Petrorhagiosides A – D, New γ-Pyrone Derivatives from *Petrorhagia saxifraga* LINK

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Four novel γ-pyrone (=4*H*-pyran-4-one) metabolites, petrorhagiosides A – D, along with four known analogs, have been isolated from the MeOH extract of *Petrorhagia saxifraga*, a perennial herbaceous plant typical of Mediterranean vegetation. The structures of the new compounds were established on the basis of extensive spectroscopic analyses including 1D- an 2D-NMR (^{1}H,^{1}H-DQ-COSY, TOCSY, HSQC, CIGAR-HMBC, and HSQC-TOCSY) experiments.

Introduction. – *Petrorhagia* is a small genus of annual and perennial plants, belonging to the Caryophyllaceae family, mostly native to the Mediterranean region. The flowers are pink, lilac, or white, and similar to those of the members of the genus *Dianthus.*

To the best of our knowledge, the only phytochemical investigations of this genus has been carried out on *Petrorhagia velutina* (GUSS.) P.W.BALL. et HEYWOOD. A series of flavonoid *C*-glycosides from this plant has been reported, and their antiproliferative activity against human hepatoblastoma cancer cell line HepG2 has been evaluated [1]. From the same species, a new pheophorbide, as well as other nine chlorophyll derivatives, were isolated. A significant photoinduced phytotoxicity was ascribed to these compounds [2]. Furthermore, a new dimeric *p*-coumaroyl glucose, and two monomeric *cis-p*-coumaroyl and feruloyl derivatives with radical-scavenging properties [3], as well as a new drimane glycoside were reported [4].

This work deals with the phytochemical study of *Petrorhagia saxifraga* LINK, known as coat flower or saxifrage pink, an eurimediterranean perennial herbaceous species, typical of woodland and macchia vegetation. The study led to the isolation and structural characterization of four novel γ-pyrone (=4*H*-pyran-4-one) glucosides, named petrorhagiosides A – D.

Results and Discussion. – The phytochemical investigation of the MeOH extract of *Petrorhagia saxifraga* led to isolation and identification of eight γ-pyrone derivatives, four of them, **1** – **4**, isolated and identified for the first time (*Fig. 1*).

Compound **1**, named petrorhagioside A, had the molecular formula $C_{18}H_{26}O_{14}$, as deduced from the NMR data and HR-ESI-mass spectrum. The ^{1}H-NMR spectrum (*Table 1*) showed, in the downfield region, signals for two H-atoms (δ(H) 8.10 (*d*, H–C(6)) and 6.49 (*d*, H–C(5)); J = 5.7). Moreover, two *doublet*s at δ(H) 4.87

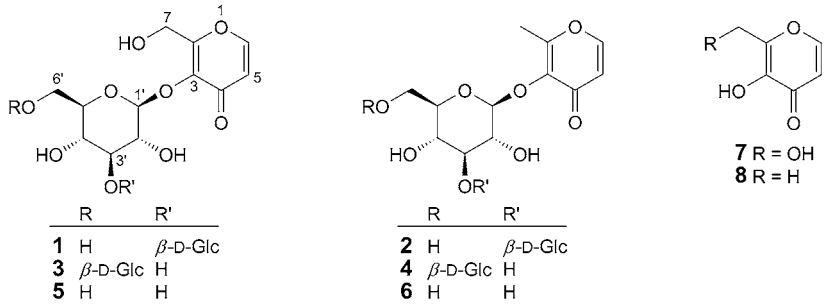

Fig. 1. *Structures of petrorhagiosides A – D* (**1** – **4**, resp.) *and compounds* **5** – **8**, *isolated from* P. saxifraga

Table 1. *^{1}H-NMR Data of Petrorhagiosides A – D* (**1** – **4**, resp.) *in CD_3OD.* δ in ppm, *J* in Hz. Atom numbering as indicated in *Fig. 1*.

Position	**1**	**2**	**3**	**4**
5	6.49 (*d*, *J* = 5.7)	6.44 (*d*, *J* = 4.9)	6.50 (*d*, *J* = 5.4)	6.44 (*d*, *J* = 4.8)
6	8.10 (*d*, *J* = 5.7)	8.00 (*d*, *J* = 4.9)	8.01 (*d*, *J* = 5.4)	7.99 (*d*, *J* = 4.8)
7	4.73 (*d*, *J* = 13.5), 4.59 (*d*, *J* = 13.5)	2.45 (*s*)	4.77 (*d*, *J* = 14.1), 4.64 (*d*, *J* = 14.1)	2.46 (*s*)
1′	4.87 (*d*, *J* = 7.2)	4.88 (*d*, *J* = 7.5)	4.81 (*d*, *J* = 7.2)	4.85[a])
2′	3.60[b])	3.60[b])	3.42[b])	3.41[b])
3′	3.63[b])	3.62[b])	3.30[b])	3.30[b])
4′	3.43[b])	3.46[b])	3.27[b])	3.28[b])
5′	3.35[b])	3.35[b])	3.42[b])	3.43[b])
6′	3.87 (*dd*, *J* = 11.1, 2.2), 3.66 (*dd*, *J* = 11.1, 5.7)	3.87 (*dd*, *J* = 11.1, 2.1), 3.66 (*dd*, *J* = 11.1, 4.5)	4.16 (*d*, *J* = 11.1), 3.75 (*dd*, *J* = 11.1, 6.6)	4.11 (*d*, *J* = 11.4), 3.75 (*dd*, *J* = 11.4, 6.3)
1″	4.60 (*d*, *J* = 7.0)	4.59 (*d*, *J* = 7.8)	4.30 (*d*, *J* = 7.8)	4.30 (*d*, *J* = 8.0)
2″	3.27 (*dd*, *J* = 9.3, 7.0)	3.24[b])	3.15 (*dd J* = 8.4, 8.1)	3.15 (*dd*, *J* = 8.4, 8.1)
3″	3.35[b])	3.35[b])	3.35[b])	3.30[b])
4″	3.27[b])	3.27[b])	3.38[b])	3.38[b])
5″	3.35[b])	3.42[b])	3.43[b])	3.42[b])
6″	3.83 (*dd*, *J* = 11.1, 2.2), 3.66 (*dd*, *J* = 11.1, 5.7)	3.83 (*dd*, *J* = 11.1, 2.2), 3.66 (*dd*, *J* = 11.1, 4.5)	3.86 (*d*, *J* = 11.7), 3.65 (*dd*, *J* = 11.7, 4.2)	3.85 (*d*, *J* = 11.1), 3.65 (*dd*, *J* = 11.1, 4.4)

[a]) Obscured. [b]) Overlapped signals, reported without designated multiplicity.

(H–C(1′)) and 4.60 (H–C(1″)), and an *AB* system at δ(H) 4.73, 4.59 (2*d*, *J* = 13.5, CH_2(7)), as well as additional H-atom signals between 3.9 and 3.2 ppm were evident. The ^{13}C-NMR spectrum (*Table 2*) showed 18 signals, identified on the basis of a DEPT experiment, as those for three CH_2 and twelve CH groups, and three quaternary C-atoms (δ(C) 177.5 (C(4)), 164.0 (C(2)), and 142.6 (C(3)).

The HSQC experiment showed correlations between the H-atoms at δ(H) 8.10 (*d*, H–C(6)) and 6.49 (*d*, H–C(5)) and the C-atoms at δ(C) 157.6 (C(6)) and 117.7 (C(5)), respectively. Furthermore, the *AB*-system H-atoms correlated with the CH_2 C-atom at δ(C) 57.6 (C(7)), while the two *doublet*s at δ(H) 4.87 (*d*, H–C(1′)) and 4.60 (*d*, H–C(1″)) showed cross-peaks with the C-atoms at δ(C) 104.0 (C(1′)) and 105.1

Table 2. *^{13}C-NMR Data of Petrorhagiosides A – D* (**1**–**4**) *in CD_3OD.* δ in ppm. Atom numbering as indicated in *Fig. 1*.

C-Atom	**1**	**2**	**3**	**4**
C(2)	164.0	164.5	164.0	164.5
C(3)	142.6	143.3	142.8	143.3
C(4)	177.5	177.1	177.5	177.1
C(5)	117.7	117.4	117.8	117.4
C(6)	157.6	157.1	157.7	157.1
C(7)	57.6	15.8	57.9	15.8
C(1′)	104.0	104.8	104.6	104.6
C(2′)	74.7	74.9	75.2	75.2
C(3′)	87.1	87.5	77.9	77.8
C(4′)	69.8	69.6	71.5	71.5
C(5′)	78.1	78.2	77.6	77.6
C(6′)	62.5	62.4	69.9	69.9
C(1″)	105.1	105.2	104.7	104.7
C(2″)	75.4	75.6	75.0	74.9
C(3″)	77.9	78.2	77.9	77.8
C(4″)	71.5	71.6	71.2	71.1
C(5″)	77.8	77.8	77.4	77.5
C(6″)	62.6	62.4	62.7	62.5

(C(1″)). These signals, as well as the remaining carbinol signals present in the ^{13}C-NMR spectrum, established the presence of two sugar moieties.

The CIGAR-HMBC experiment showed correlations between the *doublet* at δ(H) 8.10 (H–C(6)) and the C-atom signals at δ(C) 177.5 (C(4)), 164.0 (C(2)), and 117.7 (C(5)); between the H-atom signal at δ(H) 6.49 (H–C(5)) and the C-atom signals at δ(C) 177.5 (C(4)), 157.6 (C(6)), and 142.6 (C(3)); between the *AB*-system H-atom signals and the C-atom signals at δ(C) 164.0 (C(2)) and 142.6 (C(3)). These data were in good agreement with the presence of a pyran-4-one structure with an CH_2OH group at C(2) (δ(C) 160.4) and an OH group at the C(3) (δ(C) 142.6). Furthermore, this C-atom showed correlations, in the HMBC experiment, with the anomeric H-atom (δ(H) 4.87 (H–C(1′))).

The C-atoms of both the monosaccharide units were identified on the basis of a HSQC-TOCSY experiment (*Fig. 2*). In fact, the signal at δ(H) 4.87 (H–C(1′)) correlated with five CH signals δ(C) 104.0 (C(1′)) (direct correlation), 87.1 (C(3′)), 78.1 (C(5′)), 74.7 (C(2′)), 69.8 (C(4′)), and with the CH_2 signal at δ(C) 62.5 (C(6′)); besides, the signal at δ(H) 4.60 (H–C(1″)) correlated with the CH signals at δ(C) 105.1 (C(1″)) (^1J correlation), 77.9 (C(3″)), 77.8 (C(5″)), 75.4 (C(2″)), 71.5 (C(4″)), and with the CH_2 signal at δ(C) 62.6 (C(6″)) (*Fig. 2*). The GC/MS analysis of alditol acetate derivatives, obtained by the hydrolysis of compound **1** with 2N CF_3COOH (TFA), reduction with $NaBH_4$, and acetylation with Ac_2O in pyridine, suggested only the presence of glucose in the molecule. The differences observed in the chemical shifts of the C-atoms revealed the presence of a disaccharide moiety. The anomeric H-atom signal at δ(H) 4.60 (H–C(1″)) correlated with the C-atom signal at δ(C) 87.1 (C(3′)) in the CIGAR-HMBC experiment. These data agreed with the presence of a glucose moiety bound to a second sugar unit through its C(3). The coupling constant value (7.2 Hz) of the H-

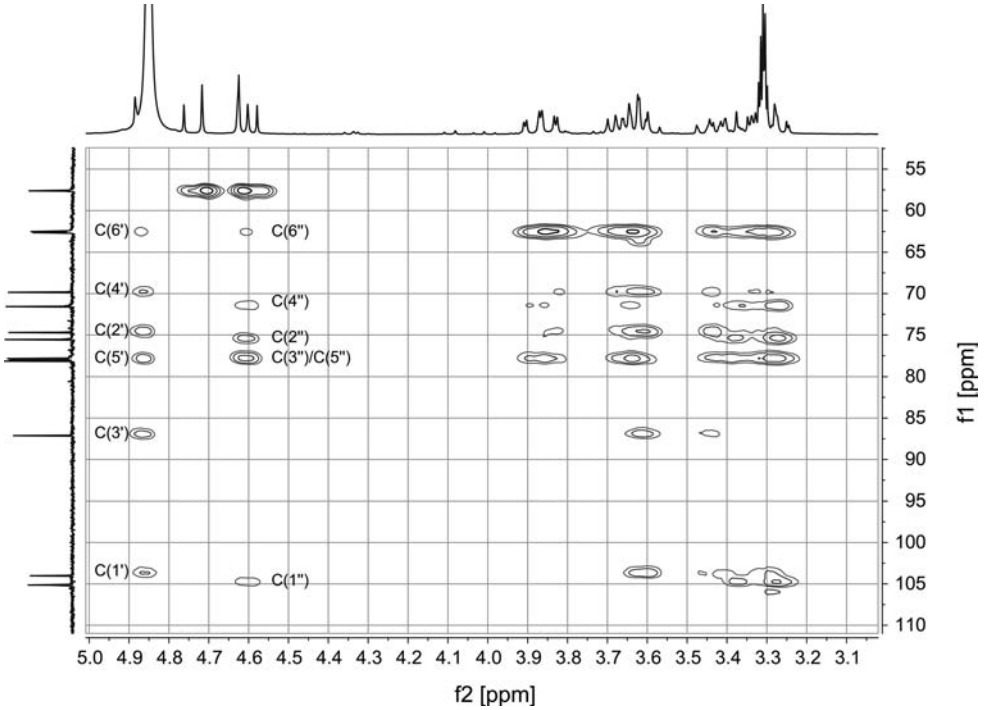

Fig. 2. *HSQC-TOCSY Spectrum of compound* **1**

atom with the signal at δ(H) 4.87 (H–C(1′)) evidenced a β-configuration at the anomeric C-atom. A similar value (7.0 Hz), obtained for the second anomeric H-atom (δ(H) 4.60), also evidenced a β-configuration for its anomeric C-atom. The absolute configuration of glucose was determined by GC/MS after reaction of hydrolyzed glucosides with L-cysteine methyl ester and acetylation. These data were in good accordance with a β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranoside moiety in the molecule. The remaining hetero- and homocorrelations detected in 2D experiments confirmed this hypothesis and allowed the structure of 2-(hydroxymethyl)-4-oxo-4*H*-pyran-3-yl 3-*O*-β-D-glucopyranosyl-β-D-glucopyranoside to be assigned to compound **1**. The enzymatic hydrolysis of **1** with β-glucosidase in acetate buffer at 37° afforded pure aglycone **7** and glucose, confirming our hypothesis.

Compound **2**, named petrorhagioside B, had the molecular formula $C_{18}H_{26}O_{13}$, as deduced by the HR-ESI-MS and NMR data. The ^{1}H-NMR spectrum showed two *doublet*s of an *AM* spin system at δ(H) 8.00 (H–C(6)) and 6.44 (H–C(5)), two anomeric *doublet*s at δ(H) 4.88 (H–C(1′)) and 4.59 (H–C(1″)), besides further overlapped signals with δ values ranging from 3.9 to 3.2 ppm, and a Me *singlet* at δ(H) 2.45. The ^{13}C-NMR spectrum showed 18 signals, identified by DEPT experiment, as those of one Me, two CH_2, and twelve CH groups, and three quaternary C-atoms (δ(C) 177.1 (C(4)), 164.5 (C(2)), and 143.3 (C(3)). The *doublet* at δ(H) 8.00 (H–C(6)) correlated, in the CIGAR-HMBC experiment, with the C=O C-atom signal at δ(C)

177.1(C(4)), and with those at δ(C) 164.5 (C(2)) and 117.4 (C(5)); the *doublet* at δ(H) 6.44 (H–C(5)) correlated with the signals at δ(C) 177.1 (C(4)), 157.1 (C(6)), and 143.3 (C(3)); The Me *singlet* correlated with the C-atom signals at δ(C) 164.5 (C(2)) and 143.3 (C(3)). These data indicated the presence of a 3-hydroxy-2-methylpyran-4-one bound to a disaccharide. The GC/MS analysis of the alditol acetate derivatives obtained by the hydrolysis of **2** afforded only glucose. The coupling constant of anomeric H-atoms revealed the β-configuration of both glucose moieties. The ^{1}H- and the ^{13}C-NMR data of the glycosidic portions were almost identical to those of compound **1**, in accordance with a β-D-glucopyranosyl-(1 → 3)-β-D-glucopyranoside moiety in the molecule. The structure of **2** was, therefore, established as 2-methyl-4-oxo-4*H*-pyran-3-yl 3-*O*-β-D-glucopyranosyl-β-D-glucopyranoside.

Compound **3**, named petrorhagioside C, had the molecular formula $C_{18}H_{26}O_{14}$, according to the NMR data and the HR-ESI-mass spectrum. The ^{1}H-NMR spectrum exhibited an *AM* spin system at δ(H) 8.01 and 6.50, two anomeric H-atom signals at δ(H) 4.81 (H–C(1′)) and 4.30 (H–C(1″)), an *AB* system at δ(H) 4.77, 4.64 (2*d*, CH_2(7)), signals of eight CH groups ranging from 3.15 to 3.44 ppm, and two diastereotopic CH_2 signals at δ(C) 4.16, 3.75 (CH_2(6′)) and 3.86, 3.65 (CH_2(6″)) bound to the C-atoms with signals at δ(C) 69.9 (C(6′)) and 62.7 (C(6″)), respectively, as evidenced in the HSQC experiment. The ^{13}C-NMR spectrum showed 18 signals assigned to three CH_2 and 12 CH groups, and three quaternary C-atoms. The NMR signals of the aglycone moiety were similar to those of compound **1**. Differences were ascribed to the saccharidic moiety. The GC analysis of the alditol acetate obtained from compound **3**, as described for the previous metabolites, indicated the exclusive presence of glucose in the molecule. The downfield shifts of the CH_2 signals at δ(H) 4.16 and 3.75 (H–C(6′)), as well as that of the C-atom at δ(C) 69.9 (C(6′)) suggested the presence of the disaccharide gentiobioside that was confirmed on the basis of heterocorrelations, evidenced in the CIGAR-HMBC, between the H–C(1″) signal at δ(H) 4.30 and C(6′). The disaccharide was bound to C(3) of the γ-pyrone based on the heterocorrelation, evidenced in the same experiment, between the anomeric H-atom signal at δ(H) 4.81 (H–C(1′)) and that of the C-atom at δ(C) 142.8 (C(3)).

Compound **4**, named petrorhagioside D, had the same molecular formula as **2** as evidenced by the HR-ESI mass spectrum and the NMR data. Its ^{1}H-NMR spectrum showed four CH *doublet*s at δ(H) 7.99 (H–C(6)), 6.44 (H–C(5)), 4.85 (H–C(1′)), and 4.30 (H–C(1″)), a Me *singlet* at δ(H) 2.46 and further twelve signals between 3.2 and 3.9 ppm. The ^{13}C-NMR and DEPT experiments indicated the presence of a Me, two CH_2, and twelve CH groups, and three quaternary C-atoms. The NMR data of the aglycone indicated the presence of a 3-hydroxy-2-methylpyran-4-one, while the 1D- and 2D-NMR experiments were in good accordance with the presence of gentiobioside in the molecule. The GC/MS analysis of alditol acetate derivatives of the sugars, obtained after hydrolysis of **4**, confirmed the presence of glucose as monosaccharide.

Spectroscopic data of the known metabolites **5**–**8** were identical to those reported in the literature: glucoside **5** and its aglycone **7** have been reported as constituents of *Pteris inaequalis* var. *aequata* (MIQ.) TAGAW [5], while glucoside **6**, known as dianthoside, has been reported as constituent of *Dianthus deltoides* L. [6] and *Derris trifoliata* LOUR. [7]. Maltol (**8**) is a widely occurring metabolite [8] used as a flavor enhancer [9]. This compound is also reported as complexant to heavy metal ions.

Maltol is reported to be an inhibitor of apoptosis of human neuroblastoma cells, induced by oxidative damage generated by H_2O_2 [10], and to be a strong enhancer of aluminum accumulation in bone and brain [11].

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Experimental Part

General. TLC: *Kieselgel 60 F_{254}* (*Merck*), *RP-18 F_{254}* (*Merck*) or cellulose (*Merck*) plates with 0.2-, 0.5-, or 1-mm film thickness. Column chromatography (CC): *Kieselgel 60* (70–240 mesh, *Merck*), *Amberlite XAD-4* (*Fluka*), *RP-18* (*Baker*), or *Sephadex LH-20* (*Pharmacia*). Prep. HPLC: *Knauer Smartline 31/40* module on *Luna* NH_2 (10 μm, 250 × 10.0 mm i.d., *Phenomenex*), equipped with *Knauer Smartline 1000* pump, UV *Knauer Smartline 2500* and *RI Knauer Smartline 2300* detectors, and PC *CromGate®* software. Optical rotation: *Perkin-Elmer 343* polarimeter in MeOH. UV Spectra: *UV-1700 Shimadzu* spectrophotometer in MeOH; λ_{max} (log ε) in nm. NMR Spectra: *Varian 300* FT-NMR spectrometer at 300 (^{1}H) and 75 MHz (^{13}C), in CD_3OD solns., at 25°; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. 1*H*-Detected heteronuclear correlations were measured by gradient Heteronuclear Single-Quantum Coherence (HSQC) optimized for $^1J(H,C) = 140$ Hz and by Constant time Inverse-detection Gradient Accordion Rescaled Heteronuclear Multiple Bond Correlation spectroscopy (CIGAR-HMBC) spectra ($8 < {}^nJ(H,C) < 5$) with the spectral width of 18000 Hz in *f*1 (^{13}C) and 3000 Hz in *f*2 (^{1}H). GC/MS: *HP 6890 GC* instrument equipped with a *5975B VL MSD* detector. HR-ESI-MS: Electrospray hybrid quadrupole orthogonal acceleration time-of-flight mass spectrometer (Q-TOF) fitted with a Z-spray electrospray ion source (*Waters S.p.A.*, Manchester, UK); in *m*/*z*.

Plant Material. Plants of *Petrorhagia saxifraga* LINK (Caryophyllaceae) were collected in June 2009 in Castel Volturno, near Caserta (Campania, Italy), and identified by Dr. *Assunta Esposito* at the Department of Scienze della Vita of Second University of Naples (SUN). A voucher specimen (CE0112) has been deposited with the Herbarium of the Dipartimento di Scienze della Vita of SUN.

Extraction and Isolation. Dried whole plants (370 g) were extracted with MeOH (5.0 l) for 5 d in the dark at 4°, to obtain 25.5 g of residual material. The MeOH extract was dissolved in dist. H_2O (2.0 l) and shaken with AcOEt (2.0 l); the aq. fraction was chromatographed on *Amberlite XAD-4*, eluting with H_2O first, to eliminate sugars, peptides, free amino acids, and other small primary metabolites, and then with MeOH. The alcoholic eluate furnished 6.4 g of residual material which was chromatographed on *Sephadex LH-20* (2.5 × 120 cm) eluting with H_2O and collecting 20-ml fractions. *Frs. 1–15* (316 mg) purified by TLC on SiO_2, eluting with the org. phase of the biphasic soln. constituted by $CHCl_3$/MeOH/H_2O 13:7:2, furnished pure compound **3** (36 mg); *Fr. 16* (77 mg) was re-chromatographed (TLC on cellulose) eluting with the upper phase of the biphasic soln. constituted by $CHCl_3$/MeOH/H_2O 13:7:2, to give pure compound **2** (8 mg) and a the second spot which was purified by HPLC (NH_2, MeCN/MeOH/H_2O 7:2:1) to yield compound **4** (49 mg). *Frs. 17–19* (350 mg) were subjected CC (*RP-18*; MeOH/H_2O 7:3) to give a fraction of 20-ml volume; *Fr. 10* was purified by HPLC (NH_2, MeCN/MeOH/H_2O 7:2:1) to give compounds **1** (38 mg) and **3** (36 mg). *Fr. 20* (60 mg) purified by flash CC (SiO_2; the org. phase of the biphasic soln. constituted by $CHCl_3$/MeOH/H_2O 13:7:2) furnished pure compounds **5** (124 mg) and **6** (165 mg). *Fr. 26* (51.7 mg) re-chromatographed (TLC on SiO_2), eluting with the org. phase of the biphasic soln. constituted by $CHCl_3$/MeOH/H_2O 13:7:3, gave pure compounds **7** (15 mg) and **8** (10 mg).

Petrorhagioside A (=*2-(Hydroxymethyl)-4-oxo-4*H-*pyran-3-yl 3*-O-*β*-D-*Glucopyranosyl-β*-D-*glucopyranoside*; **1**). White amorphous powder. $[\alpha]_D^{25} = -13.0$ ($c = 0.10$, MeOH). UV (MeOH): 258 (3.85), 202 (4.07). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 489.3792 ($[M+Na]^+$, $C_{18}H_{26}NaO_{14}^+$; calc. 489.3804).

Petrorhagioside B (=*2-Methyl-4-oxo-4*H-*pyran-3-yl 3*-O-*β*-D-*Glucopyranosyl-β*-D-*glucopyranoside*; **2**). White amorphous powder. $[\alpha]_D^{25} = -8.9$ ($c = 0.13$, MeOH). UV (MeOH): 256 (3.47), 202 (3.76). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 473.3878 ($[M+Na]^+$, $C_{18}H_{26}NaO_{13}^+$; calc. 473.3810).

Petrorhagioside C (=*2-(Hydroxymethyl)-4-oxo-4*H-*pyran-3-yl 6*-O-*β*-D-*Glucopyranosyl-β*-D-*glucopyranoside*; **3**). White amorphous powder. $[\alpha]_D^{25} = -73.5$ ($c = 0.20$, MeOH). UV (MeOH): 258 (4.06), 202 (4.36). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 489.3802 ($[M+Na]^+$, $C_{18}H_{26}NaO_{14}^+$; calc. 489.3804).

Petrorhagioside D (=*2-Methyl-4-oxo-4*H-*pyran-3-yl 6*-O-*β*-D-*Glucopyranosyl-β*-D-*glucopyranoside*; **4**). White amorphous powder. $[\alpha]_D^{25} = -12.0$ ($c = 0.02$, MeOH). UV (MeOH): 256 (4.14), 202 (4.27). ^{1}H- and ^{13}C-NMR: *Tables 1* and *2*, resp. HR-ESI-MS: 473.3996 ($[M+Na]^+$, $C_{18}H_{26}NaO_{13}^+$; calc. 473.3810).

Identification of the Sugar Moieties. Compounds **1**–**4** (0.5 mg) were subjected to acid hydrolysis with 2N TFA (150 µl). The hydrolysis reaction was conducted at 120° for 1 h. The mixture was then dried after ^{i}PrOH addition. The sugars were identified by GC analysis of their alditol acetate derivatives and comparison with eight reference monosaccharides treated under the same conditions. The reduction of the sugars was achieved by dissolving the sample in MeOH (150 µl) and by adding $NaBH_4$ (1.0 mg). The soln. was incubated at r.t. for 1 h and then dried under N_2 flow after treatment with glacial AcOH and MeOH. Finally, the obtained alditol was acetylated, in anh. pyridine (200 µl), by addition of Ac_2O (200 µl). After 20 min at 120°, 500 µl of H_2O were added, the product was extracted with CH_2Cl_2 (500 µl) in centrifuge (3500 rpm for 5 min), and the org. phase was analyzed by GC/MS.

Enzymatic Hydrolyses of **1**–**4**. To a soln. of the pure compound (2 mg) in acetate buffer (0.5M, pH 5.0, 5 ml), 40 mg of *β*-glucosidase (*β*-glucosidase from almonds, *Sigma* EC 3.2.1.21) were added. After stirring for 24 h at 37°, the mixture was extracted with AcOEt (5 ml × 2), dried (Na_2SO_4), and evaporated *in vacuo*. The residue was subjected to TLC for aglycone identification. After concentration of the aq. layer, the sugar residue was identified by RI-HPLC by comparison with authentic standards.

Determination of Absolute Configuration of Monosaccharides of **1**–**4**. Compounds **1**–**4** (2 mg each) were hydrolyzed with 2N TFA (250 µl) at 120° for 1 h. The mixture was then dried under N_2 flow, and the residue was dissolved in dry pyridine (100 µl). To pyridine solns. of the hydrolysis products **1**–**4**, D-glucose, L-glucose (0.04 mol/l), and 100 µl of pyridine soln. of L-cysteine methyl ester hydrochloride (0.06 mol/l) were added, and the mixtures were warmed at 60° for 1 h. Afterwards, Ac_2O (150 µl) was added, and the mixtures were kept at 120° for 20 min, then dried under N_2, redissolved in 500 µl of H_2O, and extracted with CH_2Cl_2 (500 µl) in a centrifuge (3500 rpm for 5 min). The org. phase was dried under N_2 flow, dissolved in CH_2Cl_2 (500 µl), and analyzed by GC/MS. Temp. conditions were as follows: injector port, 250°; initial oven temp., 45°, then increased linearly to 300° at 20°/min, and held for 25 min. Sample solns. were injected using the split mode. The retention times were: D-glucose, 14.97 min; and L-glucose, 15.22 min [12].

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