DOI: 10.1002/ejoc.200600221

6-Deoxy-α-L-talopyranosides from *Streptomyces* sp.

Jens Bitzer[a] and Axel Zeeck*[a]

Dedicated to Professor Hartmut Laatsch on the occasion of his 60th birthday

Keywords: Carbohydrates / 6-Deoxy-α-L-talopyranosides / Deoxysugars / Natural products / Precursor-directed biosynthesis / Streptomyces

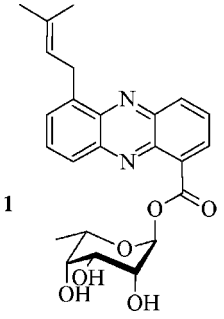
Streptomyces sp. strain GöM1 was found to produce seven novel glycosides (**2**–**8**) containing the rare deoxysugar 6-deoxy-α-L-talose. The aglycones are small phenols, isovaleric acid or aromatic carboxylic acids. By precursor-directed biosynthesis, the yields of the compounds could be increased significantly. Feeding of 4-hydroxybenzoic acid led to the production of both acyl and aryl glycosides, and of compound **9** with both structural elements. Pyrrol-2-ylcarbonyl 6-deoxy-α-L-talopyranoside (**6**) shows remarkable growth inhibition of some parasites.

Introduction

6-Deoxyhexoses are essential building blocks of many secondary metabolites. Usually they are linked as *O*-glycosides to an aglycon and are intrinsically associated with the biological activity of the resulting metabolite.[1] 6-Deoxysugars are derived from D-glucose by well-known biosynthetic pathways, during which epimerization of distinct carbon atoms takes place.[2] The most common 6-deoxyhexoses in nature are fucose and rhamnose.[3]

Various strains of streptomycetes are known to produce acyl α-L-rhamnopyranosides.[4–6] The aglycones mostly are single aromatic carboxylic acids, linked via an ester bond to the anomeric hydroxy group of the deoxysugar. The biological function of the acyl rhamnopyranosides remains unclear. Some of them inhibit the enzyme 3α-hydroxysteroid-dehydrogenase, which is involved in inflammation pathways.[6] Acyl pyranosides are discussed as energy rich intermediates, which enable acyl transfers, because the level of activation is similar to that of acyl-CoA thioesters.[7]

Unlike fucose and rhamnose, 6-deoxytalose is a rare deoxyhexose.[3] Among more than 30,000 microbial secondary metabolites, only phenazoviridin (**1**), a phenazine derivative from *Streptomyces* sp.,[8] is described in the literature as a 6-deoxy-α-L-talopyranoside. A better source are the strophantidines, a family of heart glycosides isolated from African medical plants of the genus *Strophantus*,[9] and some glycolipids from mycobacteria, pseudomonae, and rhodopseudomonae.[10] The enantiomer 6-deoxy-D-talose is found in lipopolysaccharides (LPS) of the cell wall of some Gram-negative bacteria[11] and in glycolipids of mycobacteria.[12]



In our work, we describe the isolation, structure elucidation, and biological activities of novel acyl and aryl 6-deoxy-α-L-talopyranosides. Precursor-directed biosynthesis was used to increase the amount of the native glycosides and to generate analogues by taking advantage of the poor specificity of the enzyme which performs the talosylation.

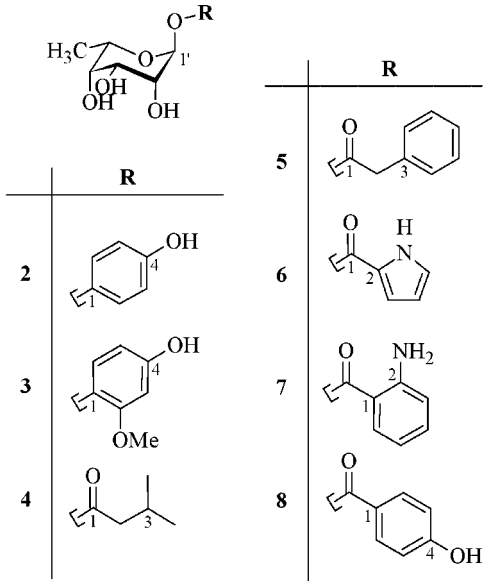
Results and Discussion

Production and Isolation

Streptomyces sp. strain GöM1 was cultivated in a medium based on malt extract and glucose. The culture filtrate contained seven metabolites giving an intense brown or black colour reaction on a TLC plate after staining with orcin. Column chromatography and preparative HPLC led to the pure compounds **2**–**8** with yields of 19 mg/ L (com-

[a] Institute of Organic and Biomolecular Chemistry, University of Göttingen,
Tammannstr. 2, 37077 Göttingen, Germany
Fax: +49-551-3912593
E-mail: azeeck@gwdg.de
Supporting information for this article is available on the WWW under http://www.eurjoc.org or from the author.

pound **5**), 6 mg/ L (compounds **2** and **4**) and 0.25–2 mg/ L (compounds **3** and **6**–**8**).



Structure Elucidation of the Sugar Unit

The NMR spectra indicated that all compounds contained the same sugar unit. Its structure elucidation is representatively discussed for compound **2**. The ^{1}H NMR spectrum (Figure 1) exhibited the signals of one methyl group (δ_H = 1.21) and four oxygen-bound methine groups (δ_H = 3.65, 3.86, 3.90, 4.01). Another methine group at δ_H = 5.33 was identified as an anomeric centre by its ^{13}C chemical shift (δ_C = 102). These groups were mapped by ^{1}H,^{1}H-COSY correlations (Figure 2) to a hexose bearing a methyl group in position 6′. NOE correlations between 3′-H and 5′-H indicated an axial position of these protons. As no diaxial $^3J_{HH}$ couplings were observed (all coupling constants are smaller than 6.5 Hz), both 2′-H and 4′-H are in equatorial positions. This was supported by a W-coupling between these protons ($^4J_{HH}$ = 1.5 Hz, see Figure 2). The geminal coupling constant of the anomeric centre ($^1J_{CH}$ = 170.9 Hz) established the α-configuration.[13] Thus, the sugar unit could be identified as 6-deoxy-α-talopyranoside.

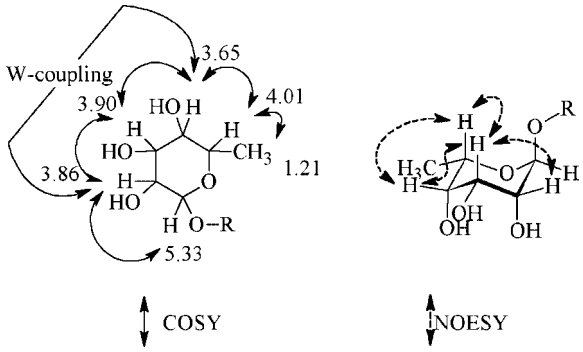


Figure 2. Important COSY and NOESY correlations of the 6-deoxytalopyranoside moiety in **2**. The numbers indicate the chemical shift in ppm.

Because the isolated compounds exhibit negative optical rotation values, their sugar units should have the same absolute stereochemistry. For determination, compound **8** was selected, because a larger amount of it (50 mg) could be subjected to acidic methanolysis. The resulting anomeric methyl 6-deoxytalopyranosides were separated and purified by silica gel chromatography. Comparison of the optical ro-

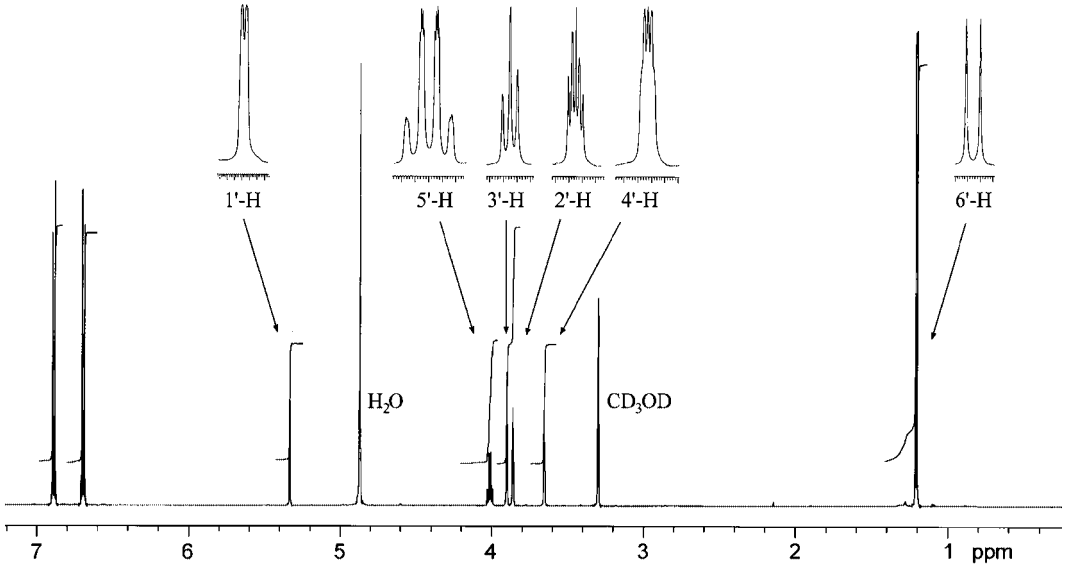


Figure 1. ^{1}H NMR spectrum of 4-hydroxyphenyl 6-deoxy-α-L-talopyranoside (**2**) with expansion of signals of the deoxyhexose (600 MHz, CD_3OD).

tation value in H_2O of the α-form ($[\alpha]_D^{20}$ = –98) with literature data[14] ($[\alpha]_D^{20}$ = –104) proved that the 6-deoxytalose belongs to the L-series. The β-anomer exhibits a more positive optical rotation value ($[\alpha]_D^{20}$ = +62 in MeOH), as predicted by Hudson's rule of isorotation.[15] The analysis agrees with the empirical Klyne rule,[16] which describes the observation that deoxyhexopyranosides of bacterial secondary metabolites occur either in the α-L- or in the β-D-form.

Structure Elucidation of the 6-Deoxy-α-L-talopyranosides

Apart from the signals of the deoxysugar, the ^{1}H NMR spectrum of compound **2** shows two aromatic 2H signals. Both appear as doublets ($^3J_{HH}$ = 9.0 Hz), indicating a *para*-substituted aromatic ring. Their upfield chemical shifts (δ_H = 6.70, 6.94) point to a twofold substitution with oxygen. Together with the molecular formula $C_{12}H_{16}O_6$, which was achieved from high resolution mass spectrometry, compound **2** could be identified as 4-hydroxyphenyl 6-deoxy-α-L-talopyranoside.

The aglycon of compound **3** exhibits three aromatic protons and an additional methoxy group (δ_H = 3.77). The latter is reflected in the molecular formula $C_{13}H_{18}O_7$ (*MW* = 286.28), which is 30 units higher than that of **2**. The substitution pattern of the aromatic system was deduced from COSY and 1D-NOESY correlations (Figure 3). The anomeric proton (δ_H = 5.23) is in spatial proximity to 6-H (δ_H = 6.87). The second aromatic proton (δ_H = 6.28) is in an *ortho* position ($^3J_{HH}$ = 8.5 Hz), and exhibits an additional *meta* coupling ($^4J_{HH}$ = 2.8 Hz) to 3-H (δ_H = 6.46). The methoxy group showed a NOESY correlation to 3-H but not to 5-H, hence compound **3** was determined as 4-hydroxy-2-methoxyphenyl 6-deoxy-α-L-talopyranoside.

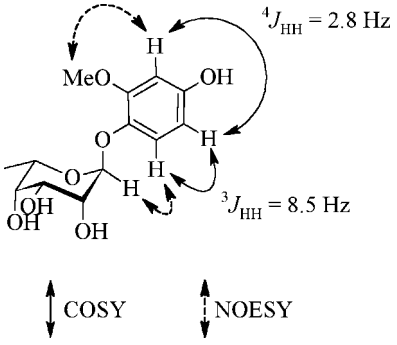
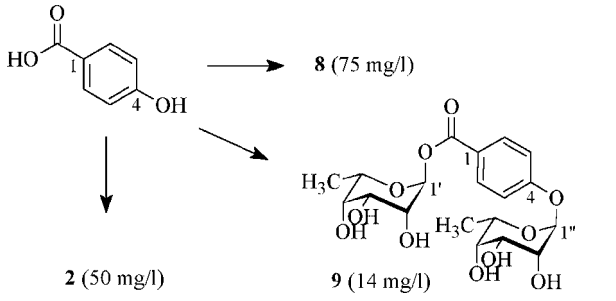


Figure 3. Selected COSY and NOESY correlations for compound **3**.

The ^{1}H NMR spectra of compounds **4**–**8** exhibit a remarkable downfield shift of the anomeric proton (δ_H = 6.00–6.24), typical for an esterification of the anomeric hydroxy group.[4,5] In agreement with this, the ^{13}C NMR spectra show in each case an ester carbonyl signal. The analysis of the NMR spectra in combination with the molecular formulae, which were established by HRESI-MS, confirm the structures of the natural products as isovaleryl, phenylacetyl, pyrrol-2-ylcarbonyl, 2-aminobenzoyl and 4-hydroxybenzoyl 6-deoxy-α-L-talopyranoside (**4**–**8**).

Precursor-Directed Biosynthesis

In the case of the well-known acyl α-L-rhamnopyranosides, precursor-directed biosynthesis proved to be a versatile technique for the production of novel glycosides and enhanced the yields of known metabolites.[5] Adopting this approach, aromatic carboxylic acids and a phenol were added as precursors to a growing culture of *Streptomyces* sp. strain GöM1. The production of both acyl and aryl glycosides was improved considerably. The addition of phenylacetic acid or pyrrole-2-carboxylic acid led to increased production of the 6-deoxy-α-L-talopyranosides **5** (from 19 to 60 mg/L) and **6** (from 1 to 33 mg/L), respectively. Supplementation of the culture medium with 4-hydroxybenzoic acid resulted in the corresponding acyl 6-deoxy-α-L-talopyranoside **8** (Scheme 1); its production was substantially enhanced from 0.5 mg/L up to 75 mg/L. Additionally, compound **9** (14 mg/L) with the deoxyhexose in both acyl and phenolic positions was found. The ^{1}H NMR spectrum showed, in addition to the signals of a *para*-disubstituted benzene, the presence of two units of the 6-deoxytalopyranoside. One of the anomeric protons has a downfield shift (δ_H = 6.24), indicating an ester bond (δ_C = 165.6). The other (δ_H = 5.65) is typical of a normal glycosidic linkage. High resolution mass spectrometry revealed the molecular formula $C_{19}H_{26}O_{11}$ and confirmed the structure as 6-deoxy-α-L-talopyranosyl 4-(6-deoxy-α-L-talopyranosyloxy)benzoate (**9**). Surprisingly, a third metabolite (50 mg/L) was isolated from this feeding experiment and identified as compound **2**. Obviously, the precursor is partly transformed into hydroquinone before or after glycosylation of the phenolic hydroxy group. A similar transformation of 4-hydroxybenzoic



Scheme 1. 6-Deoxy-α-L-talopyranosides produced by addition of 4-hydroxybenzoic acid as precursor.

Table 1. Cytotoxic activity of compound **6** against different human tumour cell lines: HM02 (gastric adenocarcinoma), HepG2 (hepatocellular carcinoma), and MCF7 (breast adenocarcinoma). Actinomycin D was used as reference. The GI_{50} and TGI values are given in μg/mL.

Cell line	HM02		HepG2		MCF7	
	GI_{50}[a]	TGI	GI_{50}	TGI	GI_{50}	TGI
6	0.68	1.0	1.8	5.5	1.3	2.4
Actinomycin D	0.002	0.008	0.0015	0.065	0.0024	0.011

[a] GI50 is the concentration at which half of the cells were inhibited in their growth; TGI is the concentration at which a total inhibition of cell growth was observed.

acid is known from plants, e.g. during the biosynthesis of shikonin.[17]

Biological Activity

The 6-deoxy-α-L-talopyranosides **2**–**9** showed no antimicrobial activity. Compound **6** was the only metabolite which exhibited a weak cytotoxicity (Table 1); additionally it was active against the parasites *Plasmodium falciparum* (IC_{50} = 0.61 μg/L) and *Trypanosoma brucei rhodesiens* (IC_{50} = 2.68 μg/L).

Conclusion and Discussion

6-Deoxy-L-talose is a rare deoxyhexose. Seven novel acyl and aryl α-glycosides (**2**–**8**) containing this sugar moiety were obtained from *Streptomyces* sp. (strain GöM1) when cultivated under standard conditions. Supplementation of the culture medium with aromatic carboxylic acids and phenols led to an enhanced production of the respective glycosides and the formation of the novel diglycosylated compound **9**.

The glycosylated phenylacetic acid **5** (19 mg/L) is the main compound of our strain. The same result is known from the production of acyl rhamnopyranosides by *Streptomyces griseoviridis* (strain Tü 3634). This is a hint that phenylacetic acid is a common intermediate, which is scavenged by the talosylation system. Strain Tü 3634 further produces acyl rhamnosides that are analogues of the acyl 6-deoxytalopyranosides **6**–**8**.[4,5] In contrast, no rhamnosides similar to the aryl glycosides **2** and **3** are observed. In both cases, the glycosylation of aromatic carboxylic acids is preferred and gives insight into the appearance of these acids during the cultivation of the strains. The low selectivity concerning the aglycon goes along with a high specificity for the 6-deoxyhexose; no glycosides other than 6-deoxytalopyranosides have been detected. In conclusion, strain GöM1 proved to be a valuable source of the rare deoxysugar 6-deoxy-L-talose and the respective glycosides. Its powerful talosylation activity should be useful for selective biotransformations of various aglycones, thereby altering their solubility and biological activity.

Experimental Section

General Experimental Procedures: NMR spectra were recorded in CD_3OD with Varian Inova 600, Varian Unity 300, and Varian Mercury 300 spectrometers at 298 K. Chemical shifts were determined relative to the solvent as internal standard (δ_C = 49.00, δ_H = 3.30). Assignments of the ^{13}C NMR resonances were done by HSQC spectra and chemical shift analysis. $^1J_{CH}$ coupling constants were obtained from coupled ^{13}C NMR spectra or coupled HSQC experiments. Optical rotation values were measured with a Perkin–Elmer 241 polarimeter. UV and CD spectra were obtained in methanol with a Varian Cary 3E and a Jasco J-500 spectrometer, respectively. Infrared spectra were recorded with a Perkin–Elmer FTIR 1600 spectrometer as KBr pellets. EI-MS spectra were obtained with a Finnigan MAT 95 (70 eV, relative intensities in parenthesis) spectrometer, ESI-MS spectra with a Finnigan LC-Q spectrometer, and high resolution ESI-MS spectra with a Bruker Apex-Q III (field strength 7 Tesla) spectrometer. TLC was carried out on silica gel 60 F_{254} plates (Merck, 0.2 mm); staining reagent: 100 mL orcinol solution (6% in ethanol) combined with a solution of 1 g $FeCl_3$ in 100 mL 2 N sulfuric acid. Column chromatography was performed on silica gel <0.08 mm (Macherey–Nagel), Sephadex® LH-20 (Sigma–Aldrich). Preparative HPLC was performed with a Jasco PU-1587 pump and a Jasco UV-1575 UV detector using a Nucleodur 100 C18 (5 μm, 250×16 mm, endcapped, Macherey–Nagel) column and a mobile phase of H_2O/CH_3CN including 0.1% TFA; detection was achieved at 254 nm.

Biological Material and Fermentation: The producing microorganism *Streptomyces* sp. strain GöM1 was isolated from a sand sample. It has been classified as a novel species of streptomycetes by morphological and physiological data as well as 16SrRNA gene sequencing.[18] Strain GöM1 was maintained on agar plates containing malt extract (1%), D-glucose (0.4%), and yeast extract (0.4%). Fermentations were carried out in 300 mL Erlenmeyer flasks containing three baffles and filled with 100 mL medium consisting of oatmeal (2%) and trace element solution (0.25%) in deionized water. The pH was 7.0 prior to sterilization. The trace element solution contained $CaCl_2 \cdot 2H_2O$ (3 g/L), Fe^{III}-citrate (1 g/L), $MnSO_4$ (0.2 g/L), $ZnCl_2$ (0.1 g/L), $CuSO_4 \cdot 5H_2O$ (25 mg/L), $Na_2B_4O_7 \cdot 10H_2O$ (20 mg/L), $CoCl_2$ (4 mg/L), and $Na_2MoO_4 \cdot 2H_2O$ (10 mg/L). The flasks were inoculated with a 1 cm^2 piece of agar plate and cultivated for 72 h at 28 °C on a rotary shaker (180 rpm). At harvest time, the cultures had become slightly yellow and the pH was 4.7.

Sample Workup and Isolation: 2.0 litres of fermentation broth were centrifuged (4500 rpm, 10 min). The mycelium was discarded, and the supernatant was adsorbed on Amberlite® XAD-2 (500 mL). After washing with water (500 mL), the metabolites were eluted with methanol (1000 mL). The solvent was removed by evaporation. The residue was purified by chromatography on silica gel (column 25×3 cm, CH_2Cl_2/MeOH, gradient 0–25% MeOH), yielding two fractions, A and B. Fraction A was separated into A1–A3 by gel permeation chromatography (column 100×2.5 cm, acetone). Further purification of fractions A1 and A2 by repetition of the gel permeation chromatography yielded **4** (12 mg) and **5** (38 mg). Fraction A3 was applied to preparative HPLC. A gradient elution from 40% to 75% CH_3CN in 25 min led to **7** (2 mg). Fraction B was further purified by gel permeation chromatography (column 100×2.5 cm, methanol). Separation was achieved by preparative HPLC; a gradient elution from 10% to 20% CH_3CN led to recovery of **2** (12 mg), **3** (2 mg), **6** (1.2 mg), and **8** (0.5 mg).

Precursor-Directed Biosynthesis: Feeding experiments were carried out in a 1-litre stirred vessel bioreactor (Biostat M, Braun-Diessel, Melsungen, Germany). The precursor (7 mmol, 994 mg of phenylacetic acid, 811 mg of pyrrole-2-carboxylic acid, and 1008 mg of 4-hydroxybenzoic acid, respectively) was dissolved in sterile water (50 mL). The solution was adjusted to pH 7 and added continuously for twelve hours to the growing culture, starting from the 13th hour of incubation. The cultures were harvested after 72 hours and worked up as described before. Yields are given in the text or are shown in Scheme 1.

Biological Tests: For plate diffusion assays, the desired compound (50 μg) was dissolved in methanol and dropped on paper disks (Ø 6 mm, thickness 0.5 mm). These disks were dried under sterile conditions and put on agar plates inoculated with the test organism (*Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, and *Candida albicans*). The plates were cultivated at 37 °C (bacteria) or

25 °C (yeast) for 24 h. The cytotoxic activity was determined according to the NCI guidelines[19] with the human tumour cell lines HM02 (gastric adenocarcinoma), HepG2 (hepatocellular carcinoma) and MCF7 (breast adenocarcinoma). Antiparasitic activity was determined against *Plasmodium falciparum*, *Trypanosoma brucei rhodesiense*, *Trypanosoma cruzi*, and *Leishmania donovani* as described previously.[20]

4-Hydroxyphenyl 6-Deoxy-α-L-talopyranoside (2): Colourless solid. $C_{12}H_{16}O_6$ (256.25); R_f = 0.15 ($CHCl_3$/MeOH, 9:1); colour reaction with orcinol/H_2SO_4: brown. $[\alpha]_D^{20}$ = –108 (*c* = 1, MeOH). ^{1}H NMR (600 MHz, CD_3OD): δ = 1.21 (d, *J* = 6.5 Hz, 3 H, 6′-H_3), 3.65 (m, 1 H, 4′-H), 3.86 (ddd, *J* = 3.5, 2.0, 1.5 Hz, 1 H 2′-H), 3.90 (t, *J* = 3.5 Hz, 1 H, 3′-H), 4.01 (q, *J* = 6.5 Hz, 1 H, 5′-H), 5.33 (d, *J* = 2.0 Hz, 1 H, 1′-H), 6.70 (d, *J* = 9.0 Hz, 2 H, 3-H, 5-H), 6.89 (d, *J* = 9.0 Hz, 2 H, 2-H, 6-H) ppm. ^{13}C NMR: see Table 2; $^1J_{CH}$ at anomeric carbon atom = 170.9 Hz. IR (KBr): $\tilde{\nu}$ = 3367, 2962, 2928, 1510, 1474, 1444, 1379, 1327, 1219, 1102, 1034 cm^{-1}. UV/Vis (MeOH): λ_{max} (logε) = 223 (3.93), 286 (3.49) nm. CD (MeOH): λ_{max} ([Θ]) = 225 (–6800) nm. ESI-MS: pos. *m*/*z* = 279 $[M+Na]^+$, 525 $[2M+Na]^+$. HRESI-MS: *m*/*z* = 279.08371 (calcd. for $C_{12}H_{16}O_6Na$ $[M+Na]^+$: *m*/*z* = 279.08391, Δ 0.72 ppm).

Table 2. ^{13}C NMR spectroscopic data of the metabolites **2**–**6**, **8** and **9** in CD_3OD.

Atom	**2**[b]	**3**[a]	**4**[a]	**5**[b]	**6**[a]	**8**[b]	**9**[b]		
1′	101.7	103.3	95.8	96.2	95.7	96.2	96.4	1′′	100.2
2′	72.1	72.0	71.1	70.7	71.3	71.2	71.1	2′′	71.6
3′	67.2	67.2	67.1	67.0	67.2	67.3	67.3	3′′	67.1
4′	74.2	74.4	73.9	73.8	74.0	73.9	73.9	4′′	73.9
5′	68.9	69.2	70.9	70.8	70.9	71.0	71.1	5′′	69.6
6′	16.9	16.8	16.9	16.8	17.0	17.0	17.0	6′′	16.9
1	151.1	139.8	172.7	171.4	160.5	121.5	124.3		
2	119.2	153.3	44.1	42.1	122.7	133.1	132.8		
3	116.8	101.7	26.9	135.4	117.7	116.4	117.3		
4	151.7	155.3	22.6	130.3	111.1	164.1	162.8		
5	116.8	107.4	22.7	129.6	125.7	116.4	117.3		
6	119.2	121.9	–	128.2	–	133.1	132.8		
7	–	56.2	–	129.6	–	166.0	165.6		
8	–	–	–	130.3	–	–	–		

[a] 150.8 MHz. [b] 75.5 MHz.

4-Hydroxy-2-methoxyphenyl 6-Deoxy-α-L-talopyranoside (3): Colourless solid. $C_{13}H_{18}O_7$ (286.28); R_f = 0.17 ($CHCl_3$/MeOH, 9:1); colour reaction with orcinol/H_2SO_4: brown. ^{1}H NMR (600 MHz, CD_3OD): δ = 1.21 (d, *J* = 6.5 Hz, 3 H, 6′-H_3), 3.67 (m, 1 H, 4′-H), 3.77 (s, 3 H, OCH_3), 3.92 (t, *J* = 3.0 Hz, 1 H, 3′-H), 3.94 (ddd, *J* = 3.5, 2.0, 1.5 Hz, 1 H, 2′-H), 4.21 (q, *J* = 6.5 Hz, 1 H, 5′-H), 5.23 (d, *J* = 2.0 Hz, 1 H, 1′-H), 6.28 (dd, *J* = 8.5, 2.8 Hz, 1 H, 5-H), 6.46 (d, *J* = 2.8 Hz, 1 H, 3-H), 6.87 (d, *J* = 8.5 Hz, 1 H, 6-H) ppm. ^{13}C NMR: see Table 2; $^1J_{CH}$ at anomeric carbon atom = 174.0 Hz. IR (KBr): $\tilde{\nu}$ = 3420, 2935, 1684, 1608, 1509, 1458, 1437, 1384, 1301, 1212, 1163, 1103, 1029 cm^{-1}. UV/Vis (MeOH): λ_{max} (logε) = 220 (3.55), 282 (3.11) nm. CD (MeOH): λ_{max} ([Θ]) = 229 (–1800), 254 (400), 273 (–300) nm. ESI-MS: pos. *m*/*z* = 309 $[M+Na]^+$, 595 $[2M+Na]^+$. HRESI-MS: *m*/*z* = 309.09441 (calcd. for $C_{13}H_{18}O_7Na$ $[M+Na]^+$: *m*/*z* = 309.09447, Δ 0.06 ppm).

Isovaleryl 6-Deoxy-α-L-talopyranoside (4): Colourless oil. $C_{11}H_{20}O_6$ (248.27); R_f = 0.33 ($CHCl_3$/MeOH, 9:1); colour reaction with orcinol/H_2SO_4: brown-black. $[\alpha]_D^{20}$ = –84 (*c* = 1, MeOH). ^{1}H NMR (600 MHz, CD_3OD): δ = 0.96 (d, *J* = 6.8 Hz, 3 H, 4-H_3), 0.97 (d, *J* = 6.8 Hz, 3 H, 5-H_3), 1.22 (d, *J* = 6.5 Hz, 3 H, 6′-H_3), 2.07 (nonet, *J* = 6.8 Hz, 1 H, 3-H), 2.22 (dd, *J* = 14.0, 6.8 Hz, 1 H, 2-H_a), 2.25 (dd, *J* = 14.0, 7.5 Hz, 1 H, 2-H_b), 3.65 (m, 1 H, 4′-H), 3.67 (ddd, *J* = 3.5, 2.0, 1.5 Hz, 1 H, 2′-H), 3.76 (t, *J* = 3.5 Hz, 1 H, 3′-H), 3.95 (q, *J* = 6.5 Hz, 1 H, 5′-H), 6.03 (d, *J* = 2.0 Hz, 1 H, 1′-H) ppm. ^{13}C NMR: see Table 2. IR (KBr): $\tilde{\nu}$ = 3400, 2966, 2935, 1745, 1458, 1387, 1370, 1292, 1252, 1169, 1144, 1101, 1020 cm^{-1}. UV/Vis (MeOH): λ_{max} (logε) = 203 (3.15) nm. CD (MeOH): λ_{max} ([Θ]) = 213 (800), 247 (–500) nm. ESI-MS: pos. *m*/*z* = 271 $[M+Na]^+$, 519 $[2M+Na]^+$, neg. *m*/*z* = 495 $[2M-H]^-$. HRESI-MS: *m*/*z* = 271.11515 (calcd. for $C_{11}H_{20}O_6Na$ $[M+Na]^+$: *m*/*z* = 271.11521, Δ 0.22 ppm).

Phenylacetyl 6-Deoxy-α-L-talopyranoside (5): Colourless solid. $C_{14}H_{18}O_6$ (282.29); R_f = 0.43 ($CHCl_3$/MeOH, 9:1); colour reaction with orcinol/H_2SO_4: black-blue. $[\alpha]_D^{20}$ = –35 (*c* = 1, MeOH). ^{1}H NMR (300 MHz, CD_3OD): δ = 1.13 (d, *J* = 6.5 Hz, 3 H, 6′-H_3), 3.55 (m, 1 H, 4′-H), 3.63 (m, 1 H, 3′-H), 3.64 (m, 1 H, 2′-H), 3.68 (s, 2 H, 2-H_2), 3.69 (q, *J* = 6.5 Hz, 1 H, 5′-H), 6.00 (d, *J* = 2.0 Hz, 1 H, 1′-H), 7.28 (m, 5 H, 4-H–8-H) ppm. ^{13}C NMR: see Table 2; $^1J_{CH}$ at anomeric carbon atom = 175.2 Hz. IR (KBr): $\tilde{\nu}$ = 3412, 2976, 2933, 1745, 1456, 1384, 1248, 1124, 1102, 1020 cm^{-1}. UV/Vis (MeOH): λ_{max} (logε) = 206 (3.83) nm. CD (MeOH): λ_{max} ([Θ]) = 226 (4800) nm. ESI-MS: pos. *m*/*z* = 305 $[M+Na]^+$, 587 $[2M+Na]^+$. HRESI-MS: *m*/*z* = 305.09972 $[M+Na]^+$ (calcd. for $C_{14}H_{18}O_6Na$: *m*/*z* = 305.09956, Δ 0.52 ppm).

Pyrrol-2-ylcarbonyl 6-Deoxy-α-L-talopyranoside (6): Colourless solid. $C_{11}H_{15}NO_6$ (257.24); R_f = 0.19 ($CHCl_3$/MeOH, 9:1); colour reaction with orcinol/H_2SO_4: brown. $[\alpha]_D^{20}$ = –60 (*c* = 1, MeOH). ^{1}H NMR (600 MHz, CD_3OD): δ = 1.24 (d, *J* = 6.5 Hz, 3 H, 6′-H_3), 3.70 (m, 1 H, 4′-H), 3.79 (ddd, *J* = 3.5, 2.0, 1.5 Hz, 1 H, 2′-H), 3.97 (dd, *J* = 3.5, 3.0 Hz, 1 H, 3′-H), 4.07 (q, *J* = 6.5 Hz, 1 H, 5′-H), 6.19 (d, *J* = 2.0 Hz, 1 H, 1′-H), 6.21 (dd, *J* = 3.5, 2.5 Hz, 1 H, 4-H), 6.92 (dd, *J* = 3.5, 1.5 Hz, 1 H, 3-H), 7.01 (dd, *J* = 2.5, 1.5 Hz, 1 H, 5-H) ppm. ^{13}C NMR: see Table 2. IR (KBr): $\tilde{\nu}$ = 3432, 2927, 1699, 1684, 1636, 1410, 1384, 1309, 1206, 1123, 1076, 1024 cm^{-1}. UV/Vis (MeOH): λ_{max} (logε) = 231 (3.49), 267 (3.96) nm. CD (MeOH): λ_{max} ([Θ]) = 216 (–1000), 230 (1800) nm. ESI-MS: pos. *m*/*z* = 280 $[M+Na]^+$, 537 $[2M+Na]^+$. HRESI-MS: *m*/*z* = 280.07912 $[M+Na]^+$ (calcd. for $C_{11}H_{15}NO_6Na$: *m*/*z* = 280.07916, Δ 0.14 ppm).

2-Aminobenzoyl 6-Deoxy-α-L-talopyranoside (7): Colourless solid. $C_{13}H_{17}NO_6$ (283.28); R_f = 0.21 ($CHCl_3$/MeOH, 9:1); colour reaction with orcinol/H_2SO_4: brown-orange. ^{1}H NMR (600 MHz, CD_3OD): δ = 1.25 (d, *J* = 6.5 Hz, 3 H, 6′-H_3), 3.71 (m, 1 H, 4′-H), 3.82 (ddd, *J* = 3.5, 2.0, 1.5 Hz, 1 H, 2′-H), 3.93 (t, *J* = 3.0 Hz, 1 H, 3′-H), 4.06 (q, *J* = 6.5 Hz, 1 H, 5′-H), 6.24 (d, *J* = 2.0 Hz, 1 H, 1′-H), 6.56 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1 H, 5-H), 6.75 (ddd, *J* = 8.5, 1.0, 0.5 Hz, 1 H, 3-H), 7.25 (ddd, *J* = 8.5, 7.0, 1.5 Hz, 1 H, 4-H), 7.74 (ddd, *J* = 8.0, 1.5, 0.5 Hz, 1 H, 6-H) ppm. IR (KBr): $\tilde{\nu}$ = 3432, 2927, 1619, 1457, 1384, 1242, 1148, 1107, 1060, 1019 cm^{-1}. UV/Vis (MeOH): λ_{max} (logε) = 219 (3.93), 248 (3.43), 340 (3.19) nm. CD (MeOH): λ_{max} ([Θ]) = 207 (–2100), 223 (500), 252 (–2200), 289 (–1800), 345 (–1800) nm. ESI-MS: pos. *m*/*z* = 306 $[M+Na]^+$, 589 $[2M+Na]^+$. HRESI-MS: *m*/*z* = 306.09518 $[M+H]^+$ (calcd. for $C_{13}H_{18}NO_6$: *m*/*z* = 306.09481, Δ 1.14 ppm).

4-Hydroxybenzoyl 6-Deoxy-α-L-talopyranoside (8): Colourless solid. $C_{13}H_{16}O_7$ (284.26); R_f = 0.15 ($CHCl_3$/MeOH, 9:1); colour reaction with orcinol/H_2SO_4: brown. $[\alpha]_D^{20}$ = –69 (*c* = 2, MeOH). ^{1}H NMR (600 MHz, CD_3OD): δ = 1.25 (d, *J* = 6.5 Hz, 3 H, 6′-H_3), 3.72 (m, 1 H, 4′-H), 3.82 (ddd, *J* = 3.5, 2.0, 1.5 Hz, 1 H, 2′-H), 3.94 (t, *J* = 3.0 Hz, 1 H, 3′-H), 4.07 (q, *J* = 6.5 Hz, 1 H, 5′-H), 6.24 (d, *J* = 2.0 Hz, 1 H, 1′-H), 6.84 (d, *J* = 9.0 Hz, 2 H, 3-H, 5-H), 7.88 (d, *J* = 9.0 Hz, 2 H, 2-H, 6-H) ppm. ^{13}C NMR: see Table 2. IR (KBr): $\tilde{\nu}$ = 3386, 2937, 1708, 1608, 1514, 1445, 1270, 1168,

1150, 1103 sh, 1082, 1020 cm^{-1}. UV/Vis (MeOH): λ_{max} (log ε) = 211 (3.53), 259 (3.63) nm. CD (MeOH): no significant effect. ESI-MS: pos. *m/z* = 307 $[M+Na]^+$, 591 $[2M+Na]^+$; neg. *m/z* = 283 $[M-H]^-$. HRESI-MS: *m/z* = 307.07862 $[M+Na]^+$ (calcd. for $C_{13}H_{16}O_7Na$: *m/z* = 307.07882, Δ 0.65 ppm).

6-Deoxy-α-L-talopyranosyl 4-(6-Deoxy-α-L-talopyranosyloxy)benzoate (9): Colourless solid. $C_{19}H_{26}O_{11}$ (430.40); R_f = 0.13 ($CHCl_3$/MeOH, 9:1); colour reaction with orcinol/H_2SO_4: brown. $[\alpha]_D^{20}$ = –124 (*c* = 2, MeOH). ^1H NMR (600 MHz, CD_3OD): δ = 1.18 (d, *J* = 6.5 Hz, 3 H, 6′′-H_3), 1.25 (d, *J* = 6.5 Hz, 3 H, 6′-H_3), 3.67 (m, 1 H, 4′′-H), 3.72 (m, 1 H, 4′-H), 3.83 (ddd, *J* = 3.5, 2.0, 1.5 Hz, 1 H, 2′-H), 3.89 (q, *J* = 6.5 Hz, 1 H, 5′′-H), 3.90 (m, 1 H, 2′′-H), 3.94 (t, *J* = 3.0 Hz, 1 H, 3′′-H), 3.95 (t, *J* = 3.0 Hz, 1 H, 3′-H), 4.08 (q, *J* = 6.5 Hz, 1 H, 5′-H), 5.65 (d, *J* = 2.0 Hz, 1 H, 1′′-H), 6.24 (d, *J* = 2.0 Hz, 1 H, 1′-H), 7.17 (d, *J* = 9.0 Hz, 2 H, 3-H, 5-H), 7.99 (d, *J* = 9.0 Hz, 2 H, 2-H, 6-H) ppm. ^{13}C NMR: see Table 2. IR (KBr): ṽ = 3404, 2935, 1718, 1605, 1508, 1421, 1312, 1271, 1246, 1173, 1150, 1102, 1023 cm^{-1}. UV/Vis (MeOH): λ_{max} (log ε) = 252 (4.03) nm. CD (MeOH): λ_{max} ([Θ]) = 252 (–6400) nm. ESI-MS: pos. *m/z* = 453 $[M+Na]^+$, 883 $[2M+Na]^+$. HRESI-MS: *m/z* = 453.13677 $[M+Na]^+$ (calcd. for $C_{13}H_{16}O_7Na$: *m/z* = 453.13673, Δ 0.09 ppm).

Methanolysis of 8: Acetyl chloride (7.1 mL, 0.10 mmol) was slowly dissolved in methanol (25 mL) at 0 °C. Compound **8** (50 mg, 0.176 mmol) was added to the solution and stirred for 12 h at room temperature. The solvent was removed in vacuo, and the residue was purified by chromatography on silica gel (column 20 × 1.5 cm, CH_2Cl_2/MeOH, 7:1) to yield methyl 4-hydroxybenzoate (13.4 mg, 0.088 mmol, 50%), methyl 6-deoxy-α-L-talopyranoside (14.4 mg, 0.081 mmol, 46%), and methyl 6-deoxy-β-L-talopyranoside (1.5 mg, 0.010 mmol, 6%).

Methyl 4-Hydroxybenzoate: Colourless solid. $C_8H_8O_3$ (152.15); R_f = 0.81 ($CHCl_3$/MeOH, 4:1); colour reaction with orcinol/H_2SO_4: none. ^1H NMR (300 MHz, CD_3OD): δ = 3.83 (s, 3 H, OMe), 6.81 (d, *J* = 8.5 Hz, 2 H, 4-H, 6-H), 7.85 (d, *J* = 8.5 Hz, 2 H, 3-H, 7-H). ^{13}C NMR (75.5 MHz, CD_3OD): δ = 52.2 (q, OMe), 116.1 (d, C-4, C-6), 122.2 (s, C-2), 132.7 (d, C-3, C-7), 163.5 (s, C-5), 168.7 (s, C-1). EI-MS: *m/z* (%) = 152 (45), 121 (100), 93 (18), 65 (16).

Methyl 6-Deoxy-α-L-talopyranoside: Colourless solid. $C_7H_{14}O_5$ (178.19); R_f = 0.39 ($CHCl_3$/MeOH, 4:1); colour reaction with orcinol/H_2SO_4: brown. $[\alpha]_D^{20}$ = –72 (*c* = 2, MeOH). $[\alpha]_D^{20}$ = –98 (*c* = 2, H_2O). ^1H NMR (600 MHz, CD_3OD): δ = 1.24 (d, *J* = 6.5 Hz, 3 H, 6-H_3), 3.35 (s, 3 H, OCH_3), 3.58 (dd, *J* = 3.0, 1.5 Hz, 1 H, 4-H), 3.66 (ddd, *J* = 3.5, 2.0, 1.5 Hz, 1 H, 2-H), 3.69 (t, *J* = 3.5 Hz, 1 H, 3-H), 3.84 (q, *J* = 6.5 Hz, 1 H, 5-H), 4.64 (d, *J* = 2.0 Hz, 1 H, 1-H) ppm. ^{13}C NMR (150.8 MHz, CD_3OD): δ = 16.9 (q, C-6), 55.3 (q, OCH_3), 67.3 (d, C-3), 67.9 (d, C-5), 71.9 (d, C-2), 74.1 (d, C-4), 103.5 (d, C-1); $^1J_{CH}$ at anomeric carbon = 172.6 Hz. ESI-MS: pos. *m/z* = 201 $[M+Na]^+$, 379 $[2M+Na]^+$.

Methyl 6-Deoxy-β-L-talopyranoside: Colourless solid. $C_7H_{14}O_5$ (178.19); R_f = 0.32 ($CHCl_3$/MeOH, 4:1); colour reaction with orcinol/H_2SO_4: brown. $[\alpha]_D^{20}$ = +62 (*c* = 1, MeOH). ^1H NMR (300 MHz, CD_3OD): δ = 1.28 (d, *J* = 6.5 Hz, 3 H, 6-H_3), 3.57 (s, 3 H, OCH_3), 3.42 (dd, *J* = 3.5, 1.5 Hz, 1 H, 4-H), 3.55 (m, 1 H, 2-H), 3.64 (t, *J* = 3.5 Hz, 1 H, 3-H), 3.70 (q, *J* = 6.5 Hz, 1 H, 5-H), 4.37 (d, *J* = 8.0 Hz, 1 H, 1-H). ESI-MS: pos. *m/z* = 201 $[M+Na]^+$, 379 $[2M+Na]^+$.

Supporting Information (see also the footnote on the first page of this article): ^1H NMR spectra of compounds **2**–**9** are available as supporting information.

Acknowledgments

The authors would like to thank M. Klingebiel for excellent technical assistance, Prof. Dr. W. Beil (Institute of Pharmacology, Hannover Medical University) for the cytotoxicity tests and Dr. M. Kaiser (Swiss Tropical Institute, Basel) for testing the antiparasitic activity.

[1] A. C. Weymouth-Wilson, *Nat. Prod. Rep.* **1997**, *14*, 99–110.
[2] a) X. M. He, H.-W. Liu, *Annu. Rev. Biochem.* **2002**, *71*, 701–754; b) H.-w. Liu, J. S. Thorson, *Annu. Rev. Microbiol.* **1994**, *48*, 223–256.
[3] a) D. A. Johnson, H.-W. Liu, in *Comprehensive Natural Products Chemistry*, vol. 3 (Ed.: P. M. Pinto), Elsevier, Amsterdam, **1999**; b) A. Trefzer, J. A. Salas, A. Bechthold, *Nat. Prod. Rep.* **1999**, *16*, 283–299.
[4] S. Grond, H.-J. Langer, P. Henne, I. Sattler, R. Thiericke, S. Grabley, H. Zähner, A. Zeeck, *Eur. J. Org. Chem.* **2000**, 929–937.
[5] S. Grond, I. Papastavrou, A. Zeeck, *Eur. J. Org. Chem.* **2000**, 1875–1881.
[6] J.-F. Hu, D. Wunderlich, I. Sattler, A. Härtl, I. Papastavrou, S. Grond, S. Grabley, X.-Z. Feng, R. Thiericke, *J. Antibiot.* **2000**, *53*, 944–953.
[7] C. Milkowski, D. Strack, *Phytochemistry* **2004**, *65*, 517–524.
[8] S. Kato, K. Shindo, Y. Yamagishi, M. Matsuoka, H. Kawai, J. Mochizuki, *J. Antibiot.* **1993**, *46*, 1485–1493.
[9] T. Reichstein, E. Weiss, *Adv. Carbohydr. Chem.* **1962**, *17*, 65–120.
[10] a) A. P. MacLennan, *Biochem. J.* **1962**, *82*, 394–400; b) J. Weckesser, H. Mayer, I. Fromme, *Biochem. J.* **1973**, *135*, 293–297.
[11] B. Lindberg, *Adv. Carbohydr. Chem. Biochem.* **1990**, *48*, 279–318.
[12] A. P. MacLennan, *Biochim. Biophys. Acta* **1961**, *48*, 600–601.
[13] E. Pretsch, P. Bühlmann, C. Affolter, M. Badertscher, *Spektroskopische Daten zur Strukturaufklärung organischer Verbindungen*, 4th ed., Springer Verlag, Berlin, Heidelberg, New York, **2001**.
[14] a) P. M. Collins, W. G. Overend, *J. Chem. Soc.* **1965**, 1912–1918; b) M. Mori, S. Tejima, T. Niwa, *Chem. Pharm. Bull.* **1986**, *34*, 4037–4044.
[15] C. S. Hudson, *J. Am. Chem. Soc.* **1909**, *31*, 66–86.
[16] W. Klyne, *Biochem. J.* **1950**, *47*, XLI–XLII.
[17] T. Okamoto, K. Yatzaki, M. Tabata, *Phytochemistry* **1995**, *38*, 83–88.
[18] R. M. Kroppenstedt (DSMZ, Braunschweig, Germany), personal communication.
[19] M. R. Grever, S. A. Schepartz, B. A. Chabner, *Semin. Oncol.* **1992**, *19*, 622–638.
[20] S. M. Jones, J. E. Urch, M. Kaiser, R. Brun, J. L. Harwood, C. Berry, I. H. Gilbert, *J. Med. Chem.* **2005**, *48*, 5932–5941.

Received: March 13, 2006
Published Online: June 12, 2006

