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New 1-*O*-Acyl α-L-Rhamnopyranosides and Rhamnosylated Lactones from *Streptomyces* sp., Inhibitors of 3α-Hydroxysteroid-dehydrogenase (3α-HSD)

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Chemical screening with extracts of *Streptomyces* sp. (strain GT 61150) resulted in the detection, isolation, and structure elucidation of two new acyl α-L-rhamnopyranosides (**1** and **2**) and three new rhamnosyllactones A, B_1 and B_2 (**3**~**5**). Rhamnosyllactones B_1 and B_2 were obtained as a 5 : 1 mixture. The structures were confirmed by spectroscopic analysis, especially 2D-NMR techniques. The rhamnosyltransferase of our strain is able to connect the sugar moiety to heteroaromatic carboxylic acids and enols. The metabolites **1** and **4**/**5** as well as previously reported acylrhamnosides **6**~**11** inhibit the enzyme 3α-hydroxysteroid-dehydrogenase (3α-HSD).

Application of our chemical screening method to different *Streptomyces* strains gave rise to the discovery of a number of new compounds[1,2]. From a culture broth of *Streptomyces* sp. (strain GT 61150), we have now obtained two new members of the 1-*O*-acyl α-L-rhamnopyranoside family[3,4], and three new rhamnosylated lactones. In this paper we describe the production, isolation, structure analysis and biological activities of these five new secondary metabolites, 2-(1-propen-1-yl)-4-hydroxymethyl-3-furanylcarbonyl α-L-rhamnopyranoside (**1**), 3-indolylcarbonyl α-L-rhamnopyranoside (**2**), and the rhamnosyllactones A, B_1 and B_2 (**3** to **5**).

Experimental

General

For parts of instrumentation and general methods see a preceding article[2]. 1D- (^1H, ^{13}C and DEPT) and 2D- (^1H-^1H COSY, HSQC, HMBC and NOESY) NMR spectra were recorded on a Bruker DPX 300 and/or on a Bruker DRX 500 spectrometer. Chemical shifts, which δ values are expressed in parts per million (ppm) are referenced to the residual solvent signals with resonances at δ_H/δ_C 3.30/49.00 (CD_3OD). IR spectra were recorded on a Bruker FT-IR (IFS 55) spectrometer. UV spectra were scanned on a Cary 1 Bio UV-visible spectrophotometer (Varian). ESI mass spectra were obtained on a VG Quattro Micromass spectrometer, EI and FAB mass spectra on a AMD Intectra 402/2 mass spectrometer.

Production and Isolation of the Rhamnopyranosides

The cultures of strain GT 61150, obtained by standard isolation procedures for *Streptomycetes* strains, were grown on medium 2 [oatmeal 20 g/liter (Kölln, Elmshorn, Germany), trace element solution ($CaCl_2 \cdot 2H_2O$ 3 g, $FeC_6H_5O_7$ 1 g, $MnSO_4$ 0.2 g, $ZnCl_2$ 0.1 g, $CuSO_4 \cdot 5H_2O$ 25 mg, $Na_2B_4O_7 \cdot 10H_2O$ 0.02 g, $Na_2MoO_4 \cdot 2H_2O$ 0.01 g and $CoCl_2$ 4 mg per liter deionized water) 2.5 ml/liter, pH=7.8 prior to sterilization] for 4 days at 28°C. Storage of

the strain was in 50% glycerol at −20°C. The glycerol-containing storage mixture (2 ml) was used to inoculate a 300-ml Erlenmeyer flask containing 100 ml of medium 2. In the routine screening course the flask was cultivated on a rotary shaker (180 rpm) at 28°C for 6 days for the primary TLC analysis. Details of the chemical screening method (cultivation, adsorption of the culture filtrate, concentration steps, and the conditions for TLC analysis) have been reported previously[5~7]. 96-hour old cultures obtained above were used to inoculate a fermentor (20-liter working volume, inoculation volume 5%, 400 rpm, 28°C, aeration 5 liters/minute) containing medium 2. Foaming could be decreased using PPG (polypropylenglycol). Highest yields (50 mg/liter) of 2-(1-propen-1-yl)-4-hydroxymethyl-3-furanylcarbonyl α-L-rhamnopyranoside (**1**) were reached after 6 days. After harvesting, the culture broth (20 liters) was filtered, and the culture filtrate was adsorbed on Amberlite XAD-16 (3 liters of resin). The resin was washed with 6 liters of deionized water and the metabolites were eluted with 5 liters of a mixture of MeOH/H_2O (4 : 1). The solution was concentrated to an aqueous residue which was then lyophilized to yield 227 g of a dark brown crude material. The whole crude material was suspended in deionized water (1.5 liters) and extracted exhaustively with EtOAc (5 liters) at room temperature. The organic layers were dried with anhydrous sodium sulfate (Na_2SO_4), and were evaporated to yield a brown-red residue (18 g). This material was chromatographed on silica gel (column: 5.0×44 cm) by using $CHCl_3$-MeOH (10 : 1) as eluent to yield in four fractions (fr. 1~fr. 4, monitored by TLC analysis). 2-(1-Propen-1-yl)-4-hydroxymethyl-3-furanylcarbonyl α-L-rhamnopyranoside (**1**, 50 mg/liter) and rhamnosyllactone A (**3**, 0.4 mg/liter) were obtained from fr. 2 (900 mg) after purification by gel permeation chromatography on Sephadex LH-20 (column 3.0×76 cm) in MeOH. 3-Indolylcarbonyl a-L-rhamnopyranoside (**2**, 0.2 mg/liter) together with a mixture (2.2 mg/liter) of the rhamnosyllactones B_1 (**4**) and B_2 (**5**) were obtained from fr. 3 (800 mg) by gel permeation chromatography on Sephadex LH-20 (column 3.0×70 cm) in MeOH, RP-18 HPLC [GILSON, column: SP 250/21 Nucleosil 100-7 C18, Macherey-Nagel (2.2×21 cm), P: 125 bar, MeOH/H_2O (25 : 75), flow: 20 ml/minute] and finally gel permeation chromatography on Sephadex LH-20 (column 3.0×76 cm, acetone).

Biological Testing

3α-Hydroxysteroid-dehydrogenase (3α-HSD) catalyzes the reduction of 5β-dihydrocortisone under consumption of NADPH. This consumption can be monitored as a decrease of extinction at 340 nm. The cytosol and 3α-hydroxysteroid-dehydrogenase (3α-HSD) were prepared according to the method described by PENNING *et al.*[8]. In addition the enzyme assay and inhibition studies were performed as described in ref. 8 and the literature cited therein. Solutions of the compounds **1**~**11** and the reference indomethacin were used in a concentration of 10 mg/ml dissolved in DMSO. These solutions were diluted with aqueous 1 M phosphate buffer (pH=6.0) resulting in a final concentration of 3.0 μg/ml.

Physico-chemical Properties of the Rhamnopyranosides **1**~**5**

The strain GT 61150 was conspicuous because of the highly intense grey-purple spots on TLC which were obtained after staining with Ehrlich's reagent. 2-(1-Propen-1-yl)-4-hydroxymethyl-3-furanylcarbonyl α-L-rhamnopyranoside (**1**): $C_{15}H_{20}O_8$; ESI-MS (positive mode) *m/z*: 329 $[M+H]^+$, 351 $[M+Na]^+$, 679 $[2M+Na]^+$. 3-Indolylcarbonyl α-L-rhamnopyranoside (**2**): $C_{15}H_{17}NO_6$; ESI-MS (positive mode) *m/z*: 330 $[M+Na]^+$, 637 $[2M+Na]^+$, 944 $[3M+Na]^+$; ESI-MS (negative mode) *m/z*: 306 $[M-H]^-$, 613 $[2M-H]^-$. Rhamnosyllactone A (**3**): $C_{20}H_{26}O_{11}$; ESI-MS (positive mode) *m/z*: 465 $[M+Na]^+$, 907 $[2M+Na]^+$, 1349 $[3M+Na]^+$. Rhamnosyllactones $B_{1/2}$ (**4/5**): $C_{15}H_{22}O_8$; ESI-MS (positive mode) *m/z*: 353 $[M+Na]^+$, 683 $[2M+Na]^+$. FAB-MS (positive mode) *m/z*: 331 $[M+H]^+$. Other physico-chemical properties of rhamnopyranosides (**1** to **5**) are summarized in Tables 1 to 4.

Results and Discussion

Structure of 2-(1-Propen-1-yl)-4-hydroxymethyl-3-furanylcarbonyl α-L-Rhamnopyranoside (**1**)

The molecular formula was determined by HR-EIMS to be $C_{15}H_{20}O_8$ (Table 1). Its IR spectrum revealed the presence of hydroxyl (3393 cm^{-1}), carbonyl as well as olefinic groups (1710, 1652, 1426 and 1247 cm^{-1}). The UV spectrum showed absorption maxima at 211, 234 and 292 nm. These data indicated the presence of a conjugated ester and a furano-ring similar to 2,4-dimethyl-3-furanylcarbonyl α-L-rhamnopyranoside isolated from *Streptomyces griseoviridis* (Tü 3634)[4]. The presence of a 1-*O*-acyl α-rhamnose moiety was proven by comparison of the NMR-data (Table 2 and 3) of **1** with the literature[4,9,10]. In addition to the proton signals of the rhamnose moiety, the ^1H NMR (CD_3OD) spectrum of **1** presented proton signals of an *E*-configurated propenyl residue.

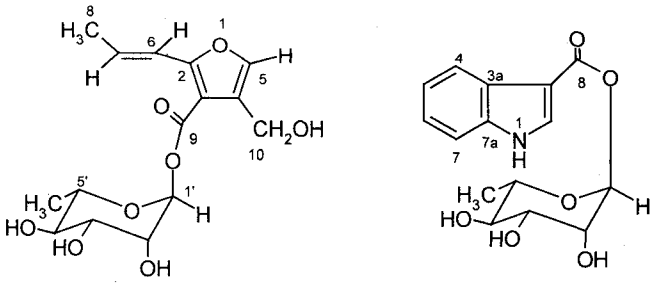
Table 1. Physico-chemical properties of **1**~**5**.

	1	**2**	**3**	**4 / 5**
Appearance	colorless powder (from $CHCl_3$-MeOH)	light yellowish powder (from $CHCl_3$-MeOH)	colorless oil	colorless oil
Molecular formula	$C_{15}H_{20}O_8$	$C_{15}H_{17}NO_6$	$C_{20}H_{26}O_{11}$	$C_{15}H_{22}O_8$
HR-MS m/z	found 328.1170[a] $C_{15}H_{20}O_8$ $[M]^+$ requires 328.1152 found 182.0569[a] $C_9H_{10}O_4$ $[M-C_6H_{10}O_4$ (aglycon)$]^+$ requires 182.0576	found 307.1062[a] $C_{15}H_{17}NO_6$ $[M]^+$ requires 307.1052 found 161.0474[a] $C_9H_7NO_2$ $[M-C_6H_{10}O_4$ (aglycon)$]^+$ requires 161.0475	found 443.1539[b] $[M+1]^+$ $C_{20}H_{27}O_{11}$ requires 443.1545	Found 331.1394[b] $[M+1]^+$ $C_{15}H_{23}O_8$ Requires 331.1386
MP (°C)	101-102	132-133		
$[\alpha]_D^{25}$	- 49.1° (*c* 0.549, MeOH)	- 36.8° (*c* 0.101, MeOH)	+ 34.7° (*c* 0.196, MeOH)	+61.8° (*c* 0.434, MeOH)
UV λ_{max} (MeOH) nm (logε)	211 (3.34), 234 (sh, 1.10), 292 (3.87)	211 (3.45), 227 (sh, 1.56), 284 (0.97)	204 (sh, 2.78), 224 (3.41), 292 (0.45)	201 (3.24), 279 (3.59)
IR ν_{max} (KBr or film) cm^{-1}	3393 (br), 2978, 2935, 1710, 1652, 1532, 1426, 1302,1247, 1212	3413 (br), 3297 (br), 2910, 1671, 1527, 1442, 1245,	3407 (br), 2978, 2934, 1761, 1714, 1674, 1448, 1382, 1287, 1219	3388 (br), 2975, 2935, 1727, 1645, 1612, 1446, 1380, 1278, 1221
TLC silica gel (R_f) *Solvent system*				
$CHCl_3$-MeOH (9:1)	0.25	0.16	0.22	0.15
$CHCl_3$-MeOH (7:3)	0.80	0.70	0.78	0.74
Ethyl acetate/MeOH (4:1)	0.77	0.72	0.63	0.57
n-BuOH/HOAc/H_2O (4:1:5, upper layer)	0.81	0.85	0.53	0.64
Staining reagent[c]				
Ehrlich's reagent[d]	grey-purple	grey-purple	grey-purple	grey-purple
Anisaldehyde/H_2SO_4	dark-green	yellow-brown	red-purple	orange
Orcinol reagent	black	grey-black	grey-black	grey-black
Blue tetrazolium	light grey	light grey	n.c.	n.c.
2-Naphthol reagent	brown	brown	brown	brown

[a] HREI-MS; [b] HRFAB-MS; [c] After spraying the plates were heated at 120°C for 5 minutes; [d] On a yellow background; n.c. No colorization.

The structure elucidation of **1** was based on 2D-NMR data analysis. In the ^1H-^1H COSY NMR spectrum a long range allylic coupling was observed between the olefinic proton H-5 and H-10 giving broadened singlets at δ 7.39 and 4.64, respectively, and indicates a $CH=C-CH_2OH$ spin system. One bond proton-carbon connectivities were determined by a heteronuclear single quantum coherence (HSQC) NMR experiment, the long range proton-carbon couplings by a heteronuclear multiple bond correlation (HMBC) NMR experiment. The aglycone of **1** does not possess stereogenic centers, so the sign of the optical rotation from **1** depends only on the α-rhamnose moiety.

Fig. 1. The structures of 1-*O*-acyl α-L-rhamnopyranosides **1** and **2** (type I).



1 **2**

The numeration of the carbon atoms is not in accordance with the IUPAC rules but allows a better comparison of the described and previously reported compounds to each other.

Thus, the L-configuration of the α-rhamnose in **1** was deduced by the negative optical rotation value of **1** ($[\alpha]_D^{25}=-49.1°$) in comparison with the literature[4]. Therefore, **1** belongs to the family of 1-*O*-acyl α-L-rhamnopyranosides[3,4].

Structure of 3-Indolylcarbonyl α-L-Rhamnopyranoside (**2**)

The molecular formula $C_{15}H_{17}NO_6$ was determined by HR-EIMS (Table 1) and supported by its ESI mass spectrum. The ^1H and ^{13}C NMR (CD_3OD) chemical shift assignments (Table 2 and 3) were done *via* 1D- and 2D-NMR which showed the aglycone of **2** to be indole-3-carboxylic acid by the number, multiplicity, and the chemical shifts of the aromatic proton and carbon atoms. In our previous paper we already reported the structure elucidation of 2-indolylcarbonyl α-L-rhamnopyranoside[9]. The main differences in the ^{13}C-NMR-data of both of these compounds can be observed in the chemical shifts of C-2 ($\Delta\delta$=6.2 ppm), C-3 ($\Delta\delta$=2.4 ppm) and C-8 ($\Delta\delta$=3.5 ppm). All the other chemical shift differences are only about ($\Delta\delta$=0~1.4 ppm) being an additional proof for the structure of **2**. Similar to **1**, the chemical shifts of the sugar signals and the small coupling constant (J=1.8 Hz) of the anomeric proton clearly indicate an α-rhamnopyranoside moiety in compound **2**. In accordance to compound **1**, the L-configuration was deduced by its negative optical rotation value ($[\alpha]_D^{25}=-36.8°$)[4,9,10]. The long range HMBC showed a cross-peak between the anomeric H-1′ (δ 6.21) and C-8 (δ 164.91) which revealed the structure of **2** to be 3-indolylcarbonyl α-L-rhamnopyranoside (Fig. 1).

Structure of Rhamnosyllactone A (**3**)

The molecular formula $C_{20}H_{26}O_{11}$ was determined by HRFAB-MS and supported by its ESI mass spectra (Table 1) together with its ^1H and ^{13}C NMR data (Table 4). The IR spectrum contained absorption bands due to hydroxy, ketone, γ-lactone, and α,β-unsaturated-γ-lactone functional groups (3407, 1761, 1714, 1674, 1448, 1219 cm^{-1}). In similarity to **1** and **2**, the ^1H and ^{13}C NMR spectral data of **3** showed the presence of an α-rhamnose sugar moiety. In addition, the ^1H NMR (CD_3OD) spectrum presented a second methyl signal at δ 1.25 (3H, d), two methine signals at δ 3.95 (1H, m) and 2.73 (1H, m), as well as five non-equivalent methylene signals at δ 4.73 (1H, d)/4.74 (1H, d), 3.05 (1H, d)/3.16 (1H, d), 2.79 (1H, dd)/2.93 (1H, dd), 3.75 (1H, dd)/4.15 (1H, dd) and 4.11 (1H, dd)/4.47 (1H, dd). In addition to the six carbon atoms in the α-rhamnose moiety, the ^{13}C and DEPT NMR (CD_3OD) spectra displayed the signals of 14 carbon atoms including one methyl (δ 20.73), five methylene (δ 71.39, 69.10, 64.99, 46.90, 29.33), two methine (δ 72.54, 45.21), one sp^3 quaternary (63.91), two sp^2 quaternary (δ 141.10, 139.99) and three carbonyl (δ 203.78, 176.61, 169.76) carbon atoms.

The structure elucidation of **3** was based on 2D-NMR analysis. Careful examination of a ^1H-^1H COSY NMR spectrum resulted in the location of the corresponding vicinal protons in the sugar moiety *via* cross peaks. In addition, the ^1H-^1H COSY NMR spectrum showed the presence of two proton spin systems: $-CH_2CH(O)CH_3$ (H-6″, H-7″, H-9″), and $-O-CH_2CHCH_2-O-$ (H-3″, H-3″a, H-4″). One bond and long range proton-carbon connectivities were determined by a HSQC and a HMBC NMR experiment (Fig. 3), respectively. The HMBC cross-peak for the anomeric proton H-1′ at δ 5.98 with the corresponding carbon atom at δ 139.99 (C-3) revealed that the rhamnose moiety is connected to a sp^2 quarternary carbon through an *O*-glycosidic bond. Detailed analysis of the ^1H-^1H COSY, HSQC and HMBC spectra proved the structure of **3** [Fig. 2; 8a-[4-(α-rhamnopyranosyl)-2-oxo-2,5-dihydro-furan-3-ylmethyl]-6-methyl-tetrahydro-furo-[3,4-*c*]oxepine-1,8-dione]. The stereochemistry of **3** remains open.

Table 2. ^{1}H NMR data (δ values, CD_3OD) for compounds **1**, **2**, **4** and **5***.

Position	1[a]	2[b]	4[a]	5[a]
2		7.21 (1H, s, overlapped)		
4		8.04 (1H, dd, J = 2.1, 8.4)	3.45 (1H, m)	3.30 (1H, m)
5	7.39 (1H, brs)	7.20 (1H, m, overlapped)	4.32 (2H, dd, J = 7.3, 14.4)	4.27 (2H, dd, J = 7.4, 14.4)
6	6.85 (1H, dd, J = 1.8, 15.9)	7.22 (1H, m, overlapped)		
7	6.55 (1H, dq, J = 6.8, 15.9)	7.46 (1H, dd, J = 2.3, 8.5)	7.01 (1H, dd, J = 1.7, 16.0)	6.23 (1H, dd, J = 1.7, 15.4)
8	1.91 (3H, dd, J = 1.8, 6.8)		6.31 (1H, dq, J = 6.8, 16.0)	6.56 (1H, dq, J = 6.9, 15.4)
9			1.90 (3H, dd, J = 1.7, 6.8)	1.92 (3H, dd, J = 1.7, 6.9)
10	4.64 (2H, brs)		3.57 (1H, dd, J = 7.8, 10.7); 3.76 (1H, dd, J = 3.4, 10.7)	3.50 (1H, dd, J = 7.8, 10.7); 3.61 (1H, dd, J = 3.4, 10.7)
1'	6.15 (1H, d, J = 1.8)	6.21 (1H, d, J = 1.8)	5.35 (1H, d, J = 1.8)	5.29 (1H, d, J = 1.8)
2'	3.92 (1H, dd, J = 1.8, 3.4)	3.97 (1H, dd, J = 1.8, 3.5)	4.05 (1H, dd, J = 1.8, 3.4)	4.14 (1H, dd, J = 1.8, 3.4)
3'	3.80 (1H, dd, J = 3.4, 9.5)	3.91 (1H, dd, J = 3.5, 9.5)	3.75 (1H, dd, J = 3.4, 9.4)	3.95 (1H, dd, J = 3.4, 9.5)
4'	3.49 (1H, dd, J = 9.5, 9.5)	3.51 (1H, dd, J = 9.5, 9.5)	3.47 (1H, dd, J = 9.5, 9.5)	3.43 (1H, dd, J = 9.5, 9.5)
5'	3.69 (1H, dq, J = 6.2, 9.5)	3.81 (1H, dq, J = 6.2, 9.5)	3.72 (1H, dq, J = 6.2, 9.4)	3.69 (1H, dq, J = 6.2, 9.5)
6'	1.26 (3H, d, J = 6.2)	1.28 (3H, d, J = 6.2)	1.28 (3H, d, J = 6.2)	1.22 (3H, d, J = 6.2)

* Assignments made by a combination of 1D (^{1}H, ^{13}C, DEPT) and 2D-NMR techniques (^{1}H-^{1}H COSY, HSQC, HMBC); J values in Hz; [a] ^{1}H NMR spectrum was recorded at 500 MHz on a Bruker DRX500 spectrometer; [b] ^{1}H NMR spectra were recorded at 300 MHz on a Bruker DPX300 spectrometer.

Structure of Rhamnosyllactones $B_{1/2}$ (**4**/**5**)

The mixture of **4** and **5** shows identical FAB- and ESI-MS spectra. With a HRFAB-MS the molecular formula of both compounds were determined as $C_{15}H_{22}O_8$ (Table 1). The signals of each proton and carbon atom emerged as a pair in the ^{1}H and ^{13}C NMR (CD_3OD) spectra, obviously due to the presence of two stereo-isomers of a given carbon skeleton. According to the integrals of the proton signals **4** is the main component (ratio **4**/**5**=5 : 1). The NMR data (Table 2 and 3) appeared to be similar to those of **1** also indicating an α-rhamnose moiety and a CH_3CH=CH (E) double bond.

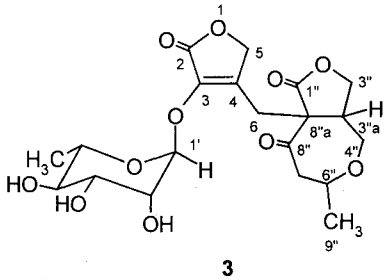
The ^{1}H-^{1}H COSY NMR showed two proton spin systems for each compound. First the α-rhamnose moiety can be clearly deduced for **4** and **5** by analyzing the chemical shifts and coupling pattern of H-1′ to H-6′. Compound **4**: –O–CH_2CHCH_2–O– (H-5: δ 4.32, H-4: δ 3.45, H-10: δ 3.57/3.76), compound **5**: H-5: δ 4.27, H-4: δ 3.30, H-10: δ 3.50/3.61, as well as compound **4**: CH_3CH=CH– (H-7: δ

Table 3. ^{13}C NMR data (δ values, CD_3OD) for compounds **1**, **2**, **4** and **5***.

Position	**1**[a] (DEPT)	**2**[b] (DEPT)	**4**[a] (DEPT)	**5**[a] (DEPT)
2	160.15 (C)	134.04 (CH)	173.11 (C)	171.38 (C)
3	111.06 (C)	107.76 (C)	110.79 (C)	109.91 (C)
3a		127.60 (C)		
4	128.24 (C)	123.94 (CH)[c]	42.63 (CH)	42.95 (CH)
5	140.75 (CH)	121.79 (CH)[c]	69.35 (CH_2)	69.12 (CH_2)
6	120.28 (CH)	122.85 (CH)[c]	163.75 (C)	161.23 (C)
7	133.29 (CH)	113.17 (CH)	123.14 (CH)	125.91 (CH)
7a		138.00 (C)		
8	18.91 (CH_3)	164.91 (C)	137.32 (CH)	138.86 (CH)
9	163.41 (C)		18.74 (CH_3)	18.69 (CH_3)
10	57.22 (CH_2)		63.32 (CH_2)	64.19 (CH_2)
1'	95.75 (CH)	94.76 (CH)	102.47 (CH)	104.20 (CH)
2'	71.30 (CH)	71.64 (CH)	71.85 (CH)	71.95 (CH)
3'	72.46 (CH)	72.40 (CH)	72.22 (CH)	72.03 (CH)
4'	73.35 (CH)	73.66 (CH)	73.36 (CH)	73.48 (CH)
5'	72.86 (CH)	72.56 (CH)	72.48 (CH)	72.48 (CH)
6'	18.10 (CH_3)	18.13 (CH_3)	18.10 (CH_3)	17.99 (CH_3)

* Assignments made by a combination of 1D (^1H, ^{13}C, DEPT) and 2D-NMR techniques (^1H-^1H COSY, HSQC, HMBC); [a] ^{13}C NMR spectrum was recorded at 125 MHz on a Bruker DRX500 spectrometer; [b] ^{13}C NMR spectra were recorded at 75 MHz on a Bruker DPX300 spectrometer. [c] Assignments may be interchanged.

Fig. 2. The structures of rhamnosylated lactones **3** to **5** (type II).



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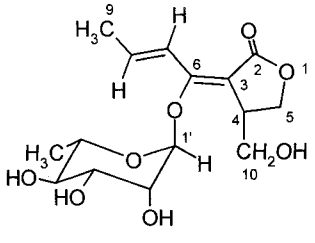
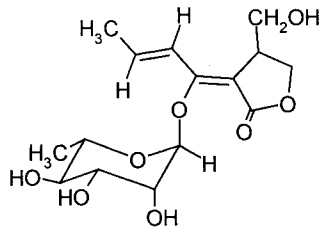


4



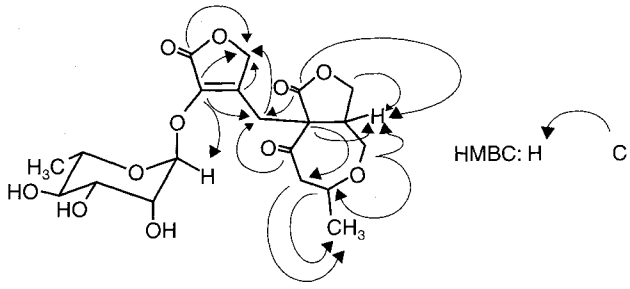
5

The numeration of the carbon atoms is not in accordance with the IUPAC rules but allows a better comparison of the described and previously reported compounds to each other.

7.01, H-8: δ 6.31, H-9: δ 1.90) and compound **5**: H-7: δ 6.23, H-8: δ 6.56, H-9: δ 1.92. A HSQC and a HMBC NMR experiment revealed the structures to be **4** and **5** as shown in Fig. 2. HMBC cross-peaks were observed for **4** between the anomeric H-1′ at δ 5.35 and C-6 (δ 163.75), and between H-1′ at δ 5.29 and C-6 (δ 161.23) for **5**, which supported that the rhamnose moiety is connected to a sp^2 quaternary carbon through an *O*-glycosidic bond in **4** and **5**. Thus, the structure of **4**/**5** is 3-(6-α-rhamnopyranosyl-but-7-enylidene)-4-hydroxymethyl-dihydro-furan-2-one.

We assume that compounds **4** and **5** are not stereoisomers in the configuration at C-4 but of the 3,6-double bond because of the difference in the chemical shifts

Fig. 3. Significant long-range correlations observed in the ^{1}H-^{13}C HMBC $^nJ_{C-H}$ (n=2 and 3) spectrum of **3** in CD_3OD. All other J_{C-H} correlations are omitted for reasons of clarity.



3

Table 4. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR data (δ values, CD_3OD) for compound **3***.

Position	^{1}H	^{13}C (DEPT)
2		169.76 (C)
3		139.99 (C)
4		141.10 (C)
5	4.73 (1H, d, J = 17.5 Hz)	71.39 (CH_2)
	4.74 (1H, d, J = 17.5 Hz)	
6	3.05 (1H, d, J = 14.8 Hz)	29.33 (CH_2)
	3.16 (1H, d, J = 14.8 Hz)	
1'	5.98 (1H, d, J = 1.8 Hz)	100.46 (CH)
2'	4.03 (1H, dd, J = 1.8, 3.4 Hz)	71.38 (CH)
3'	3.77 (1H, dd, J = 3.4, 9.5 Hz)	71.99 (CH)
4'	3.45 (1H, dd, J = 9.5, 9.5 Hz)	73.31 (CH)
5'	3.63 (1H, dq, J = 6.2, 9.5 Hz)	72.01 (CH)
6'	1.24 (3H, d, J = 6.2 Hz)	18.01 (CH_3)
1''		176.61 (C)
3''	4.11 (1H, dd, J =5.7, 9.7 Hz)	69.10 (CH_2)
	4.47 (1H, dd, J = 7.4, 9.7 Hz)	
3''a	2.73 (1H, m)	45.21 (CH)
4''	3.75 (1H, dd, J = 8.4, 13.8 Hz)	64.99 (CH_2)
	4.15 (1H, dd, J = 4.6, 13.8 Hz)	
6''	3.95 (1H, m)	72.54 (CH)
7''	2.79 (1H, dd, J = 6.1, 12.4 Hz)	46.90 (CH_2)
	2.93 (1H, dd, J = 6.0, 12.4 Hz)	
8''		203.78 (C)
8''a		63.91 (C)
9''	1.25 (3H, d, J = 6.3 Hz)	20.73 (CH_3)

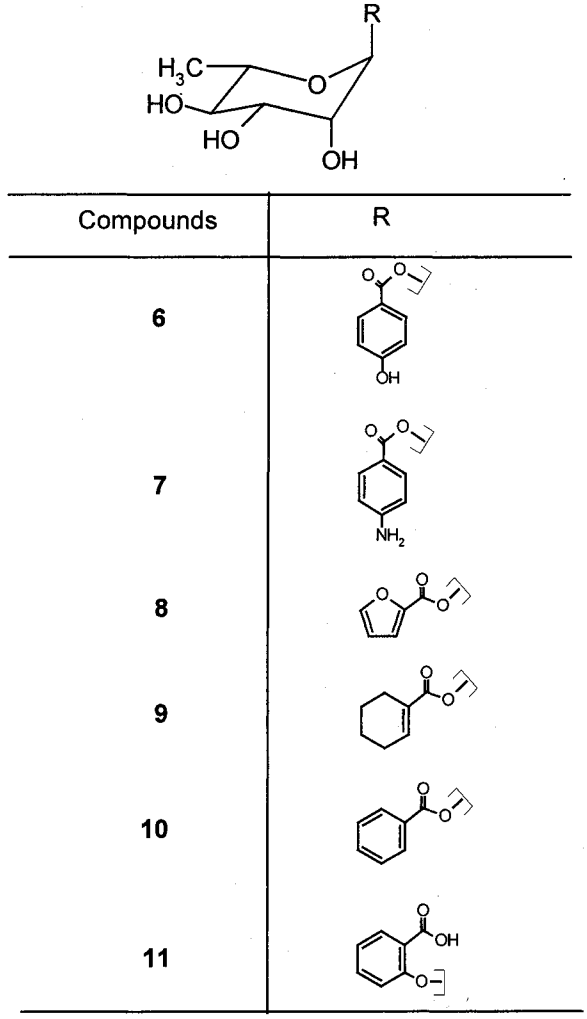
* Assignments made by a combination of 1D (^{1}H, ^{13}C, DEPT) and 2D-NMR techniques (^{1}H-^{1}H COSY, HSQC, HMBC).

Table 5. Inhibitory potencies of the rhamnopyranosides **1**~**11** on 3α-hydroxysteroid-dehydrogenase (3α-HSD) activity.

Compound	Inhibition (%)
1	41
2	0
3	0
4/5	33
6	36
7	35
8	35
9	40
10	44
11	58
Indomethacin	17

Legend: Inhibition of 5β-dihydrocortisone reduction catalyzed by rat liver cytosol-3α-HSD compared to those in controls (0% inhibition); according to the work of PENNING *et al.*[8]. All compounds are tested in a concentration of 3.0 μg/ml.

Fig. 4. The structures of the rhamnopyranosides **6**~**11** produced by *Streptomyces* sp.[4,9].



of the proton H-7 for **4** and **5**. In compound **4** the signal is shifted to a lower field (δ 7.01) in comparison to **5** (δ 6.23), while the differences of the chemical shifts of the other protons are not significant. Obviously, the reason for the dramatic shift difference is the anisotropic effect of the carbonyl group at C-2. This effect can only influence the proton H-7 in the given *E*/*E* configuration (**4**), but not in *E*/*Z* orientation (**5**). Unfortunately, a NOESY NMR experiment could not confirm this argumentation because a NOE (=nuclear overhauser effect) was not observable between H-7 and H_2-10, neither in **4** nor in **5**.

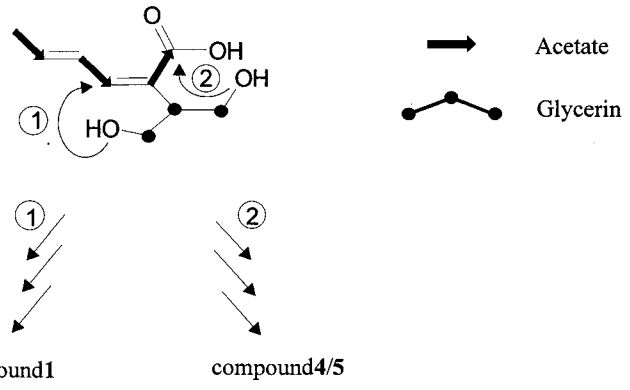
Biological Activity of the Rhamnopyranosides

3α-Hydroxysteroid-dehydrogenase (3α-HSD) is involved in the arachidonic acid biosynthesis and therefore is a useful target in the search for antiinflammatory and antiphlogistic compounds[8]. The new metabolites **1** and **4/5** were found to be inhibitors as shown in Table 5. In addition, we also observed an inhibitory effect for the α-L-rhamnopyranosides **6** to **11**[4,9].

Conclusion

We obtained five new α-L-rhamnopyranosides as secondary metabolites from *Streptomyces* sp. (strain GT 61150). The compounds **1**~**5** belong to two different classes of rhamnosides: type I) acyl α-L-rhamnopyranosides (**1** and **2**) and, type II) α-L-rhamnopyranosyllactones lacking the ester linkage where the rhamnoside is linked by an *O*-glycosidic bond to the aglycones (**3** to **5**). The anomeric H-1′ of 1-*O*-acyl rhamnopyranosides (type I) are found at the lower fields in the ^{1}H NMR spectra (**1**: δ 6.15; **2**: δ 6.21) in contrast to the

Fig. 5. Proposed biogenetic pathways for the aglyca of **1** and **4/5**.



compounds of type II (**3**: δ 5.98, **4**: δ 5.35, **5**: δ 5.29). On the other hand, the corresponding anomeric carbon C-1′ of type I substances shows resonances at higher fields (**1**: δ 95.75; **2**: δ 94.76) in comparison to **3** (δ 100.46), **4** (δ 102.47) and **5** (δ 104.20). The sign of the optical rotation of **1** and **2** is only from the α-rhamnose contribution, and not from the achiral aglycones. Thus, the L-stereochemistry in the sugar can be deduced by the negative optical rotation values known for α-L-rhamnose (Table 1). Because of the plausibility of an analogous enzymatic rhamnosylation pathway in strain GT 61150, we assume that the stereochemistry of the rhamnose moiety in all compounds described above is identical. However, there exists no prove of this assumption for **3** to **5**. Furthermore, it seems possible that compounds **1**, **4** and **5** have a common biosynthetic precursor comparable with the biosynthesis of furanylcarbonyl rhamnosides and butanolides known from the literature[4]. These precursors probably form either a lactone ring (in **4** and **5**), or a furan ring (in **1**) in dependence on the hydroxy group of the glycerin unit that is involved in the ring closure reaction (Figure 5). The compounds **1**~**5** can be classified by their possible biosynthetic origin. a) **1** is the main product with a C-15 skeleton, b) **4** and **5** also contain a C-15 backbone and seem to be biosynthetically related to **1**, c) **3** contains additional structural elements with a so far unknown biogenesis and d) **2** is a side product which documents the rhamnosylation activity of the strain GT 61150.

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