Hirtusneanoside, an Unsymmetrical Dimeric Tetrahydroxanthone from the Lichen *Usnea hirta*

Tomáš Rezanka* and Karel Sigler

Institute of Microbiology, Vídeňská 1083, Prague 142 20, Czech Republic

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Hirtusneanoside, a new *O*-deoxyglycoside of a dimeric tetrahydroxanthone, was isolated from the lichen *Usnea hirta*. Its structure, including the absolute configuration, was determined by means of extensive spectroscopic data (UV, IR, MS, CD, 1D and 2D NMR) and chemical degradation. Hirtusneanoside has a unique structure comprising L-rhamnopyranoside of a tetrahydroxanthone dimer and showed growth inhibitory activities against Gram-positive bacteria.

Xanthones are secondary metabolites with interesting pharmacological properties.[1] They show choleretic, diuretic, antimicrobial, antiviral, cardiotonic, antitubercular, and antidepressant activities, in addition to being effective as allergy inhibitors and bronchodilators in the treatment of asthma.[2,3]

The basic monomeric units of the tetrahydroxanthones are diversonolic acids and toxylxanthones. Symmetrical dimers secalonic acids A–F[4,5] exhibit toxic, antibacterial, mutagenic, fetotoxic, and teratogenic properties. Other symmetrical dimers with antibiotic properties, dicerandrols A, B, and C, structurally related to the ergochromes and secalonic acids, were isolated from the endophytic fungus *Phomopsis longicolla*.[6] *Phomopsis* sp. yielded phomoxanthones A and B,[7] structurally related to dicerandrols except for the position of the biaryl bond.[8] Unsymmetrical tetrahydroxanthone dimers, such as neosartorin from *Neosartorya fischeri*[9] and rugulotrosins A and B from *Penicillium* sp.,[10] also have antibacterial activity. Xanthonol, a novel unsymmetrical dimeric xanthone, was isolated from the fermentation broth of a nonsporulating fungal species.[11]

The *Usnea* species of lichens (family Parmeliaceae), which generally grow hanging from tree branches[12] in damp forests, have long been used medicinally. Most of these contain usnic acid, a potent antibiotic and antifungal agent.[13] *U. hirta* is distinct in that it does not droop from tree branches and trunks but grows upward and forms small shrublets. It is light green, with a gray or brown tint, and it is densely covered with black papillae.

In the course of our continuing search for novel biologically active agents from lichens,[14–19] we have isolated from *U. hirta* a new unsymmetrical glycosidic tetrahydroxanthone dimer, hirtusneanoside (**1**). In this paper, we describe the isolation and structure elucidation of **1** using extensive spectroscopic analysis, enzymatic hydrolysis, and chemical degradation. The antimicrobial activity of **1** is also reported.

A sample of 95 g of *U. hirta* was extracted with n-BuOH and subsequently separated on a Sephadex LH-20 column. The fractions were further purified by RP-HPLC to give a glycoside (**1**, 23.9 mg), which was identified by IR, UV, CD, MS, and ^{1}H and ^{13}C NMR spectroscopic data and chemical degradation. Compound **1** (Figure 1) was obtained as a pale yellow, amorphous powder with $[\alpha]_D^{20}$ −251.

Compound **1** showed absorption maxima at λ_{max} 340, 275, and 230 nm in the UV spectrum, indicating that it contained an extended chromophore. The IR spectrum displayed absorptions at 1735 cm^{-1} (aliphatic C=O), 1620 (enolic β-diketone), and 1590 cm^{-1} characteristic for aromatic ring(s) and 870 cm^{-1} for pentasubstituted benzene and showed a broad absorption band at 3290 cm^{-1} consistent with the presence of OH functionalities.

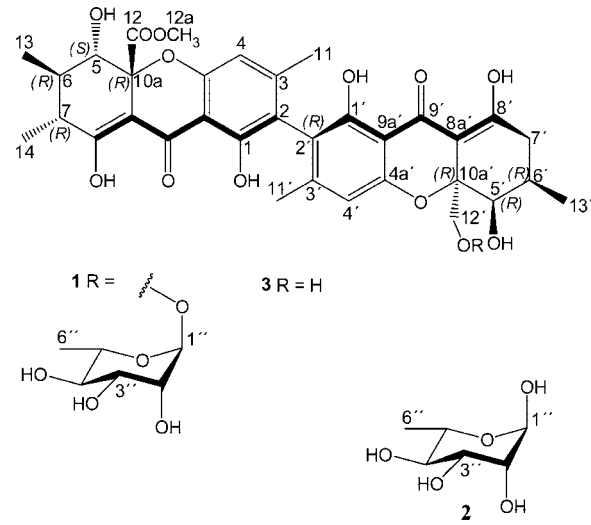

Figure 1. Hirtusneanoside (**1**), tetrahydroxanthone dimer glycoside from the lichen *Usnea hirta.*

The positive HRFABMS showed an $[M + Na]^+$ peak at *m/z* 821.2628 (calc 821.2632) corresponding to the molecular formula $C_{40}H_{46}O_{17}Na$, deduced also by ^{13}C NMR and DEPT analyses. The negative FABMS spectrum of **1** showed a molecular peak $[M - H]^-$ at *m/z* 797 that gave the fragment ions at *m/z* 651 $[M - H - 146]^-$ and *m/z* 633 $[M - H - H_2O - 146]^-$, which were formed by the loss of the deoxyhexose unit (**2**).

The NMR data of hirtusneanoside (**1**) (see Table 1) showed the presence of one *O*-glycosidic deoxyhexopyranose, i.e., one anomeric carbon resonance at δ 101.1 and one anomeric proton (δ 5.01). The key resonance is at δ 18.6, which represents C-6″ of a 6-deoxy sugar. This resonance was identified as a methyl group from the DEPT spectrum and the corresponding ^{1}H NMR chemical shift (δ 1.31). It was used as a starting point in the homonuclear correlated spectra to determine all glycosidic protons. The $J_{H\text{-}1''\text{-}H\text{-}2''}$ value (1.5 Hz) of compound **1**, and the NOE correlation between H-1″ and H-2″, H-3″ and H-5″, H-4″ and H-5″, and H-5″ and H-6″, observed in the NOESY experiment as shown in Figure 2, further confirmed that the deoxyhexose sugar was α-rhamnopyranose (**2**).[20]

The coupling constant ($^1J_{CH}$) between C-1 and H-1 in pyranose derivatives of carbohydrates is useful in the assignment of anomeric configuration since pyranoses with an axial H-1 have a $^1J_{CH}$ value that is approximately 10 Hz lower than the corresponding value in compounds with an equatorial H-1.[21] On the basis of literature data, the anomeric configuration can be seen to be α because the $^1J_{CH}$ value in our glycoside was 170.9 Hz.[22]

Enzymatic hydrolysis of **1** with hesperidinase (EC 3.2.1.40), an enzyme specifically catalyzing the hydrolysis of α-L-rhamnose, yielded a sugar (3.5 mg) that had $[\alpha]_D$ +9.0, compared to literature

* To whom correspondence should be addressed. Tel: +420 241 062 300. Fax: +420 241 062 347. E-mail: rezanka@biomed.cas.cz.

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Table 1. NMR Data (DMSO-d_6) of Hirtusneanoside (**1**)

no.	^{1}H	^{13}C	HMBC
1-OH	11.5 (brs, 1 H)	159.2 s	1, 2, 9a
2		116.7 s	
3		149.6 s	
4	6.66 (s, 1 H)	109.2 d	1, 2, 4a, 9, 11
4a		156.8 s	
5	4.02 (d, J = 9.5 Hz, 1 H)	68.8 d	7, 8a, 10a, 13
6	2.05 (ddq, J = 9.5, 10.3, 6.7 Hz, 1 H)	31.3 d	5, 8, 10a
7	2.36 (dq, J = 10.3, 6.4 Hz, 1 H)	36.7 d	5, 6, 8, 8a, 9, 10a
8-OH	13.7 (brs, 1 H)	177.8 s	7, 8, 8a
8a		101.3 s	
9		186.8 s	
9a		105.7 s	
10a		85.1 s	
11	1.94 (s, 3 H)	20.7 q	1, 2, 3, 4, 4a, 9, 10a
12		171.3 s	
12a	3.73 (s, 3 H)	54.3 q	10a, 12
13	1.09 (d, J = 6.7 Hz, 3 H)	15.6 q	5, 7, 8, 10a
14	1.01 (d, J = 6.4 Hz, 3 H)	17.3 q	5, 6, 8, 8a
1′-OH	11.5 (brs, 1 H)	159.6 s	1′, 2′, 9a′
2′		118.1 s	
3′		150.2 s	
4′	6.69 (s, 1 H)	109.3 d	1′, 2′, 4a′, 9′, 11′
4a′		156.7 s	
5′	4.07 (d, J = 1.3 Hz, 1 H)	68.9 d	6′, 7′, 8a′, 9′, 10a′, 12′
6′	2.28 (dddq, J = 1.3, 6.5, 11.3, 6.7 Hz, 1 H)	28.5 d	5′, 7′, 8′, 8a′, 10a′, 13′
7′	2.35 (dd, J = 19.2, 6.5 Hz, 1 H); 2.48 (dd, J = 19.2, 11.3 Hz, 1 H)	33.6 t	5′, 6′, 8′, 8a′, 10a′, 12′
8′-OH	13.7 (brs, 1 H)	177.6 s	7′, 8′, 8a′
8a′		101.8 s	
9′		186.6 s	
9a′		106.3 s	
10a′		84.4 s	
11′	1.96 (s, 3 H)	20.6 q	1′, 2′, 3′, 4′, 4a′, 9′, 10a′
12′	3.89 (d, J = 13.0 Hz, 1 H); 3.51 (d, J = 13.0 Hz, 1 H)	64.5 t	5′, 10a′
13′	1.04 (d, J = 6.7 Hz, 3 H)	17.7 q	5′, 7′, 8′, 10a′
1″	5.01 (d, J = 1.5 Hz, 1 H)	101.1 d	3″, 5″
2″	3.89 (dd, J = 1.5, 2.5 Hz, 1 H)	72.2 d	3″, 4″
3″	3.71 (dd, J = 2.5, 9.5 Hz, 1 H)	70.9 d	4″
4″	4.32 (t, J = 9.5 Hz, 1 H)	74.5 d	3″, 5″, 6″
5″	4.13 (dq, J = 9.5, 6.5 Hz, 1 H)	69.7 d	
6″	1.31 (d, J = 6.5 Hz, 3 H)	18.6 q	4″, 5″

data, $[\alpha]_D$ +9.1 and/or +8.9, for L-rhamnose (6-deoxy-L-mannose) (**2**) (Figure 1).[23] The hydrolysis yielded in addition the aglycone hirtusneanine (**3**) (Figure 1), which was extracted from the aqueous solution by EtOAc and further characterized (yield 17.6 mg). Compound **3** is represented by pale yellow microcrystals with the molecular formula $C_{34}H_{36}O_{13}$ (HRFABMS (*m/z*) calcd for $C_{34}H_{36}O_{13}Na$ $[M + Na]^+$, 675.2053; found, 675.2051), and the ^{13}C NMR spectrum shows 34 carbon resonances.

The maxima observed at 275 and 338 nm in the UV spectrum are typical of those of a chromanone chromophore. The absorption band at 338 nm was shifted to 353 nm in methanolic KOH,

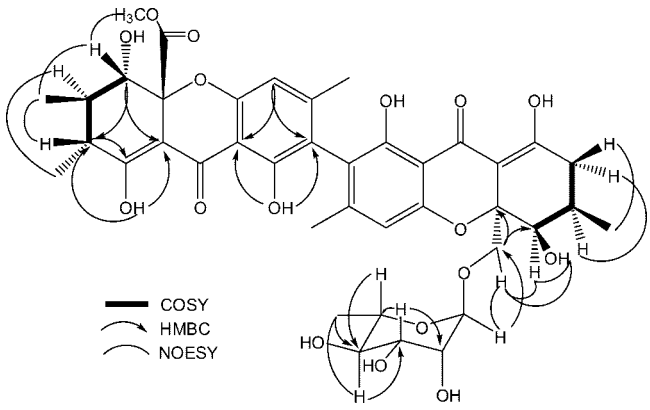

Figure 2. HMBC, ^{1}H–^{1}H COSY, and NOESY correlations of hirtusneanoside (**1**).

indicating phenolic hydroxy groups. The IR spectrum of compound **3** supported the presence of the carbonyl groups, showing strong absorptions at 1734 cm^{-1}, the presence of an enolized β-diketone being further suggested by a red-brown ferric chloride test and the IR absorption at 1608 cm^{-1}.

The ^{13}C NMR spectrum of **3** showed the presence of 34 carbon atoms and supported the molecular formula with 17 degrees of unsaturation. The DEPT spectrum suggested the presence of six methyls (one of which is part of the ester), two methylenes, seven methines, and 19 quaternary carbons. Two methyl groups appeared as singlets at δ_H 1.94 (δ_C 20.7) and δ_H 1.96 (δ_C 20.6) in the ^{1}H NMR spectrum, indicating that they were attached to aromatic carbons C-3 and C-3′. This was confirmed by HMBC correlations to three carbons each (Table 2). The third methyl group appeared at δ 3.73 (δ_C 54.3) and showed an HMBC correlation to the carboxylic carbon, δ_C 171.3. The ^{1}H NMR spectrum showed two H-bonded hydroxy groups (δ_H 13.7 and 11.5) in each monomer. The ^{1}H NMR spectrum of **3** in conjunction with COSY and HMQC identified two spin systems, i.e., from C-5′ to C-7′ including the C-13′ methyl group and C-5 to C-7 including the C-6 and C-7 methyl groups. The connectivity of the spin systems into the carbon skeleton of compound **3** was established using HMBC correlations, and it also suggested the presence of a tetrahydroxanthone moiety as shown in Figure 2.

The location of the biaryl bond between C-2 and C-2′ in **3** follows from the absence in the ^{1}H NMR spectrum of any resonances attributable to H-2 and H-2′ (cf. δ 6.60 and 6.59 for α-diversonolic and β-diversonolic esters, respectively), and the chemical shifts and multiplicities of H-4 and H-4′ (singlets at δ 6.66 and 6.69 in our

Table 2. NMR Data (DMSO-d_6) of Hirtusneanine (**3**)

no.	^{1}H	^{13}C	HMBC
1-OH	11.5 (brs, 1 H)	159.2 s	1, 2, 9a
2		116.7 s	
3		149.6 s	
4	6.66 (s, 1 H)	109.2 d	1, 2, 4a, 9, 11
4a		156.8 s	
5	4.02 (d, J = 9.5 Hz, 1 H)	68.8 d	7, 8a, 10a, 13
6	2.05 (ddq, J = 9.5, 10.3, 6.7 Hz, 1 H)	31.3 d	5, 8, 10a
7	2.36 (dq, J = 10.3, 6.4 Hz, 1 H)	36.7 d	5, 6, 8, 8a, 9, 10a
8-OH	13.7 (brs, 1 H)	177.8 s	7, 8, 8a
8a		101.3 s	
9		186.8 s	
9a		105.7 s	
10a		85.1 s	
11	1.94 (s, 3 H)	20.7 q	1, 2, 3, 4, 4a, 9, 10a
12		171.3 s	
12a	3.73 (s, 3 H)	54.3 q	10a, 12
13	1.09 (d, J = 6.7 Hz, 3 H)	15.6 q	5, 7, 8, 10a
14	1.01 (d, J = 6.4 Hz, 3 H)	17.3 q	5, 6, 8, 8a
1′-OH	11.5 (brs, 1 H)	159.6 s	1′, 2′, 9a′
2′		118.1 s	
3′		150.2 s	
4′	6.69 (s, 1 H)	109.3 d	1′, 2′, 4a′, 9′, 11′
4a′		156.7 s	
5′	4.07 (d, J = 1.3 Hz, 1 H)	68.7 d	6′, 7′, 8a′, 9′, 10a′, 12′
6′	2.28 (dddq, J = 1.3, 6.5, 11.3, 6.7 Hz, 1 H)	28.5 d	5′, 7′, 8′, 8a′, 10a′, 13′
7′	2.35 (dd, J = 19.2, 6.5 Hz, 1 H); 2.48 (dd, J = 19.2, 11.3 Hz, 1 H)	33.6 t	5′, 6′, 8′, 8a′, 10a′, 12′
8′-OH	13.7 (brs, 1 H)	177.6 s	7′, 8′, 8a′
8a′		102.1 s	
9′		186.6 s	
9a′		106.3 s	
10a′		84.1 s	
11′	1.96 (s, 3 H)	20.6 q	1′, 2′, 3′, 4′, 4a′, 9′, 10a′
12′	4.14 (dd, J = 7.0, 13.0 Hz, 1 H); 3.75 (dd, J = 4.7, 13.0 Hz, 1 H)	68.7 t	5′, 10a′
12′-OH	3.28 (m, 1H)		
13′	1.04 (d, J = 6.7 Hz, 3 H)	17.7 q	5′, 7′, 8′, 10a′

compound, compared to the doublets at δ 6.67 and 6.66 with J = 2.4 Hz in α-diversonolic and β-diversonolic esters, respectively).[24] The biaryl connectivity is also fully consistent with the HMBC experiment shown in Figure 2. Correlation from the H-bonded phenolic proton (OH-1; δ 11.5) to the quaternary carbon at δ 116.7 (C-2) was observed. A weak four-bond correlation (^4J) from an aromatic proton (H-4) to a carbonyl (C-9) and also a ^4J correlation of H-4 to C-2′ and H-4′ to C-2 over the terminal biaryl bond were also observed. Collectively, the data support the structure of **3**. This established the planar structure of the aglycone.

The relative configuration of compound **3** was determined on the basis of ^{1}H–^{1}H coupling constants. The proposed relative configuration of **3** was confirmed by the ^{1}H NMR spectrum of **3** since the resonances for H-6′ and the methylene protons (H-7′) do not overlap, appearing at δ 2.28 as dddq and 2.35 (dd, J = 19.2, 6.5 Hz) and 2.48 (dd, J = 19.2, 11.3 Hz) ppm, respectively. Due to the large vicinal coupling constant of $J_{6',7'}$ = 11.3 Hz, the δ 2.48 resonance was assigned as the pseudoaxial H-7′, and the δ 2.35 resonance $J_{6',7'}$ = 6.5 Hz, as the pseudoequatorial H-7′. On the basis of the $J_{6',7'}$ coupling, H-6′ must occupy a pseudoaxial orientation. NOE correlations between H-6′ and one of the H-12′ methylene resonances at δ 3.51 indicate that the C-10a′ hydroxymethyl group and H-6′ are cofacial (Figure 2). NOESY correlation between H-12′ (δ 3.51) and H-5′ was consistent with the established relative configuration, where the hydroxymethyl group and H-5′ are cofacial, hence the pseudoequatorial orientation of H-5′.

The large coupling constant, $J_{6,7}$ = 10.3 Hz, suggests that H-6 is axial. The C-13 methyl group must therefore be in the equatorial position. The H-6 resonance is split by a 9.5 Hz coupling constant to proton H-5, which is therefore axial. The C-10a carboxymethyl moiety is in a *cis*-position with respect to H-5, as shown by the presence of a NOESY between CH_3-12a and H-5. The coupling constants agree with the coupling constants of model compounds (2*R*,3*R*,4*S*)-4-hydroxy-2,3-dimethylcyclohexanone and (2*S*,3*R*,4*S*)-4-hydroxy-2,3-dimethylcyclohexanone.[25]

The NOEs between CH_3-12a and CH_3-11′ and between CH_2-12′ and OH-1 suggested that the two aromatic rings are not coplanar.

The relative configuration of our compound must be either 5*S*, 6*R*, 7*R*, 10a*R*, 5′*R*, 6′*R*, 10a′*R*, or 5*R*, 6*S*, 7*S*, 10a*S*, 5′*S*, 6′*S*, 10a′*S*.

The absolute configuration was determined by several independent methods. The first step involved determining the chirality of C-10a and C-10a′. Secalonic acids contain almost the same chromophore system as our aglycone, and they have a 2,2′-biaryl linkage. Their (10a*R*,10a′*R*) absolute configurations were correlated with an intensive positive Cotton effect at about 330 nm in their CD spectra.[5,26] If the same configurational assignment for secalonic acids would be applied to the aglycone (**3**), the C-10a and C-10a′ stereogenic centers of the aglycone should be homochiral with those of secalonic acids D and F since the CD spectrum of the aglycone also shows a positive CE at 334 nm ($\Delta\varepsilon$ = +15.4 in dioxane). This would imply that the aglycone has the (10a*R*,10a′*R*) absolute configuration.

When the aglycone (**3**) was subjected to oxidation with potassium permanganate,[5] TLC gave (*R*)-methylsuccinic acid (**8**), $[\alpha]_D$ +8.0, and 2*R*,3*R*-dimethylsuccinic acid (**9**), in which the specific rotation is $[\alpha]_D$ +7.8. Acid **8** was commercially obtained, while acid **9** was commercially available only as a mixture of racemic and meso forms. This value is very similar to literature values,[27] i.e., +7.8 or +8.0.[28] The absolute configuration of the tetrahydroxanthone ring is therefore 6′*R* (*R*-methylsuccinic acid) and 6*R* and 7*R* (2*R*,3*R*-dimethylsuccinic acid).

The aglycone can be viewed as an *ortho*-disubstituted biaryl derivative that, in contrast to secalonic acids, has bulky *ortho* substituents, and thus the rotation along the biaryl axis is restricted, which leads to atropisomers. The absolute configuration at the chiral axis in our hirtusneanine (**3**) was determined from the CD data,

Table 3. Biological Activities of Hirtusneanoside (**1**)[a]

test organism	LD_{50} (μM)
Staphylococcus aureus	0.0034
Bacillus subtilis	0.0140
Escherichia coli	0.0
Saccharomyces cerevisiae	0.0

[a] Samples (10 μg) were applied on 50.8 mm paper disks; values are diameters (mm) of inhibitory zones.

which are described in the Experimental Section. The data show that the compound exhibits a strong positive Cotton effect at a longer wavelength (262 nm) and a stronger negative one at a shorter wavelength close to 220 nm. This is in accord with a counterclockwise helical twist between the long axes of the aromatic chromophores,[29] as is depicted in the structure. This configuration corresponds to *R* (*M*) chirality according to the Prelog–Helmchen rules.[30] Our conclusions are further supported by the CD data of a similar model biaryl compound, i.e., (*M*)-3,3′-diacetyl-4,4′-dihydroxy-6,6′-dimethoxy-2,2′-dimethyl-1,1′-biphenyl, and kotanin[31,32] and also by CD data and spectra of a similar compound, phomoxanthone A.[7]

Therefore, the absolute configuration of all eight chiral centers in compound **3** has been elucidated to be (2*R*, 5′*R*, 6′*R*, 10a′*R*, 5*S*, 6*R*, 7*R*,10a*R*).

On the basis of these data, the structure of hirtusneanoside (**1**) is (2*R*, 5*S*, 5′*R*, 6*R*, 6′*R*, 7*R*, 10a*R*,10a′*R*)-methyl 1,1′,5,5′,8,8′-hexahydroxy-3,3′,6,6′,7-pentamethyl-9,9′-dioxo-10a′-(α-L-rhamnosyloxymethyl)-6,6′,7,7′,9,9′,10a,10a′-octahydro-5*H*,5′*H*-2,2′-bixanthene-10a-carboxylate.

Compound **1** inhibits the growth of the Gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. However, it was inactive against Gram-negative bacteria and yeast; see Table 3.

Hirtusneanoside is the first xanthone dimer *O*-glycoside, the derivatives isolated so far including only bisxanthone *C*-glycosides from the genus *Swertia*.[33,34]

Experimental Section

General Experimental Procedures. UV–vis spectra were measured in MeOH within the range of 220 to 550 nm in a Cary 118 (Varian) apparatus. A Perkin-Elmer (Norwalk, CT) model 1310 IR spectrophotometer was used for scanning IR spectroscopy as neat films. Optical rotations were recorded with a Perkin-Elmer 243 B polarimeter. Circular dichroism (CD) measurement was carried out under dry N_2 on a Jasco-500A spectropolarimeter at 24 °C. NMR spectra were recorded on a Bruker AMX 500 spectrometer (Bruker Analytik, Karlsruhe, Germany) at 500.1 MHz (^{1}H) and 125.7 MHz (^{13}C). High- and low-resolution MS were recorded using a VG 7070E-HF spectrometer (70 eV). HRFABMS (positive ion mode) were obtained with a PEG-400 matrix. The reference compounds were purchased from Sigma-Aldrich (Prague, Czech Republic).

Usnea hirta (L.) Weber ex F.H.Wigg (Parmeliaceae) material, old man's beard (95 g), was collected in July 2006 near Javorová skála, Sedlec-Prčice, Czech Republic.

The lichen was extracted with n-BuOH (×3), and the extracts were further chromatographed by means of Sephadex LH-20 columns with $CHCl_3$–MeOH (1:2) and then separated by RP-HPLC on a Discovery C18 column (Supelco), particle size 5 mm, length × i.d. (250 mm × 21.2 mm), using a linear gradient from 10% H_2O and 90% MeCN to 90% H_2O and 10% MeCN over 50 min, with a flow rate of 9.0 mL/min and monitored by a variable-wavelength detector at 254 nm. The yield was 23.9 mg (0.025%) of compound **1**.

A solution of glycoside (22.4 mg) in acetate buffer (pH 4.4, 10 mL) was treated with hesperidinase (EC 3.2.1.40) for 48 h at 37 °C. The solution was extracted with a mixture of CH_2Cl_2–MeOH (90:10) and was chromatographed on a column of silica gel (10 g), using CH_2Cl_2–MeOH–H_2O (90:10:1) to provide 17.6 mg (96.1 %) of compound **3**. The aqueous phase was lyophilized, and the residue was purified on a Sepharon SGX NH_2 column (7 μm, 3 × 150 mm) eluted with 90 % MeCN (flow rate 0.7 mL/min) to yield 3.5 mg (76.1 %) of L-rhamnose (**2**) ($[\alpha]_D^{22}$ +9.0 (equilib)).

Aglycone (16.0 mg) was dissolved in 2 mL of 2 M NaOH. The solution was cooled to 0 °C and added to 1 mL of saturated $KMnO_4$ solution at 0 °C. The mixture was maintained at 0 °C for 48 h and then clarified at 0 °C with SO_2. Extraction with EtOAc (3 × 5 mL) gave crude reaction products, which were chromatographed on 0.1 mm cellulose on TLC-PET foils with a 254 nmfluorescent indicator (solvent 96% EtOH–H_2O–25% NH_3, 25:3:4). The desired bands were eluted from the cellulose with 4 mL of MeOH–H_2O (2:1). Optical rotations were measured in water, and the following values were obtained for (*R*)-methylsuccinic acid (**8**) (2.1 mg, 65.0 %) and 2*R*,3*R*-dimethylsuccinic acid (**9**) (1.9 mg, 53.1%): $[\alpha]_D$ +8.0 (*c* 0.09, H_2O) for **8** and $[\alpha]_D$ +7.8 (*c* 0.08, H_2O) for **9** (lit.[27] $[\alpha]_D$ +8.4 (*c* 4, H_2O); lit.[28] $[\alpha]_D$ +7.8); ^{1}H NMR (DMSO) δ 12.10 (s, 2H); 2.61 (m, 2H); 1.23 (d, J = 6.9 Hz, 6H); EIMS *m/z* 146 (M^+, 0.4), 128 (M – H_2O, 1.9), 100 (7.1), 87 (4.9), 74 (9.4), 56 (100)55 (18.3), 41 (53.7).

Hirtusneanoside (1): amorphous, pale yellow powder (23.9 mg); $[\alpha]_D^{20}$ −251 (*c* 0.02, MeOH); UV λ_{max} (MeOH, nm) (log ε) 230 (4.52), 275 (4.01), 340 (3.24); IR (KBr, cm^{-1}) ν_{max} 3290, 1735, 1620, 1590, 870; HRFABMS (*m/z*) 821.2628 $[M + Na]^+$, calcd for $[C_{40}H_{46}O_{17}Na]^+$ 821.2632; negative FABMS $[M - H]^-$ at *m/z* 797, *m/z* 651 $[M - H - 146]^-$, *m/z* 633 $[M - H - H_2O - 146]^-$; NMR data, see Table 1.

Hirtusneanine (3): pale yellow microcrystals (MeOH); mp 251–253 °C; yield 17.6 mg; $[\alpha]_D^{23}$ −232 (*c* 0.01, MeOH); UV λ_{max} (MeOH, nm) (log ε) 231 (4.43), 275 (4.04), 338 (3.97); IR (KBr, cm^{-1}) ν_{max} 3290, 1734, 1608, 1590, 870; CD (*c*, 0.3 dioxan) λ ($\Delta\varepsilon$) 400 (0), 334 (15.4), 300 (0.2), 290 (0), 277 (−4.1), 262 (1.4), 248 (−14.6), 220 (−38.0), 210 (−43.7) nm; HRFABMS (*m/z*) 675.2051 $[M + Na]^+$, calcd for $[C_{34}H_{36}O_{13}Na]^+$ 675.2053; NMR data see Table 2.

The test organisms were *Bacillus subtilis*, *Staphyloccocus aureus*, *Escherichia coli*, and *Saccharomyces cerevisiae* (Czechoslovak Collection of Microorganisms, Brno). Antibacterial assays were carried out according to the literature.[35] The amounts used were 10 μg of compound per test disk (see Table 3).

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