separated on RP18: in the H_2O fraction we detected HMG and glucose; the 40% aq. MeOH fraction contained rests of unhydrolysed glycosides. The aglycones were eluted with pure MeOH and analysed by TLC and HPLC.

F. gibbosa and *F. arecae*

The procedures regarding the extraction and isolation of the flavonoids of these species are described in Yuzawa *et al.*, 1987.

TLC of all compounds was performed on precoated sheets: cellulose F 1440 (Schleicher and Schüll); polyamide-6 Polygram (Macherey and Nagel); polyamide-11 (self-made plates of Prof. Wollenweber); silica gel 60 F_{254} (Merck); RP18 F_{254s} (Merck); solvent systems: on cellulose: H_2O, mixtures of HOAc with H_2O, BAW = *n*-BuOH–HOAc–H_2O = 4:1:5 (upper phase), BEW = BuOH(2)–HOAc–H_2O = 14:1:5, AEW = *n*-pentanol–HOAc–H_2O = 2:1:1, EtOAc–C_5H_5N–HOAc–H_2O = 36:36:7:21 (for sugars), *n*-pentanol–HCOOH–H_2O = 20:20:1 (for aliphatic acids); on polyamide-6: WEMA = H_2O–MeCOEt–

MeOH–acetylaceton = 13:3:3:1, toluene–MeCOEt–MeOH = 4:3:3; toluene–petrolether–MeCOEt–MeOH = 60:30:10:5; MeOH–H_2O–25%NH_3 = 4:2:1; on silica gel: EtOAc–HOAc–HCOOH–H_2O = 100:11:11:27, toluene–MeOH–HOAc = 90:6:4, toluene–ethylformate–HCOOH = 5:4:1, EtOAc–MeOH–HOAc–H_2O = 12:3:3:2 (for sugars), *n*-BuOH–C_5H_5N–H_2O = 50:33:17 (for hexuronic acids); ethylformate–$CHCl_3$–HCOOH = 2:7:1, di-*iso*propylether–HCOOH–H_2O = 13:5:2 (for aliphatic acids), *iso*-PrOH–$CHCl_3$–MeOH–10%NH_3 = 9:9:4:2, *n*-BuOH–*iso*-PrOH–0.5%H_3BO_3–HOAc = 15:25:5:1 (for glycerol); on RP18: mixtures of MeOH or CH_3CN with 5% HOAc; spray reagents: Naturstoffreagenz A (NA, 0.1% in MeOH), Benedicts Reagenz (BR), anilinephthalate (for sugars), naphthoresorcin 0.2 g in 100 ml EtOAc + 2 ml conc. H_2SO_4 (for sugars); dichlorofluorescin/$Pb(OAc)_4$ (for glycerol); acridine or bromocresol green (for aliphatic acids). HPLC: Waters, two M-45 pumps with "automated gradient controller", variable wavelength detector 450; Bischoff, two pumps model 2200, UV/VIS detector Lambda 1000; columns: Nucleosil 5 C_{18} 250×4 mm (Macherey and Nagel), Nucleosil 7 C_{18} 250×10 mm (Macherey and Nagel), Spherisorb ODS II (5 μm) 250×20 mm (Bischoff); isocratic or gradients using mixtures of MeOH or CH_3CN and 1% H_3PO_4; detection at 280, 320 or 340 nm.

Hydrolysis methods

Acid hydrolysis: for **6** and **8**: 0.05 N TFA, 15 min, reflux and afterwards 1 N TFA, 30 min, reflux; for **7, 9, 9a, 10, 11, 13** and **14a**: 1 N TFA, 45 min or 1 h, reflux; for cleavage of neohesperidose from **6**: 1% H_2SO_4, 20 min, 70 °C.

Alkaline hydrolysis: for **10, 16** and **17** according to Markham (1982).

Enzymatic hydrolysis: for **14**: β-glucuronidase ("β-Glucuronidase pulv.", Fluka, Switzerland, No. 49320), 24 h, deion. H_2O, 30 °C.

Separation of sugars or acids from glycosides or aglycones by solid phase extraction on RP18 (self made columns or Chromabond C18 ec, Macherey and Nagel).

Spectroscopic methods

UV/VIS spectroscopy: Shimadzu UV-160 A, according to Markham 1982; NMR spectroscopy: Bruker AM 400, DMSO-d_6, ambient temperature, 400 MHz (^{1}H), 100 MHz (^{13}C); mass spectroscopy: EI, Varian MAT 311, 70 eV; FAB, Finnigan MAT 90, xenon, 5–8 keV, glycerol as matrix; for GC/MS: see Kraut *et al.*, 1994.

Spectroscopic data

1: λ_{max}; MeOH: 242–248–268–289sh–340; NaOMe: 284–303sh–365; $AlCl_3$: 262–276–295sh–359–383; $AlCl_3$/HCl: 258–277–289sh–350–379; NaOAc: 248–268–340; NaOAc/H_3BO_3: 248–268–340. ^{1}H NMR (DMSO-d_6), δ (ppm): 7.01 (s, H-3), 6.36 (d, J = 2.2 Hz, H-6), 6.79 (d, J = 2.1 Hz, H-8), 7.58 (d, J = 2.1 Hz, H-2′), 7.70 (dd, J = 2.1/8.5 Hz, H-6′), 7.13 (d, J = 8.7 Hz, H-5′), 3.85, 3.87, 3.88 (each s, 7-, 3′- and 4′-OMe respectively; assignments exchangeable). ^{13}C NMR (DMSO-d_6), δ (ppm): 163.5 (C-2), 103.9 (C-3), 181.8 (C-4), 161.1 (C-5), 97.9 (C-6), 165.1 (C-7), 92.7 (C-8), 157.2 (C-9), 104.6 (C-10), 122.7 (C-1′), 109.6 (C-2′), 149.0 (C-3′), 152.2 (C-4′), 111.7 (C-5′), 120.1 (C-6′), 55.7, 56.0, 55.9 (7-, 3′- and 4′-OMe, assignments exchangeable). EI-MS: *m/z* (rel. intensities, %) 328 (100, M^+), 299 (12, $[M-H-CO]^+$), 285 (7, $[M-CH_3-CO]^+$), 167 (11, $[A_1+H]^+$), 162 (6, B_1^+).

2: λ_{max}; MeOH: 243sh–250–266–290sh–345; NaOMe: 262sh–296sh–387; $AlCl_3$: 261–275–295sh–362–385; $AlCl_3$/HCl: 261–275–295sh–355–380sh; NaOAc: 249sh–266–361sh–408; NaOAc/H_3BO_3: 258–266sh–347. ^{1}H NMR (DMSO-d_6), δ (ppm): 6.94 (s, H-3), 6.36 (d, J = 2.1, H-6), 6.79 (d, J = 2.1 Hz, H-8), 7.57 (brs, H-2′), 7.59 (dd, J = 2.1/8.4 Hz, H-6′), 6.93 (d, J = 8.8 Hz, H-5′), 3.87, 3.89 (each s, 7- and 3′-OMe, assignments exchangeable). ^{13}C NMR (DMSO-d_6), δ (ppm): 163.8 (C-2), 103.2 (C-3), 181.8 (C-4), 161.1 (C-5), 97.8 (C-6), 165.0 (C-7), 92.5 (C-8), 157.1 (C-9), 104.6 (C-10), 121.3 (C-1′), 110.3 (C-2′), 150.8 (C-3′), 147.9 (C-4′), 115.7 (C-5′), 120.4 (C-6′), 55.9 (7- and 3′-OMe). EI-MS: *m/z* (rel. intensities, %) 314 (100, M^+), 285 (15, $[M-H-CO]^+$), 271 (12, $[M-CH_3-CO]^+$), 167 (11, $[A_1+H]^+$), 151 (4, B_2^+), 148 (6, B_1^+), 133 (6, $[B_1-CH_3]^+$).

3: λ_{max}; MeOH: 245sh–269–337.

4: λ_{max}; MeOH: 267–335; NaOMe: 272–301sh–343sh–380; $AlCl_3$: 276–300–350–382; $AlCl_3$/HCl: 276–300–340–378; NaOAc: 267–350sh–387; NaOAc/H_3BO_3: 267–339.

5: λ_{max}; MeOH: 270–329.

6: λ_{max}; MeOH: 254–265sh–345; NaOMe: 262–308–398; $AlCl_3$: 273–292sh–328–426; $AlCl_3$/HCl: 269–289sh–358–381; NaOAc: 258–401; NaOAc/H_3BO_3: 258–373. FAB-MS: *m/z* 593 ($[M-H]^-$), 447 ($[M-H-rhamnose]^-$), 285 ($[M-H-rhamnose-glucose]^-$ = aglycone fragment).

7: λ_{max}; MeOH: 249–262sh–295–346; NaOMe: 268–300sh–388; $AlCl_3$: 238–271–295sh–359–420; $AlCl_3$/HCl: 250sh–277sh–295sh–357–380sh; NaOAc: 250sh–263sh–294sh–342–412; NaOAc/H_3BO_3: 250sh–342sh–409 (**8**: almost identical UV spectroscopic data as **7**).

9: λ_{max}; MeOH: 254–272–346; NaOMe: 269–295sh–345sh–400; $AlCl_3$: 275–304sh–339–426; $AlCl_3$/HCl: 266sh–278–288sh–366; NaOAc: 275–336sh–412; NaOAc/H_3BO_3: 276–419 (**10**: almost identical UV spectroscopic data as **9**).

11: λ_{max}; MeOH: 254–272–345; NaOMe: 244sh–265–300sh–393; $AlCl_3$: 265sh–300sh–314sh–364; $AlCl_3$/HCl: 260sh–295sh–362; NaOAc: 272–298sh–366sh–430; NaOAc/H_3BO_3: 264sh–276–356.

12: λ_{max}; MeOH: 257–272–346; NaOMe: 269–281sh–334sh–411; $AlCl_3$: 278–300sh–331–425; $AlCl_3$/HCl: 278–300sh–359–388sh; NaOAc: 275sh–282–324sh–385; NaOAc/H_3BO_3: 266–378. ^1H NMR: 6.64 (s, H-3), 7.44 (m, H-2′,6′), 6.86 (d, J = 7.6 Hz, H-5′), 13.72 (s, 5-OH), 4.95, 4.75 (each brs, H-1″ and H-1‴, assignments exchangeable). FAB-MS: *m/z* 609 ($[M-H]^-$).

13: λ_{max}; MeOH: 254sh–274–297sh–339; NaOMe: 267–283sh–391; $AlCl_3$: 273–305sh–368–427; $AlCl_3$/HCl: 281–300–340–398sh; NaOAc: 281–332–383sh; NaOAc/H_3BO_3: 262–364; FAB-MS: *m/z* 609 (fragment peak).

14: λ_{max}; MeOH: 285–339; NaOMe: 250sh–317sh–382; $AlCl_3$: 298–363; $AlCl_3$/HCl: 296–360; NaOAc: 286–350sh–391; NaOAc/H_3BO_3: 291–333.

16: ^1H NMR: δ (ppm): aglycone: 7.45 (d, J = 1.9 Hz, H-2′), 7.37 (dd, J = 2.0/8.2 Hz, H-6′), 6.86 (d, J = 8.4 Hz, H-5′), 6.84 (s, H-8 or H-3), 6.67 (s, H-3 or H-8), glucose: 5.05 (d, J = 7.2 Hz, H-1), 4.35 (d, J = 10.6 Hz, H-6a), 4.08 (dd, J = 7.5/11.9 Hz, H-6b), HMG: 2.60 (d, J = 14.0 Hz, H-4a), 2.49 (H-4b, overlapped with DMSO-signal), 2.32 (s, H-2a/H-2b), 1.12 (s, 3-Me).

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