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1-Acyl-3-*O*-[β-glucopyranosyl-(1''→6')-β-glucopyranosyl]-glycerols and Cordycedipeptides B and C, New Metabolites from *Bacillus pumilus*

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Dedicated to the Publisher and Editors of Natural Product Communications in honor of the 10th anniversary of this journal.

Four 1-monoacyl-3-*O*-[β-glucopyranosyl-(1→6)-β-glucopyranosyl]-glycerols (**1**) and four 1,2-diacyl-3-*O*-[β-glucopyranosyl-(1→6)-β-glucopyranosyl]-glycerols (**2a**) with acyl residues consisting of 1:1 mixtures of 1-*iso*-pentadecanoyl- and 1-*anteiso*-pentadecanoyl residues and the respective heptadecanoic acid isomers s as main components, have been characterized in the extracts of *Bacillus pumilus* strain DKS1. Twenty-seven further metabolites, among them the diketopiperazines cordycedipeptide A (**3**), B (**4**), and C (**5**), were obtained. All compounds were elucidated by NMR and MS techniques and fully characterized and tested for antimicrobial activity against *Legionella pneumophila*.

Keywords: Glycolipid, *Bacillus pumilus*, *Legionella*, Cordycedipeptide, Diketopiperazine.

Glycolipids belong to the large family of glycoconjugates, which further include glycoproteins, glycopeptides, proteoglycans, peptidoglycans, and lipopolysaccharides [1]. Glycolipids, especially glycosyl diglycerides, occur widely as membrane components of plants [2-4], animal cells [5,6], and microorganisms. They exist in Nature as complex mixtures of homologs due to the various chain lengths of fatty acid residues and their branching and varying number of double bonds [7]. Monoglycosyl diacylglycerols and diglycosyl diacylglycerols are most common among them [8]. More complex glycolipids of many bacteria possess antigenic properties and determine the serological characteristics of a microorganism or serve as markers for cell-to-cell communication.

During the study of *Bacillus pumilus* strain DKS1, which produced pumilacidins with highly selective activity against *Legionella* spp. [9,10], we obtained also the new monoacyl-diglucosyl-glycerolipid **1** with mainly *iso*- and *anteiso*-pentadecanoic acid (1:1) and *iso*-/*anteiso*-heptadecanoic acid (1:1) as acyl residues. In addition to the diacyl derivative **2a** (Figure 1) and four pumilacidins, *B. pumilus* produced additionally a number of diketopiperazines, among them the rare cordycedipeptid A (**3**), and the new isomers cordycedipeptide B (**4**) and C (**5**). The present work deals with the isolation and structure elucidation, by chemical and spectroscopic methods, of glycolipids **1** and **2a**, and of the diketopiperazines **4** and **5**. The biological activity of these compounds was investigated.

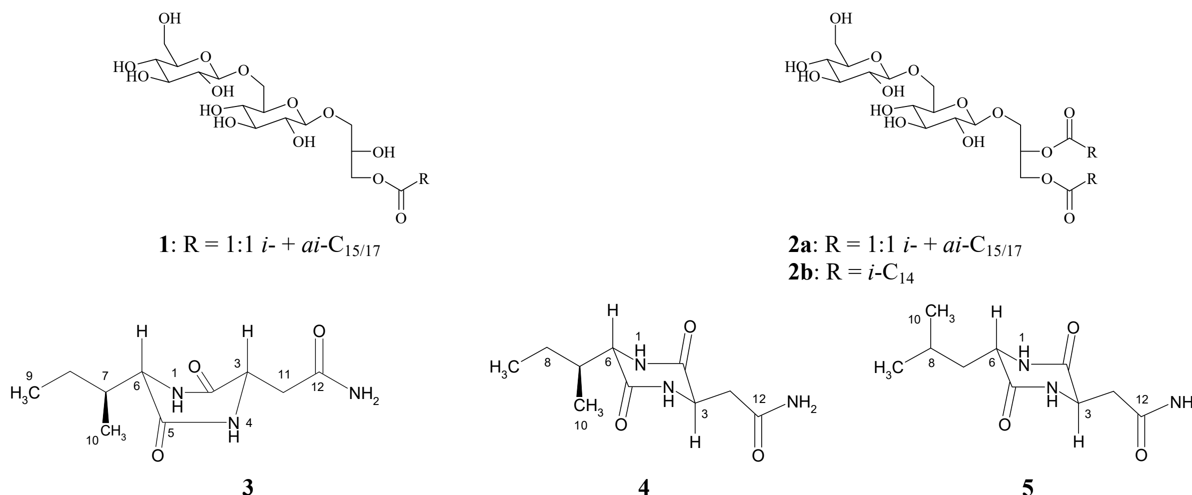

Figure 1: Structures of compounds **1**–**5**.

After fermentation of *B. pumilus* on LB medium and separation of the extracts on silica gel, a substantial amount of an amorphous sparingly soluble solid was obtained and identified as diacyl-gentiobiosyl glycerols **2a** [11]. Chromatography of the more readily soluble part on LiChroprep® DIOL phase and silica gel delivered a related compound as white amorphous solid, which turned out to be an inseparable mixture of diacyl homologues and isomers of the monoacyl-gentiobiosyl glycerol **1**.

The polar properties and the green color reaction with anisaldehyde/sulfuric acid suggested sugar derivatives. The molecular formula of the main component was established as $C_{30}H_{56}O_{14}$ by a *pseudo*molecular ion peak at *m/z* 663.3563 $[M+Na]^+$ in the ESI HR mass spectrum, in combination with the NMR data.

(+)-LC-ESI-MS/MS analysis of **1** showed prominent fragment ions at *m/z* 645 $[M-H_2O+Na]^+$, 501 $[M-Hex+Na]^+$, 421 $[M-FA+Na]^+$, 347 $[M-FA-Gol+Na]^+$, and 340 $[M-Hex-Hex+Na]^+$, interpreted as the sequence Hex-Hex-Gol-FA of two hexopyranosyl residues (Hex), a glycerol unit (Gol), and a C15 carboxylic acid (FA).

The COSY and TOCSY spectra revealed two separated spin systems for the sugar part, as expected for two hexopyranosyl residues: The anomeric proton H-1' at $\delta_{H/C}$ 4.29/104.8 coupled with four oxymethines H-2', H-3', H-4', and H-5' (at δ_H 3.22, 3.36, 3.35, 3.48), and weakly with the oxymethylene multiplet of H_2-6' at $\delta_{H/C}$ 3.76, 4.18/69.9. The second anomeric proton H-1" at $\delta_{H/C}$ 4.36/104.7 coupled with three oxymethines H-2", H-3", H-4" (at δ_H 3.22, 3.36, 3.28), and weakly with an oxymethylene signal at $\delta_{H/C}$ 3.67, 3.87/62.6; the latter coupled additionally with the oxymethine H-5" at $\delta_{H/C}$ 3.28/77.9. The anomeric proton H-1" (δ_H 4.36) showed HMBC cross-peaks with C-6' (δ_C 69.9) and *vice versa*, which established unambiguously the 1"→6' linkage of the two hexopyranosyl residues as hexopyranosyl-(1→6)-hexopyranoside.

The ^1H NMR signals were assigned by COSY and TOCSY spectra: The doublet of the anomeric proton H-1" (δ_H 4.36) coupled with a triplet (J = 7.9 Hz) assigned to H-2", so that H-1" and H-2" must be in diaxial positions. In the NOESY spectrum, H-1" showed correlations with H-3" (δ_H 3.36) and H-5" (δ_H 3.28); the proton H-3" correlated with H-5" as well, and H-2" (δ_H 3.22) correlated with H-4" (δ_H 3.28). This indicated that all oxymethine protons in this hexopyranosyl residue were in an axial position, and C-1" is β-configured (see Figure 2) [12-14]. Although the shifts of H-4 and H-5 are identical (δ_H 3.28), there is no other option for their sterical orientation so that glucose resulted. A proof of the absolute configuration e.g. by hydrolysis and OR measurements was not possible due to the lack of material.

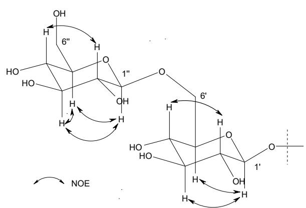

Figure 2: NOESY correlations in **1** (double-headed arrows).

In the same way, the proton H-1' (δ_H 4.29) showed NOESY correlations with H-3' (δ_H 3.36) and H-5' (δ_H 3.48); a weak correlation between H-2' (δ_H 3.22) and H-4' (δ_H 3.35) was also seen. This and the coupling constant of H-1' (J = 7.9 Hz) pointed to another glucose residue. The identity of both sugars as glucose was further confirmed by GC/MS analysis of the silylated hydrolyzate and comparison with authentic reference sugars (see Supplementary Data), so that the disaccharide gentiobiose resulted. The structure was further confirmed by the identity of the sugar NMR signals in the diacylgentiobioside **2a** with previously published data for **2b** (see below). The absolute configuration of the sugar could not be further confirmed, for reasons as before.

Table 1: ^1H (600 MHz) and ^{13}C NMR data (125 MHz) of **1** in methanol-d_4.

	1			
Pos.	δ_C	δ_H (J in Hz)	H,H COSY	HMBC
1	66.5, CH_2	4.15, m	H-2	C-2, 3, 1'''
2	69.7, CH	4.00, m	H-1, 3	C-1, 3
3	72.6, CH_2	(CH_{2a}) 3.86, m	H-2, 3b	C-1, 1', 2
		(CH_{2b}) 3.73, m	H-2, 3a	C-1, 1', 2
1'	104.8, CH	4.29, d (7.9)	H-2'	C-3, 3', 5'
2'	74.9, CH	3.22, m	H-1', 3'	C-1', 3'
3'	77.8, CH	3.36	H-2'	C-2', 4', 5'
4'	71.4, CH	3.35, m	H-5'	C-3', 5'
5'	76.9, CH	3.48, m	H-4', 6a'	C-3',4',6'
6'	69.9, CH_2	($CH_{2a'}$) 3.76, m	H-5', 6b'	C-1",5'
		($CH_{2b'}$) 4.18, m	H-6a'	C-1",4', 5'
1"	104.7, CH	4.36, d (7.9)	H-2"	C-6', 3", 5"
2"	75.0, CH	3.22, m	H-1", 3"	C-1", 3"
3"	77.7, CH	3.36, m	H-2"	C-2", 4"
4"	71.5, CH	3.28, m		C-2", 5"
5"	77.9, CH	3.28, m	H-6"	C-4", 6"
6"	62.6, CH_2	($CH_{2a"}$) 3.67, m	H-5", 6b"	C-4", 5"
		($CH_{2b"}$) 3.87, m	H-6a"	C-4", 5"
1'''	175.5, C			
2'''	34.9, CH_2	2.36, t (7.3)	H-3'''	C-1''', 3''', 4'''
3'''	25.9, CH_2	1.62, m	H-2''', 4'''	C-1''', 2''', 4'''
4'''	30.2, CH_2			
5'''-10'''	30-31, $(CH_2)_8$	16H, 1.25-1.35, m		
11'''	28.5, CH_2	1.29 m	H-12'''	
12'''	40.2, CH_2	1.17, m	H-11''', 13'''	C-14''', 15'''
13'''	29.1, CH	1.52, m	H-12''', 14''', 15'''	C-11''', 12''', 14''', 15'''
14''', 15'''	23.0, $2CH_3$	6H, 0.88, d (6.7)	H-13'''	C-12''', 13''', 14'''/15'''

Because of signal overlapping, the assignment of C-2'/2" and C-3'/3" may be exchanged

The methine group at δ_H 4.00 showed a COSY correlation with one proton of a methylene AB signal at δ_H 3.86/3.73 (CH_2-3) and a correlation with a second methylene multiplet at δ_H 4.15 (CH_2-1); it was assigned to C-2 of a glycerol unit (Gol). The anomeric proton H-1' at δ_H 4.29 showed an HMBC correlation with the oxymethylene carbon C-3 of the glycerol at δ_C 72.6 and *vice versa*, interpreted as an acetal bond between C-1' and C-3 (Hex-Gol, Figure 3).

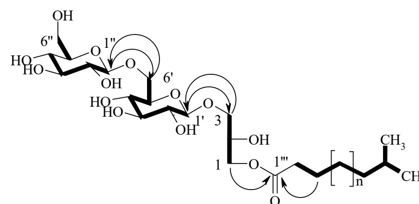

Figure 3: H-H COSY/TOCSY (bold lines) and selected HMBC correlations (arrows) of **1**.

The ^1H and ^{13}C NMR spectra of **1** showed for the main component a 6H methyl signal at $\delta_{H/C}$ 0.88/23.0 and signals of an aliphatic chain between δ_H 1.2 and 2.3 (δ_C 26–35), integrating for 1 methine and 11 methylene groups. Additionally, a carboxyl signal at δ_C 175.5 was found.

In the HMBC spectrum, the oxymethylene multiplet of CH_2-1 (δ_H 4.15) showed a correlation with this carboxyl group, indicating a glycerol ester bond between C-1 and C-1''' (Gol-FA). Further HMBC correlations confirmed the connection with a polymethylene chain, which was terminated by an isopropyl group: The 6H methyl doublet at δ_H 0.88 showed a COSY correlation with the methine multiplet at δ_H 1.52, and the carbon shift of δ 23.0 is typical for the methyl carbons of an *iso*-branching [15], resulting finally in an *iso*-pentadecanoyl residue.

Additional cross signals with carbons at δ 19.6 and 11.7 pointed to a mixture with the respective and/or homologous *anteiso*-fatty acids. As usually, it was not possible to separate these isomers, but we confirmed the mixture by GC/MS analysis after hydrolysis and esterification with diazomethane: The acids were identified as an 80:20 mixture of mainly pentadecanoic and heptadecanoic acids, respectively. Both acids occurred as *iso*- and *anteiso*-isomers in a ratio close to 1:1; the respective *n*-acids or further acid homologues occurred only in traces (see Figure 4).

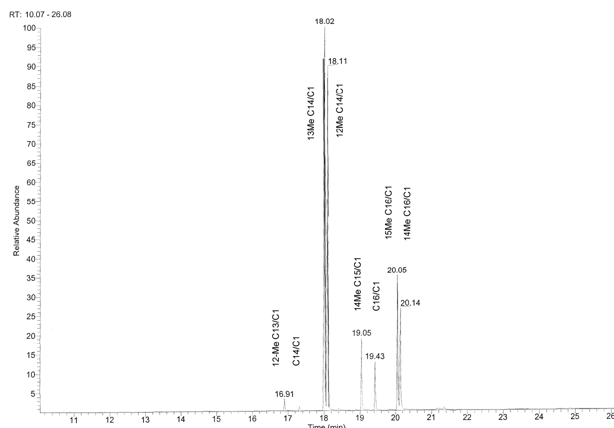

Figure 4: Total ion current (TIC) chromatogram of methyl esters obtained by hydrolysis of **1** and subsequent methylation with diazomethane.

The second compound **2a** showed the mass and the properties of a previously described gentiobiosyl 1,2-dipentanoyl-glyceride **2b**, which was reported, however, as having solely *iso*-branched acyl residues [11, 16]. GC/MS analysis indicated for our sample the same mixture of C_{15} and C_{17} acyl residues as in **1** (see Figure 5), so that **2a** was a mixture of mainly four inseparable compounds.

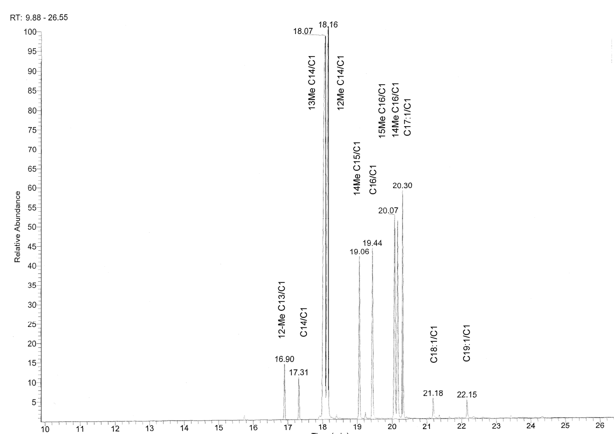

Figure 5: Total ion current (TIC) chromatogram of methyl esters obtained by hydrolysis of compound 2a and subsequent methylation with diazomethane.

A search in the literature afforded only 17 monoacyl-diglyco-pyranosyl-glycerides, among them four with saturated even-numbered fatty acids, but all with at least one galactose unit. According to these data, compound **1** is rather unique. Hydrolysis of **1** delivered mainly a 40:25:5:4:12:9 mixture of *i*-C15(0), *ai*-C15(0), *i*-C16(0), C16(0), *i*-C17(0) and *ai*-C17(0) acids, while the diacylglycerolipid **2a** yielded a similar mixture, and additionally traces of unsaturated acids. This is in contrast to the interpretation of MS data [11], that acyl variations occur solely at C-2 of the glycerol unit.

Besides *cyclo*(Pro,Val), *cyclo*(Pro,Tyr), *cyclo*(Pro,Thr), *cyclo*-(Leu,Thr), *cyclo*(dehydroAla,Leu), and the recently described (2*E*/*Z*,5*Z*)-2-[(4-methoxyphenyl)methylene]-5-(2-methylpropylide-ne)-3,6-piperazinedione [17], the rare diketopiperazine cordycedi-peptide A [*cis-cyclo*(L-Asn,L-Ile), **3**], and the new analogues cordycedipeptide B [*trans-cyclo*(D-Asn,L-Ile), **4**] and C [*trans-cyclo*(D-Asn,L-Leu), **5**] were obtained.

The UV inactive white amorphous solids **3**, **4**, and **5** turned green on TLC in the chlorine/*o,o*'-dianisidin reaction, indicating peptide-like structures [18]. According to ESIHRMS, all three compounds had the composition $C_{10}H_{17}N_3O_3$. Compound **3** had been isolated previously from the liquid culture of an ascomycete, *Cordyceps sinensis* (Berk.) Sacc [19] and is reported here for the first time from a *Bacillus* sp.

An identical planar structure of a minor component **4** (*trans-cyclo*(D-Asn,L-Ile), together with **3** (1:2.4), was obvious from the similarity of the spectra and with one exception the identical 2D correlations (see Figure 6 and Table 2): In contrast to **3**, no NOESY correlation was found between H-3 (δ_H 4.08) and H-6 (δ_H 3.65), so that a *trans*-diketopiperazine of Ile and Asn was assumed. This indicated that one of the two amino acids (whose identification will be described below) had been isomerized and must have the D-configuration.

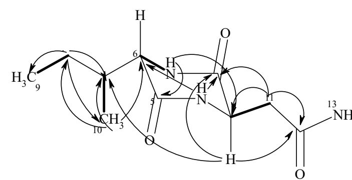

Figure 6: COSY (bold lines) and selected HMBC correlation (arrows) of cordycedipeptide B (**4**).

Table 2: ^{1}H and ^{13}C NMR data (600 and 125 MHz, resp.) of cordycedipeptide B (**4**) in DMSO-d_6.

Pos.	δ_C	δ_H (J in Hz)	H,H COSY	HMBC
1		NH, 7.98, s	H-6	C-3, 5
2	167.8*, C			
3	50.6, CH	4. 08, t (5.3)	H-11a, 11b	C-2, 7, 12
4		NH, 7.81, s		C-2, 6
5	167.1*, C			
6	59.1, CH	3.65, t (3.0)	H-1	
7	37.1, CH	1.86, m	H-8, 10	
8	24.2, CH_2	(CH_{2a}) 1.42, m	H-7, 8b, 9	C-7, 9, 10
		(CH_{2b}) 1.20, m	H-7, 8a, 9	C-7, 9, 10
9	11.6, CH_3	0.85, t (7.3)	H-8a, 8b, 10	
10	15.0, CH_3	0.91, d (7.1)	H-7, 9	C-6, 7, 8
11	38.6, CH_2	(CH_{2a}) 2.47, dd (15.9, 6.3)	H-3, 11b	C-3, 12
		(CH_{2b}) 2.62, dd (15.9, 4.6)	H-3, 11a	C-2, 3, 12
12	171.3, C			
13		(NH_{2a}) 7.34, br s	H-13b	
		(NH_{2b}) 6.85, br s	H-13a	

* δ_C values of C-2 and C-5 are interchangeable

Cordycedipeptide C [*trans-cyclo*(D-Asn,L-Leu), **5**] was obtained in a second fermentation as another 1:2.3 mixture with cordycedipeptide A (**3**), again inseparable by TLC and by HPLC due to the low solubility in methanol/water. Comparison of the ^{1}H NMR spectrum with that of **3** showed a close similarity as well. The ^{13}C NMR spectrum displayed ten carbon signals as for **3**, however, the two upfield methyls (δ 11.8 and 14.9) and the methylene signal (δ 24.1) of **3** were substituted by CH/CH_3 signals near δ 22. The COSY spectrum showed a correlation between a methine group at δ 1.84 and two methyls at δ 0.88 and 0.86, indicating an isopropyl residue.

In the same way as for **3**, a leucine and an asparagine residue were assigned by HSQC, HMBC and COSY spectra, resulting in the diketopiperazine *cyclo*(Asn,Leu) (**5**, see Figure 7 and Table 3).

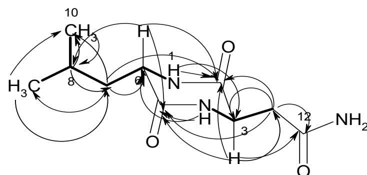

Figure 7: COSY (bold lines) and selected HMBC correlations (arrows) of cordycedipeptide C (**5**).

Table 3: ^{1}H and ^{13}C NMR data (600 and 125 MHz, resp.) of **5** in DMSO-d_6.

	Cordycedipeptide C (**5**)			
Pos.	δ_C	δ_H (J in Hz)	H,H COSY	HMBC
1		NH, 8.08, s	H-6	C-2, 3
2	168.4, C			
3	51.3, CH	4.14, m	H-4, 11a, 11b	C-2, 5, 11, 12
4		NH, 7.78, s	H-3	C-5, 6
5	167.7, C			
6	52.5, CH	3.77, m	H-1, 7a, 8	C-2, 5, 8
7	42.2, CH_2	(CH_{2a}) 1.53, m	H-6, 7b, 8	C-2, 6, 9, 10
		(CH_{2b}) 1.63, m	H-7a, 8	C-6, 9, 10
8	23.6, CH	1.85, m	H-6, 7, 9, 10	C-7, 10
9	22.9, CH_3	0.85, d (7.1)	H-8a, 8b	C-7, 9, 10
10	21.7, CH_3	0.92, d (7.1)	H-8a, 8b	C-8
11	38.1, CH_2	(CH_{2a}) 2.40, dd (16.3, 8.2)	H-3, 11b	C-2, 3, 5, 12
		(CH_{2b}) 2.62, dd (11.9, 4.4)	H-3, 11a	C-2, 3, 5, 12
12	171.1, C			
13		(NH_{2a}) 7.42, br s	H-13b	
		(NH_{2b}) 6.91, br s	H-13a	

* δ_C values of C-2 and C-5 are interchangeable

NOESY experiments were not reliable in this case, as the 3-/6-H signals of **3** and **5** were overlapping.

The absolute configuration of the amino acids in **4** and **5** was determined by HPLC-MS of the FDAA-amino acid derivatives, obtained after total hydrolysis and reaction with Marfey's reagent [20]. The extracted ion chromatogram (EIC), obtained by HPLC/MS of the derivatized hydrolysis products from the **3**/**4** mixture, gave only one signal with *m/z* 384 and the retention time for Marfey's L-Ile derivative, but two signals with *m/z* 386 for FDAA-Asp (see Figure 8 and Table 4). As **4** is the minor component in mixture with **3**, the minor D-Asp signal must be assigned to component **4**, which hereby was identified as *trans-cyclo*(D-Asn,L-Ile).

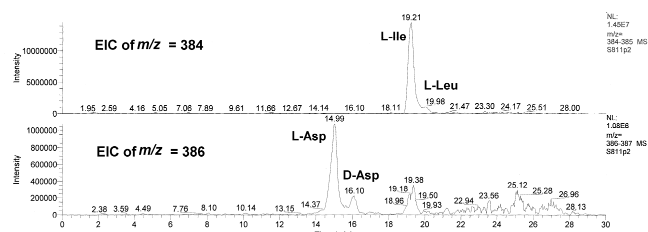

Figure 8: Extracted ion chromatogram (EIC) of Marfey's derivatives obtained from the hydrolysis products of a cordycedipeptide A/B (**3**/**4**) mixture; HPLC/MS scan for Ile/Leu with *m/z* = 384 and for Asp with *m/z* = 386.

Table 4: Retention times of FDAA-amino acid derivatives, obtained from reference amino acids or by total hydrolysis of **3**/**4** and **3**/**5** mixtures, respectively, and reaction with Marfey's reagent. For the experimental conditions, see the Supplementary Data.

Amino acid	L-Ile	D-Ile	D-allo-Ile	L-Leu	L-Asp	D-Asp
Standard amino acids	19.27	21.23	21.17	19.95	15.03	16.04
Cordycedipeptide A (**3**)	19.22	-		-	14.96	-
Cordycedipeptide A+B (**3**+**4**)	19.21	-	-	(19.98)	14.99	16.10
Cordycedipeptide A+C (**3**+**5**)	19.22	-	-	20.06	14.99	16.10

in brackets: trace component.

The EIC chromatogram obtained by HPLC/MS of FDAA-amino acids from the **3**/**5** mixture gave, in the same way, two signals at *m/z* 384 for L-Ile (main) and L-Leu (minor), and two signals at *m/z* 386 for D-Asp (minor) and L-Asp (main) (see Figure 9 and Table 4). Hereby it follows, that **5** is *trans-cyclo*(D-Asn,L-Leu), named cordycedipeptide C. The (L,L)-form of this *cyclo*-dipeptide was

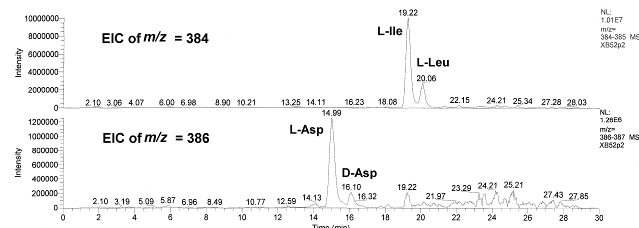

Figure 9: Extracted ion chromatogram (EIC) of Marfey's derivatives obtained from the hydrolysis products of a cordycedipeptide A/C (**3**/**5**) mixture; HPLC/MS scan for Ile/Leu with *m/z* = 384, and for Asp with *m/z* = 386.

isolated from the fungus *Phellinus igniarius* [21] and has been obtained by synthesis [22] as well; the NMR data have not been assigned in the literature, although published ^{13}C shifts were identical with our values within a range of ±0.1 ppm.

In the biological tests, the glucolipid **1** showed weak bactericidal activity against *Legionella pneumophila* with an MIC value of 25 μg/mL. Due to the insolubility of **2a**, its activity could not be measured. Cordycedipeptide A (**3**) alone or in mixtures with **4** or **5**, respectively, was inactive at 200 μg/mL.

The cordycedipeptides were antibiotically inactive (MIC > 200 μg/mL), and for the activity of the extract against *Legionella* spp., mainly the content of the pumilacidins was made responsible. It has been found, however, that cyclic dipeptides and simple derivatives thereof have a biological function as signaling compounds [23], e.g. as sporulation factors.

Proline containing diketopiperazines are sometimes formed as trace contaminants of bacterial fermentations, especially on prolonged heating of the broth or sterilization at higher temperature. In our investigations of hundreds of strains, however, less than 1% formed proline-diketopiperazines in such a variety and concentration as *B. pumilus*: We found five saturated and unsaturated known diketopiperazines with and without proline [*cyclo*(Pro,Val), *cyclo*(Pro, Tyr), *cyclo*(Pro,Thr), *cyclo*(Leu,Thr), *cyclo*(dehydroAla,Leu)]. Furthermore, four rare derivatives were characterized as (2*E*/*Z*,5*Z*)-2-[(4-methoxyphenyl)methylene]-5-(2-methylpropylidene)-3,6-piperazinedione, cordycedipeptide A [*cyclo*(L-Asn,L-Ile), **3**], and the new corycedipeptides *trans-cyclo*(D-Asn,L-Ile) (**4**), and *trans-cyclo*(D-Asn,L-Leu) (**5**). It can be assumed therefore, that their presence is due to biosynthetic capabilities of the producing strain and may have a functional reason as described for other compounds of this type [23].

Additionally, N^{β}-acetyltryptamine, *N*-acetyltryptophan, decenoic acid, palmitic acid, harman, nonactic acid methyl ester, homononactic acid methyl ester, 3-hydroxyacetyl-indole, indole-3-carboxylic acid, *N*-(2-indol-3-yl-2-oxoethyl)-acetamide, 3-(3,3-bisindolyl)-propane-1,2-diol, the acylpeptide lactones pumilacidin A, B, C, and E, triethylamine (as ammonium salt), tryptophane, and turbomycin A were isolated. All known compounds were identified by means of their NMR and mass data with the aid of AntiBase [16], and confirmed with the original references cited therein.

Experimental

General experimental procedures: Optical rotations were measured on a Perkin-Elmer, model 243 polarimeter, NMR spectra on Varian Unity 300 (300 MHz/ 75.5 MHz) and Varian Inova 500 (500 MHz/ 125 MHz) spectrometers, and high-resolution mass spectra (HRMS), recorded by ESI MS, on an Apex IV 7 Tesla Fourier-Transform Ion Cyclotron Resonance Mass Spectrometer (Bruker Daltonics, Billerica, MA, USA). HPLC-ESI-MS and MS/MS

spectra were recorded on a Finnigan LCQ with a Rheos 4000 quaternary pump (Flux Instruments) and a Phenomenex Synergi Max-RP 150 × 2.00 mm 4 μm RP-C12 column, solvent A: MeOH + 0.05% HCOOH, solvent B: H_2O + 0.05% HCOOH, flow rate 300 μL/min, gradient: start 10% or 20% of A, 0-20 min from 10% or 20% to 100% of A, 20-30 min at 100% A, UV/VIS-Diode-Array-Detector (200-800 nm ever 5 nm). ESI-MS/MS was performed with normalized collision energy of 35%. TLC was performed on Polygram SIL G/UV_{254} (Macherey-Nagel & Co.). R_f values were measured on Polygram SIL G/UV_{254} (Macherey-Nagel & Co.) using CH_2Cl_2/20% MeOH. Spots on chromatogram were detected under UV light and by spraying with anisaldehyde/sulfuric acid. PTLC was performed with silica gel P/UV_{254} (Macherey-Nagel & Co.) on 20 × 20 cm glass plates. Size exclusion chromatography was conducted on Sephadex LH-20 (Lipophilic Sephadex; Amersham Biosciences, Ltd., purchased from Sigma-Aldrich Chemie, Steinheim, Germany). Column chromatography was performed using MN silica gel 60 (0.05-0.2 mm, 70-270 mesh; Macherey-Nagel & Co); diol-phase 40-63 μm (LiChroprep DIOL, Merck KGaA, Darmstadt, Germany). Amberlite XAD-16 resin was obtained from Rohm and Haas, France. GC/MS was performed on a Thermo Finnigan Trace GC+MS system (Autosampler AS 2000), using a Varian column CP-Sil 8CB for amines (30 m × 0.25 mm i.d., 0.25 μm film thickness). The temperature was programmed from 40°C (held for 1 min) to 300°C (held for 4 min), ramping the temperature up at 10°C/min. Filter press: Schenk Niro 212 B40.

Fermentation, extraction and isolation: For the starter culture, 15 1 L Erlenmeyer flasks filled with 300 mL of LB medium (5 g/L NaCl, 5 g/L yeast extract 10 g/L tryptone) were inoculated with *Bacillus pumilus* strain DKS1 from well-grown agar plates and shaken over night at 28°C. A Biostat U fermenter (70 L) was filled with 45 L LB medium, autoclaved and then inoculated with the starter culture. Fermentation conditions: 15 h at 35°C, stirring rate 200 rpm, aeration 20 L/min, pH 6.5 ± 1.5; pH regulation by automatic addition of 2 N NaOH or 2 N citric acid, Niax (polypropylenglycol) was used as an antifoaming agent. The culture broth was filtered to separate biomass and water phase. From the EtOAc+acetone extract FFM1 (6.73 g) of the biomass, 800 mg **2a** separated as a sparingly soluble white solid. The culture filtrate was adsorbed onto an XAD-16 resin column (7 × 120 cm), which was washed with water to remove inorganic salts and other polar compounds, and eluted with methanol. After removing the methanol, the remaining water residue was re-extracted with ethyl acetate to give 8.47 g of a crude extract FFX (first fermentation) and 14 g XAD extract S (second fermentation). The latter was separated by chromatography, affording **1** (1.3 mg), **3** (60 mg), **3**+**4** (4.3 mg) and **3**+**5** (2.6 mg) (see Supplementary Data, Figure S1). The peptide fraction from FFXB31 delivered the peptide lactones pumilacidin A (12 mg), pumilacidin B (3.4 mg), pumilacidin C (2.3 mg), and pumilacidin E (1.3 mg). Further fractions yielded the following known compounds: (2*E*/*Z*,5*Z*)-2-[(4-methoxyphenyl)methylene]-5-(2-methylpropylidene)-3,6-piperazinedione [17] (1.1 mg), *cyclo*-(Thr,Leu) (2.5 mg), *cyclo*(Pro,Val) (14.4 mg), homononactic acid methylester, 3-(hydroxyacetyl)indole, *N*-(2-indol-3-yl-2-oxoethyl)acetamide, turbomycin A, 3-(3,3-bisindolyl)-propane-1,2-diol. A further eight known compounds were obtained in a second fermentation and are listed in the workup diagram (Supplementary Data, Figure S1).

Mixture of 1-*i*-/1-*ai*-pentadecanoyl-3-*O*-[β-glucopyranosyl-(1''→6')-(β-glucopyranosyl)]-glycerol and 1-*i*-/1-*ai*-heptadecanoyl-3-*O*-[β-glucopyranosyl-(1''→6')-(β-glucopyranosyl)]-glycerol (1)

White amorphous powder.
R_f: 0.3 (CH_2Cl_2-MeOH, 5:1).
$[\alpha]_D^{20}$: –17.5 (*c* 1.60, MeOH); due to the structural similarity of the *i*-C_{15} and *i*-C_{17} acids and of the low chiral contribution (if any) of the *ai*-acids, the optical rotation of the mixture may be similar to that of the pure components.
^{1}H and ^{13}C NMR: see Table 1.
(+)-HRESIMS *m/z* $[M+Na]^+$ calcd. for $C_{30}H_{56}O_{14}Na$: 663.3562; found: 663.3563. High resolution for the signal of the C17-acyl homologue observed at *m/z* = 691.39 was not performed.

Mixture of 1,2-Di-*i*-/*ai*-pentadecanoyl-3-*O*-[β-glucopyranosyl-(1''→6')-β-glucopyranosyl]-glycerol and 1,2-Di-*i*-/*ai*-heptadecanoyl-3-*O*-[β-glucopyranosyl-(1''→6')-β-glucopyranosyl]-glycerol (2a)

Colorless solid.
R_f: 0.2 (CH_2Cl_2/MeOH, 4:1).
^{1}H NMR (300 MHz, pyridine-d_5): δ 5.68 (1H, m, H-2), 5.04 (1H, d, *J* = 7.7 Hz, H-1''), 4.79 (1H, d, *J* = 7.7 Hz, H-1'), 4.75 (1H, m, H-6'a), 4.71 (1H, dd, *J* = 12.2, 3.1 Hz, H-1a), 4.55 (1H, dd, *J* = 11.9, 6.8 Hz, H-1b), 4.46 (1H, m, H-6''a), 4.40 (1H, dd, *J* = 11.0, 5.5 Hz, H-3a), 4.27 (1H, m, H-6''b), 4.25 (1H, m, H-6'b), 4.16 (1H, m, H-4''), 4.16 (1H, m, H-3'), 4.14 (1H, m, H-3''), 4.12 (1H, m, H-4'), 4.00 (1H, m, H-3b), 4.00 (1H, m, H-5''), 3.99 (1H, m, H-2''), 3.90 (1H, m, H-2'), 3.88 (1H, m, H-5'), 2.37 (4H, m, H-2''' and 2''''), 1.64 (4H, m, H-3''' and 3''''), 1.49 (2H, m, H-13''', 13''''), 1.23-1.42 (36H, m, H-4''' to 11''', 4'''' to 11''''), 1.15 (4H, m, H-12''' and 12''''), 0.87 (12H, d, *J* = 6.9 Hz, H-14''', 15''', 14'''', and 15'''').
^{13}C NMR (125 MHz, pyridine-d_5): δ 173.7 (C-1'''), 173.5 (C-1''''), 105.9 (CH-1''), 105.2 (CH-1'), 78.7 (CH-5', 3', 3''), 77.6 (CH-5''), 75.5 (CH-2''), 75.1 (CH-2'), 72.0 (CH-4''), 71.8 (CH-3'), 71.4 (CH-2), 70.5 (CH_2-6'), 68.6 (CH_2-3), 63.7 (CH_2-1), 63.1 (CH_2-6''), 39.6 (CH_2-12''', 12''''), 34.6 (CH_2-2''', 2''''), 30.4-27.7 (CH_2-4''' to 11''', 4'''' to 11''''), 28.5 (CH-13''', 13''''), 25.6 (CH_2-3''', 3''''), 23.1 (CH_3-14''', 15''', 14'''', 15'''').
(+)-HRESIMS *m/z* $[M+Na]^+$ calcd. for $C_{45}H_{84}O_{15}Na$: 887.57024; found: 887.56988.

Cordycedipeptide A (3)

White amorphous powder.
R_f: 0.3 (CH_2Cl_2-MeOH, 9:1).
$[\alpha]_D^{20}$: –89.2 (*c* 0.65 in DMSO).
^{1}H and ^{13}C NMR data, see Supplementary Data, Table S1.
(+)-ESI MS *m/z*: 228 $[M+H]^+$, 250 $[M+Na]^+$, 455 $[2M+H]^+$, 477 $[2M+Na]^+$.
(-)-ESI MS *m/z*: 226 $[M-H]^-$, 262 $[M+Cl]^-$, 272 $[M+HCOO]^-$, 453 $[2M-H]^-$.
(+)-HRESIMS *m/z*: $[M+H]^+$ calcd. for $C_{10}H_{17}N_3O_3$: 228.1343; found: 228.1344.

Cordycedipeptide B (4, in mixture with 3)

White amorphous powder.
R_f: 0.3 (CH_2Cl_2-MeOH, 9:1).
^{1}H and ^{13}C NMR: see Table 2.
ESI MS (+) *m/z*: 228 $[M+H]^+$, 250 $[M+Na]^+$, 455 $[2M+H]^+$, 477 $[2M+Na]^+$.
(-)-ESI MS *m/z*: 226 $[M-H]^-$, 262 $[M+Cl]^-$, 453 $[2M-H]^-$.
(+)-HRESIMS *m/z*: $[M+H]^+$ calcd. for $C_{10}H_{17}N_3O_3$: 228.1343; found: 228.1346.

Cordycedipeptide C (5, in mixture with 3)

White amorphous powder.
R_f: 0.3 (CH_2Cl_2-MeOH, 9:1).
^{1}H and ^{13}C NMR: see Table 3.
(+)-ESI MS *m/z*: 228 $[M+H]^+$, 250 $[M+Na]^+$, 455 $[2M+H]^+$, 477 $[2M+Na]^+$.

(-)-ESI MS *m/z*: 226 [M-H]$^-$, 262 [M+Cl]$^-$, 272 [M+HCOO]$^-$, 453 [2M-H]$^-$, 489 [2M+Cl]$^-$.
(+)-HRESIMS *m/z*: [M+H]$^+$ calcd. for $C_{10}H_{17}N_3O_3$: 228.1343; found: 228.1346.

Identification of the sugar part of 1 and 2a [12]: A solution of approximately 0.1 mg of either **1** or **2a** in 50 µL of MeOH and 50 µL of 1 N HCl was hydrolyzed at 80°C for 4 h in a sealed tube. The sample was brought to dryness at 0.1 mbar, and the residue was treated with 50 µL of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) at 40°C for 60 min. GC/MS analysis showed 4 peaks with retention times of 18.09, 18.56, 19.02 and 19.85 min. Sugars as standards: D-Glucose: 18.05, 18.48, 19.06, 19.85 min; D-mannose: 18.10, 19.15, 19.61, 19.83 min; D-galactose: 18.22, 18.61, 18.71, 19.14 min (see Supplementary data).

Determination of amino acid configurations [24]**:** Each 0.5 mg of **3**/**4** and **3**/**5** mixtures was hydrolyzed by heating with 0.2 mL conc. HCl (37.5%) in a sealed tube for 14 h at 100°C. After cooling, the solution was evaporated to dryness and the residue re-dissolved in 50 µL H_2O. To each of these hydrolyzate solutions, or to a solution of the reference amino acids (50 µL, 50 mM of L-Ile; D,L-Ile; D-*allo*-Ile; mixture of L-Ile and D-allo-Ile; D,L-Asp; L-Asp; L-Leu), a solution of FDAA (Marfey's reagent, N^{α}-(5-fluoro-2,4-dinitrophenyl)-L-alanineamide) in acetone (100 µL of 1% solution) was added. After addition of 20 µL 1 M $NaHCO_3$ solution, the mixture was incubated for 1 h at 40°C. The reaction was stopped by addition of 10 µL of 2 M HCl, the solvents were evaporated, and the residue was re-dissolved in 1 mL acetonitrile. An aliquot of this solution (20 µL) was analyzed by HPLC (Phenomenex Synergi Max-RP C12, 150 2.00 mm, 4 µm; solvents: A is H_2O + 0.05% HCOOH, B is MeOH + 0.05% HCOOH; linear gradient from 10% B in A at t = 0 min to 90% B in A within 20 min; 25°C; 300 µL/min). FDAA-D-Asp: 16.04 min; FDAA-L-Asp: 15.03 min; FDAA-L-Leu: 19.95 min; FDAA-D-*allo*-Ile: 21.17 min; FDAA-L-Ile: 19.27 min; FDAA-D-Ile: 21.23 min (see Figures 8 and 9).

Supplementary Data: Isolation procedures, NMR spectra of compounds **1**, **2a**, and **3**–**5** and details on the identification of glucose in **1**/**2a** and amino acids in **3**-**5**.

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