Tensioactive Compounds from the Aquatic Plant *Ranunculus fluitans* L. (Ranunculaceae)

by **Christian Wegner** and **Matthias Hamburger***

Institute of Pharmaceutical Biology, University of Jena, D-07743 Jena

and **Olaf Kunert** and **Ernst Haslinger**

Institute of Pharmaceutical Chemistry, University of Graz, A-8010 Graz

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In the search for the cause for the formation of persistent foam in the Rhine River below the Rhine Fall at Schaffhausen, an investigation of the tensioactive principles from the aquatic plant *Ranunculus fluitans* L. (*Ranunculaceae*) was carried out. Two new (see **1** and **2**) and four known bisdesmosidic triterpene saponins (see **4**–**6**) were isolated along with the two known diacylglycerol galactosides **7** and **8**. The saponin structures were established by the identification of the aglycon and sugar moieties by HPLC and chiral capillary zone electrophoresis (CZE), ion-spray LC/MS and extensive 1- and 2D homo- and heteronuclear NMR spectroscopy. The structures of the new oleanane-type saponins were identified as 3-*O*-[β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranosyl]-28-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl]hederagenin (**1**) and 3-*O*-[β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranosyl]oleanolic acid [α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] ester (**2**). LC/MS Studies of tensioactive fractions revealed the presence of additional glycoglycerolipids.

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1. Introduction. – For almost thirty years, a persistent foam cover has been observed in the Rhine River beneath the Rhine Fall near Schaffhausen, Switzerland, during the summer months (June to September). The foam varies from day to day in intensity, but may cover up to half of the river surface and only gradually disappears a few kilometers downstream. In the back water areas of the river, massive foam lumps may accumulate. This phenomenon is a matter of continuing concern to the authorities and the public, and it is obviously also highly disturbing in an area of considerable attraction for tourism [1]. Numerous attempts have been made over the years to elucidate the nature and origin of the tensioactive compounds responsible for the formation of the foam. As industrial pollution by detergents or other tensioactive chemicals could be ruled out, the mystery remained unresolved. Although a possible biogenic origin of the surfactants was discussed already in 1976 [2], no investigations were carried out in this direction. Only recently, the hypothesis of a possible natural origin of the phenomenon was revived.

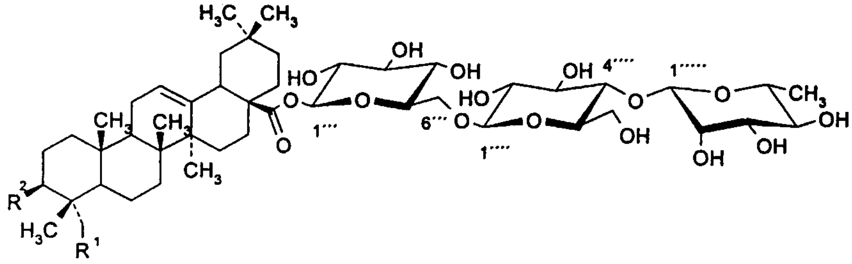
Two converging lines of evidence and reasoning are at the origin of this hypothesis. First, the occurrence of foam formation in the years 1970/71 coincided with an almost explosive growth of the aquatic plant *Ranunculus fluitans* L. (*Ranunculaceae*). Certainly, *R. fluitans* was part of the original aquatic flora of the upper Rhine River, since the earliest safe record in the area of Schaffhausen was documented in 1847/48 by *Laffon* in his 'Flora des Cantons Schaffhausen'. Up to the late 1960's, *R. fluitans* was a regularly but sparsely encountered species of an aquatic flora largely dominated by several *Potamogeton* species and *Zannichellia palustris* [3]. In the years 1970/71, however, *R. fluitans* very suddenly began to form banks of dense growth along large stretches of the river bed between Lake Constance and Schaffhausen. It is now generally accepted that the massive expansion was linked to an increase in nitrate and phosphate concentration in the upper Rhine River [3][4].

A second argument in favor of a possible causal link between *R. fluitans* and the occurrence of foam is based on chemotaxonomic considerations. The occurrence of triterpene saponins is considered to be a characteristic feature of the family *Ranunculaceae*. Oleanene-type saponins have been reported from various genera of the family, such as *Clematis*, *Pulsatilla*, *Anemone*, *Thalictrum*, and *Caltha* species [5–11]. Considering the large size of the family, however, the number of saponin structures established is surprisingly small. In the genus *Ranunculus*, for example, saponin structures have been reported only from *Ranunculus ficaria* [5][6].

In an attempt to resolve the lingering uncertainty about the nature and origin of the foam formation in the upper Rhine River, we recently embarked on a project in which we attempt to clarify the role of *R. fluitans* in this matter. As a first step in a systematic approach to the issue, we report here the occurrence and nature of tensioactive constituents in the plant.

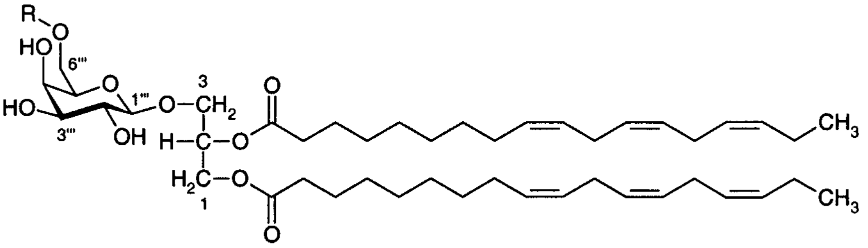
2. Results. – Preliminary TLC analysis of a MeOH extract from the herbal parts of *R. fluitans* revealed the presence of several spots exhibiting chromatographic mobility and staining properties of saponins. For the isolation of tensioactive compounds, the MeOH extract was partitioned between BuOH and H_2O. The BuOH extract was subjected to vacuum liquid chromatography (VLC) on silica gel to afford 8 fractions. The surfactant activity of the individual fractions was monitored by their ability to form a stable foam in aqueous solution. Subsequently, a saponin fraction and two glycoglycerolipid fractions were further purified by solid-phase extraction on *RP 18* and gel chromatography on *Sephadex LH 20*, followed by low-pressure reversed-phase liquid chromatography on a *RP 18* column. A total of 6 saponins, *i.e.*, **1**–**6**, and of two glycoglycerolipids, *i.e.*, **7** and **8**, was obtained. Final purification was achieved by semi-preparative reversed-phase HPLC or gel chromatography (see *Exper. Part*).

Acidic hydrolysis of the saponins **1**–**6** afforded hederagenin in case of the saponins **1**, **3**, **4**, and **5**, and oleanolic acid for **2** and **6**. The monosaccharide residues of **1**–**6** were identified by HPLC, whereas their assignment to the D- and L-series was established by capillary zone electrophoresis [12]. The sugar moieties thus were identified as D-glucose and L-rhamnose in the case of compounds **2**, **3**, and **6**, and as D-glucose, L-rhamnose, and L-arabinose for compounds **1**, **4**, and **5**. ^{1}H- and ^{13}C-NMR Data of **1**–**6** confirmed the nature of the triterpene moieties [13] (see *Table 1*). The NMR findings were confirmed by co-TLC of the aglycons with authentic samples. The ^{13}C-NMR resonances of C(3) (δ 82.3–82.7 for **1** and **3**–**5**, 89.3 for **2**, and 89.1 for **6**) and C(28) (δ 176.6–177.0) for all saponins were indicative of *O*-glycosidic and ester glycosidic linkages at these positions [14]. All saponins were thus bisdesmosides. The number of anomeric C-atom resonances and the remaining signals attributable to the carbohydrate moiety suggested that **3**, **5**, and **6** were tetraglycosides, while **1**, **2**, and **4** were pentaglycosides (*Table 2*). A Me signal (δ 18.6) in the ^{13}C-NMR spectra of **1**–**6** supported the presence of a rhamnosyl residue in all saponins.



	R^1	R^2 a)
1	OH	Glc(1→3)Ara
2	H	Glc(1→3)Glc
3	OH	Glc
4	OH	Glc(1→2)Ara
5	OH	Ara
6	H	Glc

a) Glc = β-D-glucopyranosyl; ara = α-L-Arabinopyranosyl.



	R a)
7	H
8	Gal

a) Gal = α-D-Galactopyranosyl.

The ESI-MS of **1** recorded in the positive-ion mode showed two quasi-molecular ions at m/z 1255 ($[M+NH_4]^+$) and 1238 ($[M+H]^+$). These data were confirmed by a quasi-molecular ion at m/z 1236 ($[M-H]^-$) in the negative-ion mode. Since the nature of the sugars had already been identified, fragment signals (see *Exper. Part*) suggested the consecutive loss of three glucosyl, one rhamnosyl, and one arabinosyl moieties. Basic hydrolysis of **1** followed by LC/MS (ESI, positive-ion mode) showed a quasi-molecular ion of monodesmoside **1a**¹) at m/z 768 ($[M+H]^+$) and a fragment ion at m/z 606 ($[M+H-162]^+$) indicative of a diglycosyl residue consisting of a glucosyl and an arabinosyl moiety attached at C(3) of the aglycon.

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¹) The key numbers **1a**, **3a**, **5a**, and **6a** are used for the basic-hydrolysis products obtained from **1**, **3**, **5**, and **6**, respectively, which contain the aglycon moiety.

Table 1. *^{13}C- and ^{1}H-NMR Chemical Shifts* δ [ppm] *of the Aglycon Part of* **1–6** *in (D$_5$)Pyridine.* At 600 (^{1}H) or 150 MHz (^{13}C) and 313 K; SiMe$_4$ as internal standard.

	1		**2**		**3**		**4**		**5**		**6**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
CH$_2$(1)	39.1	1.58, 1.05	39.0	1.45, 0.90	39.1	1.51, 0.99	39.0	1.59, 1.22	39.1	1.59, 1.06	39.0	1.47, 0.92
CH$_2$(2)	26.5	2.20, 1.99	26.7	2.16, 1.79	26.2	2.24, 1.93	26.5	2.14, 1.95	26.3	2.22, 2.00	26.7	2.23, 1.84
H–C(3)	82.3	4.22	89.3	3.36	82.7	4.23	4.11	82.6	82.5	4.21	89.1	3.40
C(4)	43.8		39.8		43.6		43.7		43.8		39.7	
H–C(5)	48.6	1.76	56.2	0.79	48.5	1.73	48.4	1.73	48.6	1.75	56.1	0.81
CH$_2$(6)	18.8	1.68, 1.36	18.9	1.49, 1.32	18.6	1.68, 1.37	18.8	1.67, 1.36	18.6	1.65, 1.37	18.9	1.50, 1.33
CH$_2$(7)	33.2	1.59, 1.31	33.4	1.48, 1.34	33.1	1.59, 1.31	33.0	1.55, 1.29	33.1	1.60, 1.31	33.4	1.49, 1.35
C(8)	40.2		40.2		40.1		40.1		40.2		40.2	
H–C(9)	48.0	1.65	48.4	1.62	48.2	1.61	48.2	1.57	48.0	1.64	48.2	1.63
C(10)	37.2		37.3		37.2		37.1		37.2		37.1	
CH$_2$(11)	24.1	1.94, 1.94	24.0	1.91, 1.91	24.1	1.93, 1.93	24.0	1.94, 1.94	24.1	1.94, 1.94	23.9	1.91, 1.91
H–C(12)	123.4	5.41	123.2	5.42	123.5	5.41	123.2	5.41	123.4	5.41	123.2	5.44
C(13)	144.5		144.4		144.7		144.5		144.5		144.4	
C(14)	42.3		42.4		42.4		42.4		42.5		42.3	
CH$_2$(15)	28.6	2.26, 1.09	28.6	2.29, 1.17	28.7	2.26, 1.09	28.5	2.26, 1.08	28.5	2.26, 1.09	28.4	2.30, 1.18
CH$_2$(16)	23.6	2.03, 1.91	23.7	2.09, 1.94	23.7	2.04, 1.91	23.6	2.03, 1.90	23.6	2.04, 1.90	23.5	2.09, 1.96
C(17)	47.3		47.5		47.5		n.d.		47.4		47.3	
H–C(18)	42.0	3.18	42.0	3.19	42.1	3.17	42.0	3.18	42.0	3.17	41.8	3.19
CH$_2$(19)	46.6	1.71, 1.22	46.5	1.76, 1.25	46.6	1.73, 1.23	46.6	1.72, 1.22	46.6	1.71, 1.22	46.5	1.77, 1.26
C(20)	30.9		30.9		30.9		30.9		31.0		30.9	
CH$_2$(21)	34.3	1.32, 1.12	34.3	1.35, 1.14	34.4	1.32, 1.12	34.2	1.32, 1.12	34.3	1.31, 1.12	34.2	1.36, 1.16
CH$_2$(22)	32.8	1.89, 1.75	32.8	1.91, 1.78	32.9	1.89, 1.76	32.8	1.90, 1.75	32.8	1.88, 1.74	32.7	1.91, 1.78
CH$_2$(23)	64.7	4.25, 3.68	28.4	1.29	65.4	4.28, 3.69	65.1	4.25, 3.69	65.0	4.20, 3.67	28.4	1.31
Me(24)	13.9	0.94	17.2	1.00	13.8	0.98	13.6	1.02	13.7	0.94	17.2	1.02
Me(25)	16.4	0.99	15.9	0.88	16.5	0.97	16.4	0.99	16.4	1.00	15.7	0.90
Me(26)	17.9	1.10	17.7	1.09	18.0	1.11	17.7	1.11	17.8	1.11	17.6	1.10
Me(27)	26.3	1.19	26.4	1.26	26.4	1.19	26.2	1.17	26.3	1.18	26.2	1.27
Me(28)	177.0		176.9		177.0		176.6		176.9		176.8	
Me(29)	33.5	0.88	33.4	0.90	33.5	0.88	33.3	0.88	33.4	0.88	33.4	0.91
Me(30)	23.9	0.90	24.0	0.92	23.9	0.91	23.9	0.91	23.9	0.91	23.9	0.92

A more detailed structural analysis of **1** and the complete assignments of the ^{1}H- and ^{13}C-NMR resonances (see *Tables 1* and *2*) are mainly based on 1D and 2D NMR experiments (COSY, TOCSY, HMBC, HSQC, HSQC-TOCSY and ROESY). The structure of **1** was thus determined to be 3-*O*-[β-D-glucopyranosyl-(1 → 3)-α-L-arabinopyranosyl]-28-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl]hederagenin.

Table 2. *^{13}C- and ^{1}H-NMR Chemical Shifts δ* [ppm] *of the Carbohydrate Moieties of* **1**–**6** *in (D_5)Pyridine.* At 600 (^{1}H) or 150 MHz (^{13}C) and 313 K; $SiMe_4$ as internal standard; *J* in Hz.

	1		**2**		**3**		**4**		**5**		**6**	
	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)
Ara*p*′[a])/Glc*p*′[b])	106.7	4.92	106.4	4.85	106.2	5.08	104.1	5.14	106.8	4.92	106.7	4.91
H−C(1′)		(*J* = 7.3)		(*J* = 7.7)		(*J* = 7.1)		(*J* = 6.7)		(*J* = 7.8)		(*J* = 7.6)
H−C(2′)	72.2	4.52	74.6	4.02	76.2	4.00	81.4	4.52	73.4	4.38	76.0	4.01
H−C(3′)	84.6	4.06	89.3	4.16	79.0	4.12	73.7	4.23	75.0	4.04	79.0	4.21
H−C(4′)	69.5	4.34	70.1	4.07	72.2	4.18	68.4	4.28	69.7	4.22	72.1	4.19
CH_2(5′) or	67.2	4.15,	78.0	3.88	78.5	3.87	65.2	4.16,	67.1	4.25,	78.3	3.97
H−C(5)		3.62						3.72		3.71		
CH_2(6′)			62.9	4.44,	63.3	4.46,					63.3	4.54,
				4.27		4.35						4.37
Glc*p*″[b])	106.5	5.25	106.1	5.24			106.1	5.15				
H−C(1″)		(*J* = 7.9)		(*J* = 8.5)				(*J* = 8.2)				
H−C(2″)	76.0	3.99	75.7	4.03			76.3	4.04				
H−C(3″)	78.7	4.21	78.5	4.19			78.4	4.12				
H−C(4″)	72.0	4.17	71.9	4.14			71.7	4.17				
H−C(5″)	79.0	3.95	78.9	3.99			78.4	3.80				
CH_2(6″)	63.1	4.49,	62.9	4.49,			62.8	4.42,				
		4.32		4.27				4.32				
Glc*p*‴[b])	96.0	6.19	95.9	6.19	96.1	6.19	95.9	6.19	96.0	6.18	95.8	6.20
H−C(1‴)		(*J* = 7.9)		(*J* = 8.0)		(*J* = 8.3)		(*J* = 8.1)		(*J* = 7.8)		(*J* = 8.2)
H−C(2‴)	74.4	4.08	74.2	4.10	74.3	4.09	74.4	4.08	74.3	4.08	74.0	4.11
H−C(3)‴	79.1	4.16	79.0	4.16	79.0	4.16	79.0	4.16	79.0	4.17	79.0	4.18
H−C(4‴)	71.4	4.23	71.3	4.24	71.4	4.24	71.3	4.23	71.3	4.23	71.3	4.25
H−C(5‴)	78.4	4.07	78.3	4.07	78.4	4.07	78.2	4.06	78.4	4.07	78.2	4.08
CH_2(6‴)	69.7	4.62,	69.6	4.63,	69.7	4.62,	69.6	4.62,	69.6	4.62,	69.5	4.63,
		4.29		4.30		4.29		4.30		4.30		4.31
Glc*p*⁗[b])	105.3	4.95	105.1	4.95	105.3	4.96	105.2	4.96	105.3	4.95	105.3	4.96
H−C(1⁗)		(*J* = 7.5)		(*J* = 7.9)		(*J* = 7.3)		(*J* = 7.8)		(*J* = 7.0)		(*J* = 7.6)
H−C(2⁗)	75.6	3.90	75.6	3.90	75.5	3.90	75.5	3.90	75.6	3.90	75.5	3.91
H−C(3⁗)	76.9	4.10	76.7	4.11	76.9	4.11	76.7	4.11	76.9	4.09	76.7	4.11
H−C(4⁗)	78.9	4.33	78.8	4.34	79.0	4.33	78.8	4.33	79.0	4.32	78.8	4.33
H−C(5⁗)	77.5	3.66	77.4	3.66	77.4	3.66	77.3	3.66	77.4	3.65	77.3	3.66
CH_2(6⁗)	61.7	4.19,	61.9	4.19,	61.8	4.19,	61.6	4.19,	61.7	4.18,	61.6	4.20,
		4.06		4.06		4.06		4.07		4.06		4.08
Rha*p*′′′′′[c])	103.0	5.78	102.9	5.77	103.2	5.78	102.9	5.78	103.0	5.76	102.9	5.78
H−C(1′′′′′)												
H−C(2′′′′′)	72.8	4.61	72.7	4.61	72.8	4.61	72.6	4.61	72.8	4.61	72.7	4.62
H−C(3′′′′′)	73.1	4.48	73.0	4.48	73.1	4.48	72.9	4.48	73.1	4.47	73.0	4.49
H−C(4′′′′′)	74.3	4.26	74.2	4.26	74.3	4.26	74.2	4.25	74.2	4.26	74.2	4.27
H−C(5′′′′′)	70.6	4.85	70.6	4.85	70.7	4.86	70.5	4.86	70.7	4.83	70.5	4.85
Me(6′′′′′)	18.8	1.66	18.8	1.67	18.8	1.67	18.8	1.67	18.8	1.66	18.6	1.68

[a]) Ara*p* = α-L-Arabinopyranosyl. [b]) Glc*p* = β-D-Glucopyranosyl. [c]) Rha*p* = α-L-Rhamnopyranosyl.

The point of attachment of the saccharide units of **1** to the genin and the interglycosidic linkages were established by an HMBC experiment. Long-range correlations were observed between H−C(1′) of arabinose and C(3) of hederagenin as well as between H−C(1″) of Glc*p* and C(3′) of Ara*p*. Further correlations were observed between H−C(1‴) of Glc*p* and C(28) of the genin, between H−C(1⁗) of Glc*p* and C(6‴) of Glc*p* and between H−C(1′′′′′) of Rha*p* and C(4⁗) of Glc*p*. This connection was confirmed by an HMBC correlation between H−C(4⁗) of Glc*p* and C(1′′′′′) of Rha*p*. The α-L configuration of the Ara*p* moiety and β-D configuration of all Glc*p* moieties were derived from the coupling constants of the anomeric protons, which were characteristic for pyranoses with axially oriented protons at positions 1 and 2. The α-L configuration of Rha*p* was established by comparing the δ(C) of C(3′′′′′) and C(5′′′′′) with data for methyl rhamnopyranosides [15].

The ESI-MS of **2** (positive-ion mode) showed a quasi-molecular ion at *m/z* 1269 ($[M+NH_4]^+$), which was confirmed by a signal at *m/z* 1250 ($[M-H]^-$) in the negative-ion mode. The signals and, however, weak fragment ions suggested an oleanolic acid bisdesmoside with one rhamnosyl and 4 glucosyl moieties. All remaining data identified **2** as 3-*O*-[β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranosyl]oleanolic acid [α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl].

Long-range correlations between H–C(1′) of Glc*p* and C(3) of oleanolic acid and between H–C(1″) of Glc*p* and C(3′) of Glc*p* were observed in the HMBC spectrum of **2**. The latter connectivity was confirmed by a correlation between H–C(3′) of Glc*p* and C(1″) of Glc*p*. The coupling constants indicated the axial orientation of the anomeric protons in the glucosyl moieties. The saccharide moieties attached to C(28)OO of the genin showed the same long-range correlations as reported for **1**.

The structures of compounds **3**–**6** were elucidated likewise (for details, see *Tables 1* and *2*). These saponins have been isolated previously from *Stauntonia hexaphylla* (*Lardizabalaceae*) (staunoside B (**3**)) [16], *Caulophyllum robustum* (*Berberidaceae*) (cauloside G (**4**)) [17], *Caltha polypetala* (*Ranunculaceae*) (**4**; **5**) [7], *Pulsatilla campanella* (*Ranunculaceae*) (**4**; **5**) [10], *Ranunculus ficaria* (*Ranunculaceae*) (**5**) [16], *Hedera taurica* (*Araliaceae*) (tauroside St-H_1 (**3**); tauroside St-I_1 (**4**); tauroside St-G_1 (**5**)) [18–20], *Kalopanax pictus* (*Araliaceae*) (pericarpsaponin P_{J3} (**5**)) [21], *Akebia quinata* (*Lardizabalaceae*) (pericarpsaponin P_{J2} (**5**)) [22], and *Oplopanax elatus* (*Araliaceae*) (**6**) [23]. Although the sugar residues in these saponins have been assigned the D- and L–configuration, respectively, there are no facts reported for the asserted assignment. It should be noted that certain assignments of ^{13}C-NMR signals for the sugar moieties in [8] and [16] need to be revised.

Structure elucidation of the glycoglycerolipids was carried out by means of ESI LC/MS experiments, ^{1}H- and ^{13}C-NMR spectral measurements, GC analysis of the fatty acid methyl esters, and by comparison with reference data [24][25]. The number of signals for anomeric C-atoms in the ^{13}C-NMR spectra of the glycoglycerolipids showed **7** to be a monoglycoside, while **8** was the corresponding diglycoside.

The ESI MS of **7** (positive-ion mode) exhibited quasi-molecular ions at *m/z* 793 ($[M+NH_4]^+$) and 776 ($[M+H]^+$). The signal at *m/z* 614 ($[M-162]^+$) was indicative of the loss of a hexosyl residue. These findings were corroborated by the presence of a resonance for C(1‴) (105.36 ppm) in the ^{13}C-NMR spectrum. The remaining ^{13}C-NMR data were in perfect accordance with literature data for monogalactosyl-1,2-diacylglycerols (MGDG) [24][25]. GC Analysis of the fatty acid methyl esters obtained from **7** by methanolysis showed the presence of linolenoyl residues in **7**. Thus, the structure of **7** was established as (2*S*)-3-*O*-β-D-galactopyranosyl-1,2-di-*O*-[(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoyl]-*sn*-glycerol, the optical rotation being consistent with reported data [13]. This MGDG has been identified earlier from the cyanobacterium *Phormidium tenue* and the green seaweed *Caulerpa taxifolia* [24][25].

The ESI-MS of **8** (positive-ion mode) showed a quasi-molecular ion at *m/z* 955 ($[M+NH_4]^+$), a signal at *m/z* 938 ($[M+H]^+$), and characteristic fragments at *m/z* 776 ($[M-162]^+$) and 614 ($[M-162-162]^+$). The α-D-(1 → 6) linkage of the two galactosyl residues was evidenced by ^{13}C-NMR data (C(1'''') at δ 100.62 and C(6''') at δ 67.77). Comparison of the ^{13}C-NMR spectrum with literature data and the data of **7** suggested **8** to be the digalactosyl analogue of **7** (DGDG) [24][25]. The presence of linolenoyl residues was confirmed by GC analysis after methanolysis. Thus, **8** is (2S)-3-*O*-[α-D-galactopyranosyl-(1 → 6)-β-D-galactopyranosyl]-1,2-di-*O*-[(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoyl]-*sn*-glycerol. The optical rotation of **8** was in good agreement with literature data [24]. This DGDG has also been reported from the *Phormidium tenue* and *Caulerpa taxifolia* [24][25].

3. Discussion. – A targeted search for tensioactive compounds in the aquatic plant *Ranunculus fluitans* afforded two novel and four known bisdesmosidic triterpene saponins along with two known glycoglycerolipids. The saponins follow the structural pattern of other *Ranunculaceae* saponins. Rather unexpected was the discovery that two of the three VLC fractions active in the foam test contained glycoglycerolipids. Much to our surprise, these fractions turned out to produce a foam that was even more stable than that of the saponin fraction. Besides the triterpene saponins, the high glycoglycerolipid content thus significantly contributes to the propensity of *R. fluitans* extracts to form stable foams and needs to be taken into account in further investigations. Indeed, careful ESI LC/MS and TLC analysis (data not shown) of the glycoglycerolipid fractions revealed, in addition to compounds **7** and **8**, a complex pattern of several diacylglycerol mono- and diglycosides that awaits further analysis. Diacylglycerol galactosides are ubiquitous metabolites occurring primarily in the mitochondrial membrane of higher plant cells [26]. More recently, MGDGs and DGDGs have been isolated from dinoflagellates and cyanobacteria [24][25]. The reason for their exceptionally high content in *R. fluitans*, however, is not understood at the moment.

With compounds **1**–**8** in hand, targeted search and quantitative determination of saponins and glycoglycerolipids in the Rhine River by means of ESI LC/MS is underway. In 1998/99, water and foam samples had been taken below the Rhine Fall at regular intervals. We will now attempt to clarify whether seasonal changes in qualitative and quantitative composition of these samples correlates with their propensity for foam formation. These findings will be presented in a forthcoming publication.

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Experimental Part

General. Thin-layer chromatography (TLC): silica gel *60 F_{254}* coated Al sheets (*Merck*, Darmstadt, Germany); eluents $CHCl_3$/MeOH/H_2O 70:30:3 (*A*) or $CHCl_3$/MeOH 95:5 (*B*); visualization with *Godin* reagent [27] (blue for hederagenin derivatives and purple for those of oleanolic acid). Reversed-phase HP-TLC: *RP 18 W* precoated glass plates (*Merck*, Darmstadt); eluent MeCN/H_2O 40:60 (*C*); detection as above. Solid-phase extraction: on 3 g of *Baker Bond Phase RP 18* silica gel for flash (*J. T. Baker*, Deventer Holland). Column chromatographies: vacuum liquid chromatography (VLC) and open-column chromatography (CC) on silica gel 60 (40–63 μm; *Macherey-Nagel*, Düren, Germany); low-pressure liquid chromatography (LPLC) on a *Lobar LiChroprep RP 18* column (40–63 μm; 2.5 × 31.0 cm; *Merck*, Darmstadt) equipped with a series *III* pump (*Pharma-Tech Research Corp.*, Baltimore, USA) and a UV detector *K-2500* (*Knauer*, Berlin, Germany). Semi-prep. HPLC: *LiChrosorb RP 18* column (7 μm, 2.5 × 20 cm; *Merck*, Darmstadt), pump and detector same as above. Gel chromatography. *Sephadex-LH-20* columns (2.5 × 50 cm, and 1 × 50 cm for final purification; *Pharmacia Fine Chemicals*); eluent MeOH. Anal. HPLC: *LiChrosorb RP 18* column (7 μm, 4.6 × 250 mm; *Merck*, Darmstadt; *Hewlett Packard 1050*, equipped with a *Hewlett Packard 1040 M II* detector and workstation; UV detection at 200–205 nm; eluent flow 1 ml/min, MeCN/H_2O 2:8 → 4:6 within 15 min (*D*), MeOH/H_2O 96:4 (*E*) and 98:2 (*F*). Identification of sugars: *Chrompack Valco Carbohydrates PB* column (7.8 mm × 300 mm; eluent H_2O at 80°, flow 0.4 ml/min; detection at 192 nm), *Hewlett Packard 1100* and workstation. Liquid chromatography/mass spectroscopy (LC/MS): *Hewlett Packard 1100* equipped with a *PE Sciex API 165* quadrupole mass spectrometer (turbo ion spray; positive/negative-ion mode; 350°, IS 5 kV, OR 30 eV, RNG 230); eluent MeCN/0.1N NH_4OAc 2:8 → 4:6 within 15 min, flow 1 ml/min; column as for anal. HPLC or flow injection in MeCN/0.1N NH_4OAc 1:1. Gas chromatography (GC): *Shimadzu GC 17 A*; *J. & W. DB-Wax ETR* column (0.25 mm × 30 m); temp. program 80° (5 min), 80 → 230° (5.5°/min), 230° (4 min), 230 → 240° (2.5°/min), 240° (10 min); injection temp. 250°; carrier gas H_2, flow 1.4 ml/min. Capillary zone electrophoresis: *HP 3D* instrument (*Hewlett Packard*, Waldbronn, Germany), equipped with a diode-array detector; detection at 208 nm; capillary, 41.5 cm × 50 μm. M.p.: *Kofler*-type hot-stage apparatus; uncorrected. Optical rotation: *Propol* (Dr. *W. Kernchen GmbH*, Seelze, Germany). 1D NMR Spectra: *Bruker-DRX-400* spectrometer at 400.13 (^1H) and 100.61 MHz (^{13}C) and 300 K; data processing with a *SGI Indy* workstation and *XWinNMR* software; 5-mm multinuclear probe head; the 2D NMR spectra were recorded according to [28].

Plant Material. Samples of *Ranunculus fluitans* L. were collected and identified by staff of the 'Kantonales Laboratorium für Lebensmittelkontrolle und Umweltschutz', Schaffhausen, from the Rhine River over the years 1998–99 at Schlösschen Wörth and the stretch between Schaffhausen and Büsingen. The plant material was frozen immediately after collection.

Extraction and Isolation. Material collected at Schlösschen Wörth in July 1998, was freeze-dried. An aliquot (400 g) was milled, defatted with CH_2Cl_2 at r.t. for 48 h, and then extracted with MeOH to afford 60 g of extract. The MeOH extract was dissolved in H_2O and extracted 3 × with BuOH sat. with H_2O (500 ml each). The BuOH layers were combined and evaporated to afford 12 g of an enriched extract. The BuOH extract was submitted to VLC (step-gradient elution with $CHCl_3$/MeOH 95:5, $CHCl_3$/MeOH/H_2O 80:20:2, $CHCl_3$/MeOH/H_2O 60:40:10): 8 fractions (*Fr. 1–8*).

The saponin-containing *Fr. 4* (2.2 g), upon purification by solid-phase extraction (MeCN/H_2O 4:6) and gel chromatography, afforded a saponin mixture (420 mg). This mixture was separated by LPLC (step-gradient elution with MeCN/H_2O 27:73 → 30:70, flow 4 ml/min, detection at 205 nm): 6 saponins. Final purification of the saponins by gel chromatography or semi-prep. HPLC (MeCN/H_2O 30:70, flow 10 ml/min) gave **1** (14 mg), **2** (7 mg), **3** (16 mg), **4** (9 mg), **5** (19 mg), and **6** (12 mg). The purity of all compounds was checked by anal. HPLC (*D*).

The monogalactosyl-diacylglycerol-containing *Fr. 2* (400 mg) was submitted to gel chromatography to give 138 mg, which were submitted to CC ($CHCl_3$/MeOH 9:1): 55 mg. This fraction was further purified by LPLC (MeOH/H_2O 96:4, flow 4 ml/min, detection at 205 nm) and finally by gel chromatography: **7** (6 mg).

The digalactosyl-diacylglycerol-containing fraction was submitted to solid-phase extraction (step-gradient elution with $CHCl_3$/MeOH 9:1 → $CHCl_3$/MeOH/H_2O 70:30:3) to give an enriched fraction of 900 mg. This fraction was submitted to LPLC (MeOH/H_2O 98:2, flow 4 ml/min, detection at 205 nm) and finally to gel chromatography: **8** (15 mg).

Acidic Hydrolysis. The saponin (0.5 mg) was refluxed in 2N CF_3COOH (0.5 ml) for 1 h. After evaporation, H_2O (0.5 ml) was added and the mixture extracted with $CHCl_3$. The upper layer was evaporated and the residue redissolved repeatedly in H_2O, and evaporated to remove traces of CF_3COOH. The dry residue was then dissolved in H_2O (300 μl), and this soln. was used to identify the sugars by HPLC with authentic samples. The org. layer was checked by TLC (*B*) for the aglycon.

Basic Hydrolysis. The saponin (0.5 mg) was refluxed in 0.5N KOH for 1 h. The mixture was adjusted to pH 5 with aq. HCl soln. and extracted with AcOEt. The org. layer was washed with H_2O (3 ×) and evaporated and the residue dissolved in MeOH (300 μl).

Capillary Zone Electrophoresis. To the lyophilisate (0.5 mg) of the aq. phase of acidic hydrolysis, 0.5M 5-aminonaphthalene-2-sulfonic acid (20 μl; adjusted with aq. NaOH soln. to pH 6.2) and H_2O (90 μl) were added, and the mixture was heated at 90° for 1 h. Then 2M aq. $NaBH_4$ (10 μl) was added, and the mixture was heated at 90° for 2 h. The sample was then diluted with H_2O to a concentration of 1 mM; this soln. (0.3 μl) was used for analysis. Similar reactions were carried out with authentic sugar samples. To optimize the resolution, 5 mM cyclodextrin and 10 mM borate were used. The D-configuration of glucose, the L-configuration of arabinose, and the L-configuration of rhamnose was determined using authentic samples of D- and L-glucose, D- and L-arabinose, and L-rhamnose.

Methanolysis of Glycoglycerolipids. The glycoglycerolipid (0.5 mg) was refluxed in dry 1.25N methanolic HCl (200 µl) for 35 min. The solvent was removed in a stream of dry N_2 and the residue dissolved in H_2O (200 µl) and extracted with hexane (200 µl). The solvent was removed again and the obtained fatty acid methyl ester dissolved in hexane (50 µl).

Foam Assay. Aliquots of all VLC fractions were dissolved in H_2O at a concentration of 1 mg/ml, transferred into a test tube, and vortexed for 10 min. The formation of a stable foam was assessed after 0, 30, 60, and 90 min against a dark background.

*3-*O*-[β-D-Glucopyranosyl-(1 → 3)-*O*-α-L-arabinopyranosyl]-28-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] hederagenin*[2]) (**1**): White, amorphous powder. M.p. 190–195° (dec.). $[\alpha]_D^{20} = +2.45$ ($c = 0.81$, MeOH). HPLC (*D*): t_R 8.62. TLC (*RP 18*, *C*) R_f 0.41. ESI-MS (pos.): 1255 ($[M+\mathrm{NH}_4]^+$), 1238 ($[M+\mathrm{H}]^+$), $\mathrm{C}_{59}\mathrm{H}_{97}\mathrm{O}_{27}{}^+$), 1076 ($[M+\mathrm{H}-162]^+$), 914 ($[M+\mathrm{H}-162-162]^+$), 944 ($[M+\mathrm{H}-162-132]^+$), 782 ($[M+\mathrm{H}-162-162-132]^+$), 752 ($[M+\mathrm{H}-162-162-162]^+$). ESI-MS (neg.): 1236 ($[M-\mathrm{H}]^-$), ESI-MS (pos.; **1a**[1])): 768 ($[M+\mathrm{H}]^+$), 606 ($[M+\mathrm{H}-162]^+$).

*3-*O*-[β-D-Glucopyranosyl-(1 → 3)-β-D-glucopyranosyl]oleanolic Acid [α-L-Rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] Ester*[3]) (**2**): White, amorphous powder. M.p. 210–215° (dec.). $[\alpha]_D^{20} = -5.23$ ($c = 0.42$, MeOH). HPLC (*D*): t_R 9.82. TLC (*RP 18*, *C*): R_f 0.33. ESI-MS (pos.): 1269 ($[M+\mathrm{NH}_4]^+$), 1252 ($[M+\mathrm{H}]^+$, $\mathrm{C}_{60}\mathrm{H}_{99}\mathrm{O}_{27}{}^+$), 1090 ($[M+\mathrm{H}-162]^+$), 928 ($[M+\mathrm{H}-162-162]^+$). ESI-MS (neg.): 1250 ($[M-\mathrm{H}]^-$).

*3-*O*-(β-D-Glucopyranosyl)-28-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl]hederagenin*[2]) (**3**): White, amorphous powder. M.p. 212–215° (dec.). $[\alpha]_D^{20} = -8.91$ ($c = 0.79$, MeOH). HPLC (*D*): t_R 8.24. TLC (*RP 18*, *C*): R_f 0.42. ESI-MS (pos.): 1123 ($[M+\mathrm{NH}_4]^+$), 1106 ($[M+\mathrm{H}]^+$, $\mathrm{C}_{54}\mathrm{H}_{89}\mathrm{O}_{23}{}^+$), 944 ($[M+\mathrm{H}-162]^+$, 782 ($[M+\mathrm{H}-162-162]^+$), 620 ($[M+\mathrm{H}-162-162-162]^+$). ESI-MS (neg.): 1104 ($[M-\mathrm{H}]^-$). ESI-MS (neg.; **3a**[1])): 634 ($[M-\mathrm{H}]^-$), 472 ($[M-\mathrm{H}-162]^-$).

*3-*O*-[β-D-Glucopyranosyl-(1 → 2)-α-L-arabinopyranosyl]-28-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl]hederagenin*[2]) (**4**): White, amorphous powder. M.p. 210–215° (dec.). $[\alpha]_D^{20} = -2.43$ ($c = 0.33$, MeOH). HPLC (*D*): t_R 8.78. TLC (*RP 18*, *C*): R_f 0.39. ESI-MS (pos.): 1255 ($[M+\mathrm{NH}_4]^+$), 1238 ($[M+\mathrm{H}]^+$, $\mathrm{C}_{59}\mathrm{H}_{97}\mathrm{O}_{2}27^+$), 1076 ($[M+\mathrm{H}-162]^+$), 914 ($[M+\mathrm{H}-162-162]^+$), 944 ($[M+\mathrm{H}-162-132]^+$), 782 ($[M+\mathrm{H}-162-162-132]^+$), 752 ($[M+\mathrm{H}-162-162-162]^+$). ESI-MS (neg.): 1236 ($[M-\mathrm{H}]^-$).

*3-*O*-(α-L-Arabinopyranosyl)-28-*O*-[α-L-rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl]hederagenin*[2]) (**5**): White, amorphous powder. M.p. 195–200° (dec.). $[\alpha]_D^{20} = -2.49$ ($c = 1.12$, MeOH). HPLC (*D*): t_R 9.77. TLC (*RP-18*, *C*): R_f 0.32. ESI-MS (pos.): 1093 ($[M+\mathrm{NH}_4]^+$), 1076 ($[M+\mathrm{H}]^+$, $\mathrm{C}_{53}\mathrm{H}_{87}\mathrm{O}_{22}{}^+$), 914 ($[M+\mathrm{H}-162]^+$, 944 ($[M+\mathrm{H}-132]^+$), 782 ($[M+\mathrm{H}-162-132]^+$), 752 ($[M+\mathrm{H}-162-162]^+$). ESI-MS (neg.): 1074 ($[M-\mathrm{H}]^-$). ESI-MS (neg.; **5a**[1]): 604 ($[M-\mathrm{H}]^-$), 472 ($[M-\mathrm{H}-132]^-$).

*3-*O*-(β-D-Glucopyranosyl)oleanolic Acid [α-L-Rhamnopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1 → 6)-β-D-glucopyranosyl] Ester*[3]) (**6**): White, amorphous powder. M.p. 215–220° (dec.). $[\alpha]_D^{20} = -6.07$ ($c = 0.63$, MeOH). HPLC (*D*): t_R 10.83. TLC (*RP-18*, *C*) R_f 0.26. ESI-MS (pos.): 1107 ($[M+\mathrm{NH}_4]^+$), 1090 ($[M+\mathrm{H}]^+$, $\mathrm{C}_{54}\mathrm{H}_{89}\mathrm{O}_{22}{}^+$); 928 ($[M+\mathrm{H}-162]^+$), 766 ($[M+\mathrm{H}-162-162]^+$). ESI-MS (neg.): 1088 ($[M-\mathrm{H}]^-$). ESI-MS (neg.; **6a**[1]): 617 ($[M-\mathrm{H}]^-$), 455 ($[M-\mathrm{H}-162]^-$).

*(2*S*)-3-*O*-β-D-Galactopyranosyl-1,2-di-*O*-[(9*Z*,12*Z*,15*Z*)-octadeca-9,12,15-trienoyl]-sn-glycerol* (**7**): Colorless oil. $[\alpha]_D^{20} = +2.89$ ($c = 0.55$, MeOH). HPLC (*F*): t_R 10.39. TLC (SiO_2, *A*): R_f 0.48. ^{1}H-NMR ($\mathrm{CD_3OD}$, SiMe_4 as int. standard): 4.23 (*dd*, $J(1a,2) = 7.3$, $J_{gem} = 13.6$, $\mathrm{H_a-C(1)}$); 4.44 (*dd*, $J(1b,2) = 3.0$, $J_{gem} = 12.1$, $\mathrm{H_b-C(1)}$); 5.26 (*m*, H–C(2)); 3.97 (*dd*, $J(3b,2) = 5.4$, $J_{gem} = 10.9$ $\mathrm{H_b-C(3)}$); 3.70 (*dd*, $J(3a,2) = 5.35$, $J_{gem} = 11.2$ $\mathrm{H_a-C(3)}$); 2.32 (*m*, 2 H–C(2′), 2 H–C(2″)); 1.59 (*m*, 2 H–C(3′), 2 H–C(3″)); 1.32 (*m*, 16 H, 2 H–C(4′) to 2 H–C(7′), 2 H–C(4″) to 2 H–C(7″)); 2.08 (*m*, 2 H–C(8′), 2 H–C(8″), 2 H–C(17′), 2 H–C(17″)); 5.33 (*m*, H–C(9′), H–C(10′), H–C(12′), H–C(13′), H–C(15′), H–C(16′), H–C(9″), H–C(10″), H–C(12″), H–C(13″), H–C(15″), H–C(16″)); 2.80 (*m*, 2 H–C(11′), 2 H–C(11″), 2 H–C(14′), 2 H–C(14″)); 0.97 (*t*, $J = 7.5$, Me(18′), Me(18″)); 4.23 (*d*, $J = 7.3$, H–C(1‴)); 3.52 (*dd*, $J = 7.4$, 9.8, H–C(2‴)); 3.45 (*dd*, $J = 3.3$, 10.3, H–C(3‴)); 3.83 (*dd*, $J = 2.5$, 1.0, H–C(4‴)); 3.48 (*m*, H–C(5‴)); 3.71 (*m*, 1 H–C(6‴); 3.76 (*m*, 1 H–C(6‴)). ^{13}C-NMR

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[2]) The systematic name of hederagenin is (3β,4α)-3,23-dihydroxyolean-12-en-28-oic acid.

[3]) The systematic name of oleanolic acid is (3β)-3-hydroxyolean-12-en-28-oic acid.

(CD$_3$OD): 64.00 (*t*, C(1)); 71.79 (*d*, C(2)); 68.72 (*t*, C(3)); 175.01, 174.71 (2*s*, C(1′), C(1″)); 35.12, 34.95 (*t*, C(2′), C(2″)); 26.55, 26.43, 26.02 (*t*, C(3′), C(11′), C(14′), C(3″), C(11″), C(14″)); 30.84, 30.40, 30.25, 30.20 (all *t*, C(4′) to C(7′), C(4″) to C(7″)); 28.19 (*t*, C(8′), C(8″)); 21.51 (*t*, C(17′), C(17″)); 128.25, 128.90, 129.21 (2 ×), 131.06, 132.74 (all *d*, C(9′), C(10′), C(12′), C(13′), C(15′), C(16′), C(9″), C(10″), C(12″), C(13″), C(15″), C(16″)); 21.51 (*t*, C(17′), C(17″)); 14.69 (*q*, C(18′), C(18″)); 105.36 (*d*, C(1‴)); 72.37 (*d*, C(2‴)); 74.86 (*d*, C(3‴)); 70.22 (*d*, C(4‴)); 76.80 (*d*, C(5‴)); 62.46 (*t*, C(6‴)); assignments for ^{13}C signals of the fatty-acid residues by comparison with [29]. ESI-MS (pos.): 793 ([*M* + NH$_4$]$^+$), 776 ([*M* + H]$^+$, C$_{45}$H$_{75}$O$_{10}$$^+$), 614 ([*M* – 162]$^+$). ESI-MS (neg.): 774 ([*M* – H]$^-$).

(2S)-3-O-α-D-Galactopyranosyl-(1 → 6)-β-D-galactopyranosyl-1,2-di-O-[(9Z,12Z,15Z)-octadeca-9,12,15-trienoyl]-sn-glycerol (**8**): Colorless oil. $[\alpha]^{20}_{D} = +38.1$ ($c = 1.00$, MeOH). HPLC (*E*): t_R 5.46. TLC (SiO$_2$, *B*): R_f 0.39. ^{1}H-NMR (CD$_3$OD, SiMe$_4$ as int. standard): 4.23 (*dd*, *J*(1a,2) = 7.3, J_{gem} = 13.6, H$_a$–C(1)); 4.44 (*dd*, *J*(1b,2) = 2.9, J_{gem} = 12.1, H$_b$–C(1)); 5.24 (*m*, H–C(2)); 3.88 (*dd*, *J*(3b,2) = 5.4, J_{gem} = 11.0 H$_b$–C(3)); 3.71 (*dd*, *J*(3a,2) = 6.1, J_{gem} = 9.7 H$_a$–C(3)); 2.31 (*m*, 2 H–C(2′), 2 H–C(2″)); 1.58 (*m*, 2 H–C(3′), 2 H–C(3″)); 1.31 (*m*, 16 H, 2 H–C(4′) to 2 H–C(7′), 2 H–C(4″) to 2 H–C(7″)); 2.06 (*m*, 2 H–C(8′), 2 H–C(8″), 2 H–C(17′), 2 H–C(17″)); 5.33 (*m*, H–C(9′), H–C(10′), H–C(12′), H–C(13′), H–C(15′), H–C(16′), H–C(9″), H–C(10″), H–C(12″), H–C(13″), H–C(15″), H–C(16″)); 2.79 (*m*, 2 H–C(11′), 2 H–C(11″), 2 H–C(14′), 2 H–C(14″)); 0.95 (*t*, *J* = 7.5, Me(18′), Me(18″)); 3.47 (*dd*, *J* = 3.5, 10.3, H–C(3‴)); 3.49 (*dd*, *J* = 6.8, 9.8, H–C(2‴)); 3.68 – 3.75 (*m*, 8 H); 3.86 – 3.88 (*m*, 3 H); 4.23 (*d*, *J* = 7.3, H–C(1‴)). ^{13}C-NMR (CD$_3$OD): 64.01 (*t*, C(1)); 71.74 (*d*, C(2)); 68.75 (*t*, C(3)); 175.03, 174.71 (2*s*, C(1′), C(1″)); 35.16, 34.98 (*t*, C(2′), C(2″)); 26.58, 26.47, 26.06 (*t*, C(3′), C(11′), C(14′), C(3″), C(11″), C(14″)), 30.77, 30.38, 30.28, 30.23 (all *t*, C(4′) to C(7′), C(4″) to C(7″)); 28.23 (*t*, C(8′), C(8″)); 21.55 (*t*, C(17′), C(17″)); 128.25, 128.90, 129.22 (2 ×), 131.06, 132.74 (all *d*, C(9′), C(10′), C(12′), C(13′), C(15′), C(16′), C(9″), C(10″), C(12″), C(13″), C(15″), C(16″)); 21.55 (*t*, C(17′), C(17″)); 14.72 (*q*, C(18′), C(18″)); 105.31 (*d*, C(1‴)); 72.55 (*d*, C(2‴)); 74.66 (*d*, C(3‴)); 70.04 (*d*, C(4‴)); 74.56 (*d*, C(5‴)); 67.77 (*d*, C(6‴)); 100.62 (*d*, C(1⁗)); 70.22 (*d*, C(2⁗)); 71.46 (*d*, C(3⁗)); 71.11 (*d*, C(4⁗)); 72.37 (*d*, C(5⁗)); 62.84 (*t*, C(6⁗)). ESI-MS (pos.): 955 ([*M* + NH$_4$]$^+$), 938 ([*M* + H]$^+$, C$_{51}$H$_{85}$O$_{15}$$^+$), 776 ([*M* – 162]$^+$), 614 ([*M* – 162 – 162]$^+$). ESI-MS (neg.): 936 ([*M* – H]$^-$).

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