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Parallel Chromatography in Natural Products Chemistry: Isolation of New Secondary Metabolites from *Streptomyces* sp

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Abstract: Integration of parallel chromatography on both, gel permeation and silica gel chromatography by making use of the CombiFlash^{TM} si1000s system (ISCO, Lincoln, USA) into purification process to speed up the isolation of secodary metabolites from microorganisms. As an example, we applied this approach to *Streptomyces* sp. (GT 061089) which led to the isolation and structural characterization of six 2-3-disubstituted butanoids (**1** to **6**), four 2,4-disubtituted butanoids (**7** to **10**), a monoterpenoid (**11**), two indol compounds (**12**, **13**), a furan-3-carboxylic acid (**14**), as well as two already known isocoumarins (**15**, **16**). The isolated pure compounds were characterized by spectroscopic methods and chemical transformations. The results of biological tests showed that both **15** and **16** possess medium cytotoxic activity and strong inhibiting activity on horse radish peroxidase. **15** also exhibits antiviral activity as well as a distinct inhibiting activity on 3a-hydroxysteroid dehydrogenase (3a-HSD).

Introduction

The search for new pharmacologically active agents obtained from natural sources has led to the discovery of many clinically useful drugs that play a major role in the treatment of human diseases. Numerous examples impressively demonstrated the innovative potential of natural products and their impact on the progress in the drug discovery and development[1,2,3]. However, natural products research as a part of drug discovery effort faces increasing challenges: how to improve diversity and quality of sample sources and reduce incidence of false positive and interfering material in biological screening attempts, how to accelerate dereplication; and automatic sample preparation and isolation. A new technology based on solid-phase-extraction (SPE) and the automation concept of the CyBi^{TM}-Xtract (CyBio AG, Jena, Germany) focused on the preparation of high-quality samples from natural origin which fulfilled quality and quantity of the high throughput screening (HTS)[4]. The next challenge is to speed up the subsequent isolation and structure characterization procedure of striking compounds from the crude extracts. In consequence, we used parallel chromatography approach for purification of several natural products simultaneously.

In the course of our chemical screening program of terrestrial *Streptomyces* sp. aiming at new secondary metabolites[5-7], a number of so-called “talented” strains[5] were discovered. Picking out a *Streptomyces* sp. (strain GT 061089) as an example, we approached parallel chromatography to yield a number of new secondary metabolites (**1**-**14**) along with two known compounds (**15**, **16**).

Parallel Chromatography - CombiFlash^{TM} si1000s System

The CombiFlash^{TM} si1000s system (ISCO, Lincoln, USA) is consisted of a FMI pump which can gradient solvent system, a up to 10 columns adopt system and a foxy 200 fraction collector which also serves as system controller. This system allows to run up to 10 samples simultaneously using the same solvent system. Both prepackaged and self-fulfilled columns are available allow to apply various chromatographic materials for different isolation purpose.

Screening and Fermentation

The strain GT 061089 was cultivated in a 300-ml Erlenmeyer flask containing 100 ml of medium B. The culture broth was absorbed by Amberchrom CG-161c (supelco) (1 ml resin) and eluted with methanol/water mixture (stepwise: 20%, 60%, 100%) to yield three fractions which were examined with the procedures of chemical screening[5]. The screening results (Table 1) showed that this strain produced a number of different classes compounds. Those spots were identified as new with respect to our screening database of more than 1000 natural products on retention characteristics in two elution solvents and band characterization by color, UV-absorption and staining behavior with different reagents. In order to isolate significant amounts of these compounds, cultivation of the producing organism was carried out in a 200-l fermentor containing medium B at 28 ° C for 5 d (500 rpm, aeration 10 l/min).

Table 1. Yields and properties of the isolated metabolites

Compd.	Fr.	Yield (mg/l)	Rf[a]	Rf[b]	Color reactions [c d e]		
1	I, II	0.37	0.37	0.98	-	Gray	-
2	I	0.15	0.35	0.80	-	Light green	-
3	I	0.36	0.29	0.72	-	Green	-
4	II	0.19	0.41	0.82	Dark	Green	Light purple
5	I	0.03	0.58	0.81	-	Blue gray	-
6	II	0.05	0.60	0.95	-	Blue gray	-
7	II	0.04	0.40	0.91	-	Blue	Light blue
8	II	0.13	0.39	0.91	-	Turquoise	-
9	I	0.06	0.12	0.68	-	Blue green	-
10	I	0.10	0.18	0.75	-	Brown	-
11	II	0.30	0.53	0.96	-	Blue	-
12	II	0.15	0.38	0.93	Dark	Purple	Purple
13	II	0.08	0.40	0.93	Dark	Purple	Purple
14	II	0.05	0.61	0.85	Dark	Light purple	Light purple
15	II, III	1.30	0.60	0.97	Dark	Blue gray	Light purple
16	II,	0.10	0.34	0.95	Dark	Blue gray	Light purple

a $CHCl_3$/MeOH (9:1), [b] *n*-butanol/acetic acid/water (4:1:5) upper layer, [c] UV (254 nm), [d] anisaldehyde, [e] Ehrlich's reagent.

Isolation and Parallel Chromatography Approach

After harvesting, the culture filtrate was passed through a Amberlite-XAD 16 column and eluted with water/methanol (gradient from 20% to 70% methanol, then 100% methanol) to yield three fractions. Figure 1 and Figure 2 showed the process of separation and purification of the first two fractions. After first chromatography of this two fractions on silica gel columns yielded three and two enriched fractions, respectively. This five fractions were then separated by parallel gel permeation chromatography on Sephadex LH-20 (five columns: 2.5 × 50 cm, Methanol, 0.5 ml/min) using CombiFlash^{TM} si1000s system. The combined fractions were further purified by parallel chromatography on silica gel (five columns: 1.1 × 30 cm, *n*-hexane/EtOAc, gradient from 4:1 to 2:1) or/and RP-C18 HPLC (2.5 × 25 cm, 7 mm, MeOH/H2O) (Figure 1 and 2) to obtain 0.37 mg/l of **1**, 0.15 mg/l of **2**, 0.36 mg/l of **3**, 0.19 mg/l of **4**, 0.03 mg/l of **5**, 0.05 mg/l of **6a** and **6b**, 0.04 mg/l of **7**, 0.13 mg/l of **8**, 0.06 mg/l of **9**, 0.10 mg/l of **10**, 0.30 mg/l of **11**, 0.15 mg/l of **12**, 0.08 mg/l of **13**, 0.05 mg/l of **14**, 0.30 mg/l of **15**, 0.10 mg/l of **16**. 1.0 g of **15** (1.0 mg/l) was obtain from the third fraction after extraction with EtOAc and re-crystallized in methanol.

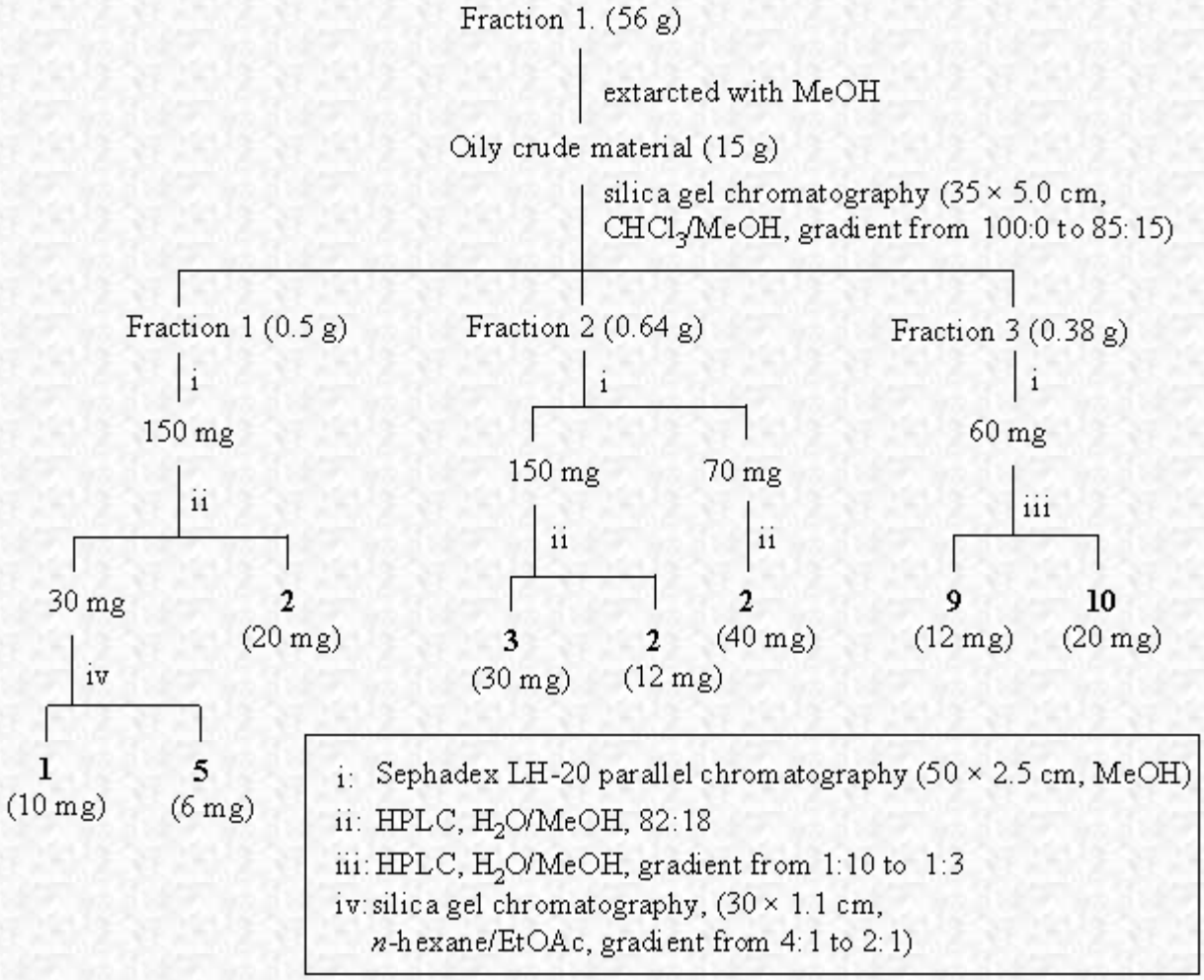


Figure 1. Isolation of compounds **1** to **3**, **5**, **9** and **10** form Fraction I.

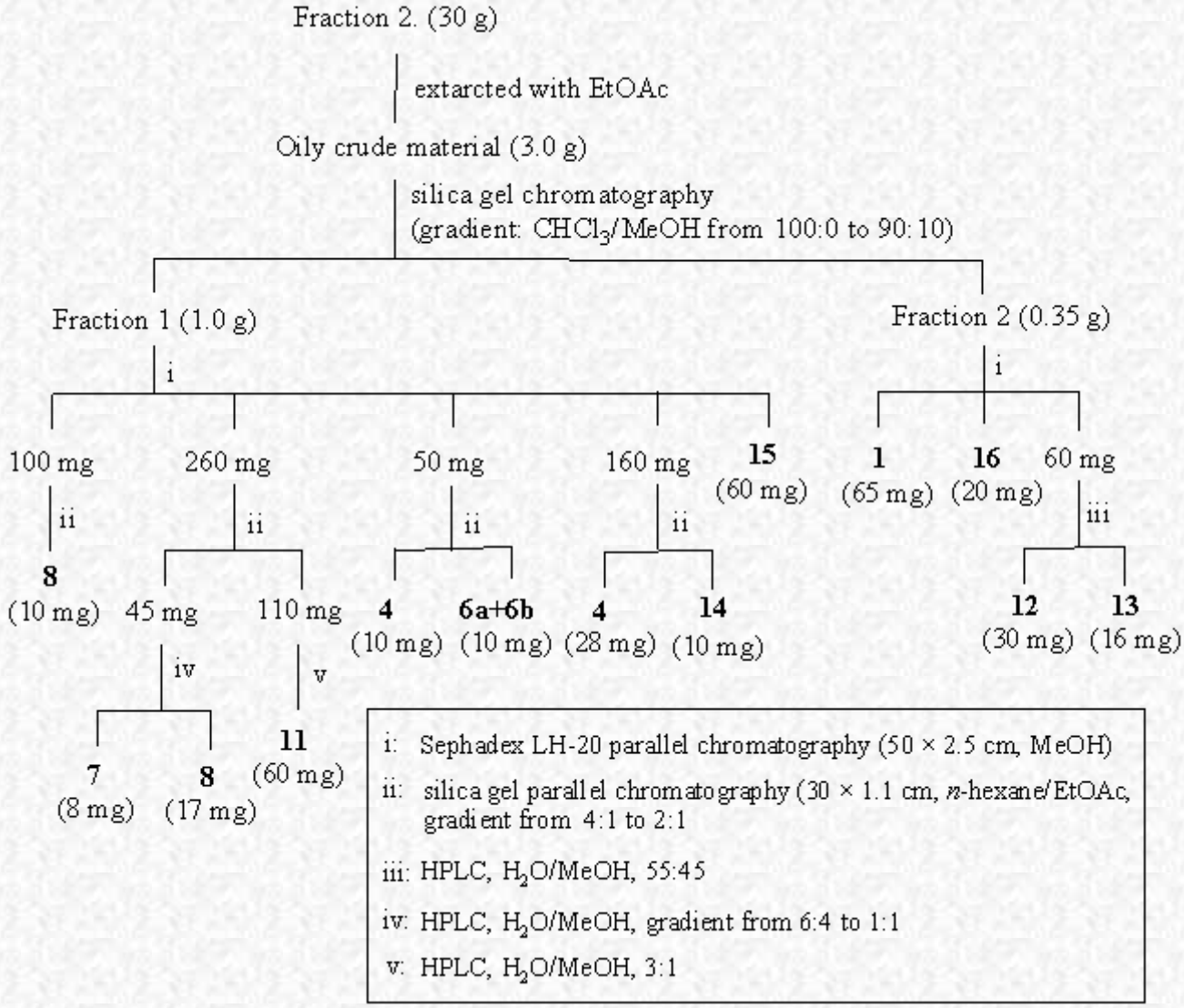


Figure 2. Isolation of compounds **1**, **4**, **6-8**, **11-16** from Fraction II.

The isolated pure compounds were characterized spectroscopically. The molecular formulae were determined by mass spectrometry and the structures were elucidated by both, detailed analysis of the ^{1}H-, ^{13}C-, ^{1}H-^{1}H-, and ^{1}H-^{13}C-shift correlation NMR-spectra, and chemical transformations.

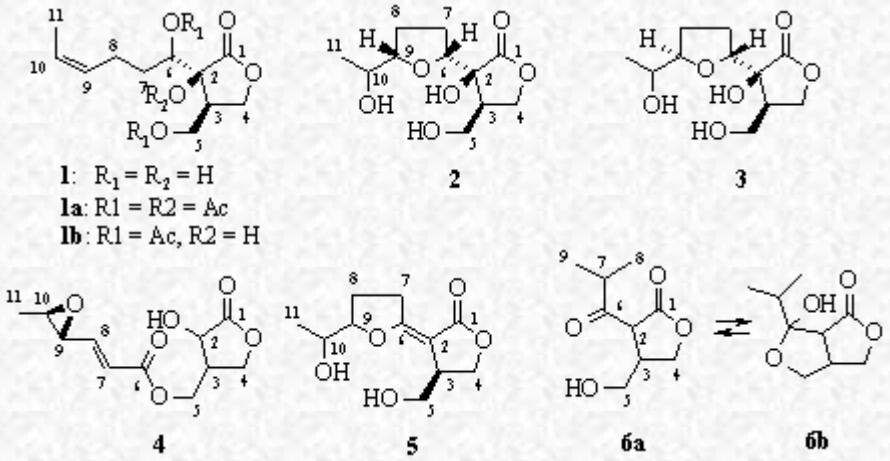
2,3-Disubstituted Butanolides

(E)-3-hydroxy-3-(1-hydroxy-hex-4-enyl)-4-hydroxymethyl-dihydro-furan-2-one (1): The molecule of compound **1** was deduced as $C_{11}H_{18}O_5$ from ESI-MS spectrometry (positive ion) (*m/z* = 230.9 $[M + H]^+$, 247.8 $[M + NH_4]^+$ and 253.0 $[M + Na]^+$ and HR-EIMS of the fragment ions at *m/z* = 212.1026 ($C_{11}H_{16}O_4$, calcd. 212.1049, $M^+ - H_2O$) and 194.0931 ($C_{11}H_{14}O_3$, calcd. 194.0943, $M^+ - 2H_2O$). The IR absorption bands at 3430 and 1761 cm^{-1} indicates the presence of hydroxyl groups and an g-lactone functionality. Upon treatment with acetic anhydride in pyridine, **1** yielded a triacetate product **1a** and a diacetate products **1b**, which indicated the presence of three hydroxyl groups in **1**.

The ^{1}H-NMR spectrum of **1** shows 15 proton signals (Table 2): one methyl group at d 1.64; two methylene groups at d 1.74 / 1.78 and 2.24 / 2.10; two methylene groups which are linkage to oxygen atoms at d 3.78 / 3.84 and 4.25 / 4.43; an aliphatic methine group at d 2.74 (dddd, *J* = 8.0, 5.7, 4.5, 4.3 Hz), two olefinic protons at d 5.48 and 5.46, as well as an additional methine bearing oxygen at d 3.74. The coupling constant of the two olefinic protons $J_{9,10}$ = 9.5 Hz indicates an *E*-configuration of the double bond. The ^{13}C-NMR (Table 3) and DEPT spectra show 11 carbon signals: one carbonyl (d 178.8), one quaternary carbon (d 78.4) two methine (d 73.9, 40.7), two olefinic carbon atoms (d 130.1, 126.3), four methylene (d 68.8, 60.9, 30.4, 28.9) and one methyl (d 17.8) groups.

The proton-proton connections arose from both a comparison of coupling constants and ^{1}H-^{1}H COSY NMR experiments, showing two segments: -O-CH_2-CH-CH_2-O- and -CH(O)-CH_2-CH_2-CH=CH-CH_3. Assignments of ^{1}H- and ^{13}C-NMR data were achieved by detailed investigation of

2D (^{1}H-^{1}H COSY, HSQC and HMBC) NMR data, which led to the structure of **1** shown in Scheme 1.



Scheme 1. Structures of 2,3-disubstituted butanolides

The correlation signals between d 5.62 (2-OH) and d 3.58 (5-Ha) as well as d 3.40 (5-Hb) in the NOESY spectrum (DMSO-d_6, 500 MHz) indicates that 2-OH and 3-hydroxymethyl group in a *syn*-facial position, which is confirmed by the NOE effects between d 2.63 (H-3) and d 3.56 (H-6), as well as d 5.18 (6-OH). Therefore, **1** is (E)-3-hydroxy-3-(1-hydroxy-hex-4-enyl)-4-hydroxymethyl-dihydro-furan-2-one.

Table 2. ^{1}H-NMR data of the compounds **1** to **5**.

Position	1[a]	2[a]	3[a]	4[a]	5 [b]
2				4.60 (d, 8.1)	
3	2.74 (dddd, 8.0, 5.7, 4.5, 4.3)	2.74 (dddd, 7.6, 5.7, 4.9, 4.3)	2.65 (dtd, 7.7, 5.7, 4.0)	2.98 (ddtd, 8.1, 7.6, 5.6, 3.9)	3.30 (dddd, 8.3, 7.1, 3.9, 3.8)
4a	4.43 (dd, 9.2, 8.0)	4.39 (dd, 9.3, 7.6)	4.38 (dd, 9.3, 7.7)	4.40 (dd, 9.7, 7.6)	4.31 (dd, 9.0, 8.3)
4b	4.25 (dd, 9.2, 4.5)	4.21 (dd, 9.3, 4.9)	4.23 (dd, 9.3, 4.0)	4.36 (dd, 9.7, 5.6)	4.25 (dd, 9.0, 3.9)
5a	3.84 (dd, 11.5, 4.3)	3.83 (dd, 11.5, 4.3)	3.85 (d, 5.7)	4.47 (dd, 11.5, 3.9)	3.74 (dd, 10.9, 3.8)
5b	3.78 (dd, 11.5, 5.7)	3.81 (dd, 11.5, 5.7)	3.85 (d, 5.7)	4.30 (dd, 11.5, 5.6)	3.61 (dd, 10.9, 7.1)
6	3.74 (br.d, 10.3)	4.12 (t, 6.7)	4.21 (dd, 7.2, 5.8)		
7	1.74 (m) / 1.78 (m)	2.27 (m) / 2.00 (m)	2.18 (m) / 1.95 (m)	6.05 (dd, 15.7, 0.8)	3.20 (m) / 2.98 (m)
8	2.24 (m) / 2.10 (m)	2.10 (m) / 1.89 (m)	1.98 (m) / 1.95 (m)	6.79 (dd, 15.7, 6.5)	2.07 (m) / 2.14 (m)
9	5.46 (m)	3.96 (td, 6.8, 2.6)	3.99 (m)	3.48 (ddd, 6.5, 4.4, 0.8)	4.40 (td, 7.1, 4.6)
10	5.48 (dq, 9.5, 5.9)	4.10 (qd, 5.1, 2.6)	3.97 (qd, 6.4, 2.8)	3.30 (qd, 5.3, 4.4)	3.90 (qd, 6.5, 4.6)
11	1.64 (dd, 5.9, 1.0)	1.13 (d, 5.1)	1.11 (d, 6.4)	1.29 (d, 5.3)	1.19 (d, 6.5)

[a]: in $CDCl_3$ (500 MHz); [b]: in CD_3OD (300 MHz).

3´-Hydroxy-5-(1-hydroxy-ethyl)-4´-hydroxymethyl-octahydro-[2,3´]bifuranyl-2´-one (2) and *Epi*-3´-hydroxy-5-(1-hydroxy-ethyl)-4´-hydroxymethyl-octahydro-[2,3´]bifuranyl-2´-one (3): Compounds **2** and **3** exhibit an identical molecular formula, $C_{11}H_{18}O_6$, resulted from the HR-EIMS, which exhibits one more oxygen atom compared to **1**. The IR spectra of both compounds also show the presence of hydroxyl group (**2**: 3430 cm^{-1} **3**: 3425 cm^{-1}) and g-lactone moiety (**2** and **3**: 1761 cm^{-1}). A

comparison of ^{13}C- and ^{1}H-NMR spectra (Table 2 and Table 3) of **2**, **3** and **1** shows an identically partial structure, the 2-hydroxy-3-hydroxymethyl-g-lactone moiety. The difference was found that the double bond between C-9 and C-10 in **1** was replaced by two methine groups linked to oxygen atoms in **2** and **3**. The correlation between C-6 (d 81.0) and H-9 (d 3.96) indicates an ether bond between C-6 and C-9, forming a tetrahydrofurane ring. Thus, the identical constituent of **2** and **3** was deduced as shown in Scheme 1, which is confirmed by detail analysis of 2D NMR data.

The relative stereochemistry of **2** and **3** was assigned by analysis of the NOESY NMR data. An NOE effect observable between H-3 (d 2.74) and H-6 (d 4.12) in the NOESY spectrum of **2** indicates a *syn*-facial position of 2-OH and 3-hydroxymethyl group. The *syn*-substituted pattern in the tetrahydrofurane ring in **2** was deduced from the NOE effect between H-6 (d 4.12) and H-9 (d 3.96).

The correlation signal between H-3 (d 2.65) and H-7 (d 1.95) in the NOESY spectrum of **3** indicates the *syn*-facial position of 2-OH and 3-hydroxymethyl group, which is identical to that in **2**. However, the NOE effect observed between H-6 (d 4.21) and the methyl group (d 1.11) points to the *anti*-substituted pattern in the tetrahydrofurane ring, which is instead of the *syn*-substituted pattern in **2** and agreement with the optical rotation values {**2**: $[a]_D$ = + 7.4 (c = 3.51, methanol); **3**: $[a]_D$ = + 15.5 (c = 0.92, methanol)}. Therefore, **3** is *epi*-3´-Hydroxy-5-(1-hydroxy-ethyl)-4´-hydroxymethyl-octahydro-[2,3´]bifuranyl-2´-one.

Table 3. ^{13}C-NMR data of the compounds **1** to **5**, **7** to **10** and **17**.

C	**1** [a]	**2** [a]	**3** [a]	**4** [a]	**5** [b]	**7** [c]	**8** [c]	**9** [b]	**10** [b]	**17** [b]
1	178.8 (s)	176.4 (s)	177.4 (s)	176.7 (s)	175.7 (s)	180.1 (s)	180.2 (s)	177.7 (s)	175.5 (s)	180.0 (s)
2	78.4 (s)	77.7 (s)	77.8 (s)	67.5 (d)	95.1 (s)	47.7 (d)	47.8 (d)	47.0 (d)	46.6 (d)	46.7 (d)
3	40.7 (d)	44.2 (d)	42.2 (d)	38.9 (d)	41.8 (d)	24.4 (t)	24.4 (t)	22.8 (t)	22.4 (t)	22.4 (t)
4	68.8 (t)	67.9 (t)	68.3 (t)	67.8 (t)	70.0 (t)	81.0 (d)	81.0 (d)	79.2 (d)	78.8 (d)	79.2 (d)
5	60.9 (t)	60.2 (t)	61.0 (t)	61.1 (t)	62.9 (t)	64.9 (t)	64.9 (t)	64.2 (t)	63.9 (t)	64.1 (t)
6	73.9 (d)	81.0 (d)	82.0 (d)	165.2 (s)	173.7 (d)	70.5 (d)	70.3 (d)	69.9 (d)	69.5 (d)	69.7 (d)
7	30.4 (t)	25.9 (t)	26.9 (t)	124.3 (d)	30.7 (t)	34.2 (t)	34.5 (t)	29.8 (t)	32.2 (t)	32.6 (t)
8	28.9 (t)	24.0 (t)	25.0 (t)	143.4 (d)	24.5 (t)	33.8 (t)	29.2 (t)	39.9 (t)	42.9 (t)	32.4 (t)
9	130.1 (d)	83.7 (d)	84.7 (d)	55.2 (d)	90.2 (d)	35.7 (d)	36.3 (d)	70.7 (s)	70.6 (s)	34.3 (d)
10	126.3 (d)	68.5 (d)	67.8 (d)	55.5 (d)	69.0 (d)	30.5 (t)	23.0 (q)	30.0 (q)	28.6 (t)	29.3 (t)
11	17.8 (q)	18.9 (q)	18.0 (q)	13.1 (q)	19.3 (q)	11.7 (q)	22.9 (q)	29.0 (q)	13.9 (q)	11.3 (q)
12						19.8 (q)			20.2 (q)	19.1 (q)

[a]: in $CDCl_3$ (125 MHz); [b]: in CD_3OD (75 MHz); [c]: in CD_3OD (125 MHz).

3-(3-Methyl-oxiranyl)-acrylic acid 4-hydroxy-5-oxo-tetrahydro-furan-3-ylmethyl ester (4): The HR-EIMS spectrum of **4** shows the molecular ion peak at *m/z* = 242.0790, pointing to molecular formulae $C_{11}H_{14}O_6$ (calcd. 242.0791). The IR spectrum of **4** shows hydroxyl group (3370 cm^{-1}) and g-lactone moiety (1766 cm^{-1}) as well as an a,b-unsaturated ester functionality (1722 cm^{-1}). As expected from mass spectrometry the ^{1}H-NMR spectrum ($CDCl_3$, 500M Hz) of **4** exhibits 14 protons signals which indicate two conjugated olefinic protons at d 6.79 (dd, *J* = 15.7, 6.5 Hz) and 6.05 (dd, *J* = 15.7, 0.8 Hz), four methine groups (d = 4.60, 3.48, 3.30 and 2.98 ppm), two methylene groups (d = 4.40/4.36 and 4.47/4.30 ppm) as well as a methyl group at d 1.29. This is agreement with the ^{13}C-NMR spectrum (125.0 MHz, $CDCl_3$) which showed the signals of eleven carbon atoms. Besides the proton attached carbon atoms, the signals of two quaternary carbon atoms are observed, a g-lactone carbonyl (d 176.7) and a conjugated carbonyl (d 165.2) (Table 3).

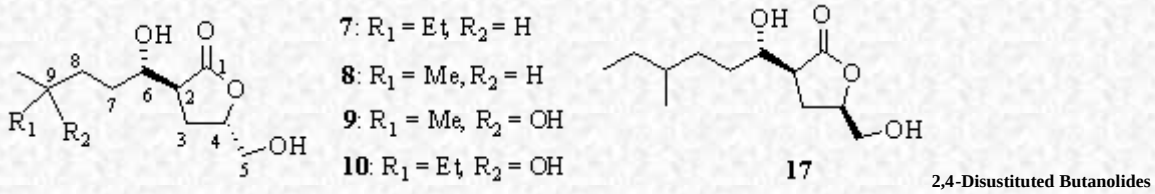
Proton-proton connections arose from both, a comparison of coupling constants, and a ^{1}H-^{1}H COSY spectrum. It reveals two segments: -O-CH_2-CH (CH-OH)-CH_2-O- and CH_3-CH(O)-CH(O)-CH=CH-. Carbon and proton signal assignments resulted from a 2D ^{1}H-^{13}C correlation spectrum. The connections of the proton attached carbon atoms with the quaternary carbon atoms are demonstrated by appropriate

correlation in the HMBC spectrum data and lead to the constitution of **4**.

The $J_{7,8}$ coupling constant of 15.7 Hz points the *E*-configuration of the double bond. The *cis*-substituted of the epoxide ring is determined by both, the coupling constant $J_{9,10}$ (4.4 Hz), and the NOE effects between the methyl group (d = 1.21) and H-8 (d = 6.68) in the NOESY spectrum (DMSO-d_6, 500 M Hz). The observable correlative signals between 2-OH (d = 6.10) and H-5 (d = 4.13/4.28) indicates the *cis* relative orientation of the substitution groups at C-2 and C-3. Thus, **4** is determined as 3-(3-methyl-oxiranyl)-acrylic acid 4-hydroxy-5-oxo-tetrahydro-furan-3-ylmethyl ester depicted in Scheme 1.

5-(1-Hydroxyethyl)-4´-hydroxymethyl-tetrahydro-[2,3´]bifuranyl-2´-one (5): The molecular formula, $C_{11}H_{16}O_5$, was determined from the HREI-MS spectrum of **5** (*m/z* = 228.1020, calcd. 228.0998) and supported by its ESI-MS spectrum. The IR spectrum shows the presence of hydroxyl group (3325 cm^{-1}) and a,b-unsaturated carbonyl group (1721, 1659cm^{-1}). The ^{1}H- and ^{13}C-NMR (300 MHz, CD_3OD) spectra show signals of 14 protons and 11 carbons, respectively (Table 2 and Table 3). A comparison of NMR data of **2** and **5** shows the closely structural similarities. The difference is the presence of a double bond between C-2 (d = 95.0) and C-6 (d = 173.7) in **5**. This causes the downfield-shift of 7-H_2 (from d_H = 2.27/2.00 in **2** shifting to d_H = 3.20/2.98 in **5**). It seems that **2** lose the 2-OH and the 6-H to form an a,b-unsaturated ester and yielded the dehydrated product **5**. Two dimensional correlation [COSY, HSQC, HMBC] allowed assignments of all proton and carbon resonance and fully confirmed this hypothesis. Thus, **5** is 5-(1-hydroxyethyl)-4´-hydroxymethyl-tetrahydro-[2,3´]bifuranyl-2´-one.

4-Hydroxymethyl-3-isobutryl-dihydro-furan-2-one / 6-hydroxy-6-isopropyl-tetrahydro-furo[3.4-c]furan-1-one (6a/6b): The ^{1}H-NMR (300 MHz, $CDCl_3$) spectrum of **6** reveals a mixture of **6a** and **6b** in a ratio of 5: 3. The structural elucidation of both compounds from the mixture is possible because of the well separated signal patterns. Both compounds possess 2,3-disubstituted g-lactone moiety. The ESI-MS (positive and negative ion) spectrum shows only one molecular weight, 186 g/mol, for both compounds. Detailed investigation of 2D (^{1}H-^{1}H COSY, HSQC and HMBC) NMR data led to the structures of **6a** and **6b** are depicted in Scheme 1. It seems that the semi-ketone **6b** was formed from **6a** via intramolecular semi-acetal reaction.



Scheme 2. Structures of isolated 2,4-disustituted butanolides **7** to **10** and related butanolide **17**

2-(1-Hydroxy-4-methyl-hexyl)-4-hydroxymethyl-butanolide (7): The molecular formula $C_{12}H_{22}O_4$ of **7**, which is identical with previously reported butanolide **17**[8], was determined by HREI-MS (*m/z*: 213.1596 [M^+ + H]). A comparison of IR, ^{13}C- and ^{1}H-NMR spectra (Table 4 and Table 3) of **7** and **17** led to the identical constitution of them. However, an observable NOE effect between H-6 (d 4.00) and H-4 (d 4.49) as well as a lack of NOE effect between H-2 (d 2.84) and H-4 (d 4.49) in the NOESY spectrum of **7** exhibit a *trans* relative orientation of the substituted groups at C-2 and C-3 in the g-lactone moiety, while a *cis*-2,4-disubstituted pattern in **17**.

Table 4. ^{1}H-NMR data of the compounds **7** to **10** and **17**.

H	**7** [a]	**8**[a]	**9** [b]	**10** [b]	**17** [b]
2	2.84 (ddd, 11.0, 9.5, 2.9)	2.84 (ddd, 10.9, 9.5, 3.0)	2.80 (td, 10.2, 2.9)	2.84 (m)	2.81 (ddd, 10.1, 9.9, 2.5)
3a	2.19 (ddd, 12.3, 9.5, 6.8)	2.20 (ddd, 12.5, 9.5, 6.7)	2.30 (ddd, 12.0, 10.2, 6.8)	2.25 (ddd, 12.0, 10.2, 6.8)	2.25 (ddd, 13.0, 10.1, 9.5)
3b	2.04 (ddd, 12.3, 11.0, 9.8)	2.05 (ddd, 12.5, 10.9, 9.8)	2.23 (ddd, 12.0, 10.2, 9.8)	2.20 (ddd, 12.0, 10.2, 9.8)	2.17 (ddd, 13.0, 9.9, 6.8)
4	4.49 (dddd, 9.8, 6.8, 6.1, 3.5)	4.50 (dddd, 9.8, 6.7, 6.1, 3.5)	4.50 (dddd, 9.8, 6.8, 5.0, 2.8)	4.57 (dddd, 9.8, 6.8, 5.1, 2.8)	4.57 (dddd, 9.5, 6.8, 5.2, 2.7)
5a	3.74 (dd, 12.3, 3.5)	3.74 (dd, 12.3, 3.5)	3.92 (dd, 12.6, 2.8)	3.92 (dd, 12.5, 2.8)	3.90 (dd, 12.7, 2.7)
5b	3.62 (dd, 12.3, 6.1)	3.62 (dd, 12.3, 6.1)	3.70 (dd, 12.6, 5.0)	3.70 (dd, 12.5, 5.1)	3.70 (dd, 12.7, 5.2)
6	4.00 (td, 7.4, 3.0)	4.00 (ddd, 7.8, 5.3, 3.0)	4.15 (m)	4.19 (m)	4.16 (td, 7.6, 2.5)
7	1.50 (m) / 1.43 (m)	1.45 (m) / 1.30 (m)	1.64 (m) / 1.64 (m)	1.65 (m) / 1.65 (m)	1.48 (m) / 1.09 (m)
8	1.48 (m) / 1.12 (m)	1.45 (m) / 1.20 (m)	1.70 (m) / 1.58 (m)	1.50 (m) / 1.50 (m)	1.48 (m)
9	1.33 (m)	1.60 (m)			1.34 (m)
10	1.36 (m) / 1.16 (m)	0.90 (d, 6.6)	1.25 (s)	1.70 (q, 6.8)	1.34 (m) / 1.16 (m)
11	0.88 (t, 7.2)	0.91 (d, 6.5)	1.27 (s)	0.90 (t, 6.8)	0.86 (t, 7.0)
12	0.89 (d, 6.5)			1.23 (s)	0.87 (d, 6.3)

[a]: in CD_3OD (500 MHz); [b]: in $CDCl_3$ (500 MHz).

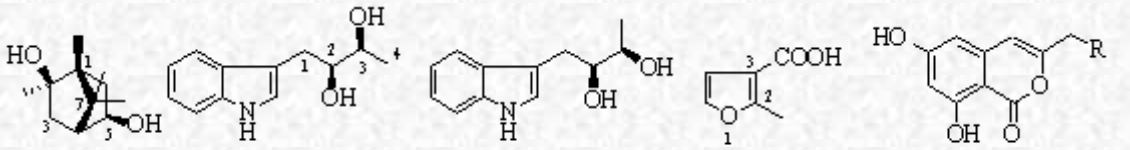
2-(1-Hydroxy-4-methyl-pentyl)-4-hydroxymethyl-butanolide (8): The HREI-MS spectrum of **8** exhibits a *pseudo*-molecular ion peak at *m/z* 217.1445 ([M^+ + H]), corresponding to the molecular formula $C_{11}H_{20}O_4$ bearing one less methylene group in comparison to **7**. The IR, ^{1}H-NMR, and ^{13}C-NMR spectra of **7** and **8** (Table 4 and Table 3) indicate their identically partial structure expect an isopropyl group in **8** instead of an isobutyl group in **7**. This is conformed by detailed interpretation of 2D NMR (^{1}H-^{1}H COSY, HSQC and HMBC) data of **8**. Identical coupling constants of H-2, H-3, H-4 and H-5 in **7** to those in **8** indicate the same relative stereochemistry, *trans*-2,4-disubstituted, in the g-lactone moiety.

2-(1,4-Dihydroxy-4-methyl-pentyl)-4-hydroxymethyl-butanolide (9): Combination of the results from HREI-MS and ESI-MS spectra of **9** revealed the molecular formula $C_{11}H_{20}O_5$, possessing one more oxygen atom in comparison to **8**. The IR, ^{1}H-NMR, and ^{13}C-NMR spectra of **8** and **9** (Table 4 and Table 3) indicate their closely structural similarities. Two tertiary methyl groups (d 1.25, s, and 1.27, s) in **9** are instead of the two secondary ones (d 0.90, d, and 0.91, d) in **8**. And one methine group (d_H 1.60, d_C 36.3 in **8**) is replaced by a quaternary carbon atom (d 70.7). The latter is linked to a hydroxyl group while the signal of the tertiary carbon at d 36.3 in **8** is missing in **9**. In comparison to **8**, the analysis of the 2D-NMR spectra led to the constitution of **9** as depicted in Scheme 2. The *trans* relative orientation of the substituent groups at C-2 and C-3 in the g-lactone ring was proposed due to the comparable coupling constants of **8** and **9**.

2-(1,4-Dihydroxy-4-methyl-hexyl)-4-hydroxymethyl-butanolide (10): Compound **10** possesses a molecular formulae $C_{12}H_{22}O_5$ which was determined by HREI-MS and ESI-MS and shows one more oxygen atom in comparison to **7**. A comparison of IR, ^{13}C- and ^{1}H-NMR spectra (Table 4 and Table 3) of **10** and **7** led to the structure of **10** as depicted in Scheme 2. This is conformed by the analysis of 2D-NMR data of **10**. From the biosynthesis view, **10** was suggested to possess a *trans*-2,4-disubstituted pattern in the g-lactone ring, which is supported by the comparable coupling constants of **7** and **10**.

Indoles and Miscellaneous Compounds

***Threo*-1-(1H-indol-3-yl)-butane-2,3-diol (12) and *Ethro*-1-(1H-indol-3-yl)-butane-2,3-diol (13)**: Compounds **12** and **13** possess the same molecular formula $C_{12}H_{15}NO_2$ which were determined by HREI-MS spectra. The IR spectra of both compounds show the absorption peaks for indol skeleton (**12**: 1616, 1452 cm^{-1}; **13**: 1600, 1452 cm^{-1}) and hydroxyl group (**12**: 3405 cm^{-1}; **13**: 3410 cm^{-1}). The ^{1}H-NMR and ^{13}C-NMR ($CDCl_3$) of **12** exhibits the signals of a secondary methyl group (d_H 1.30, d_C 17.5), a methylene group (d_H 3.03 / 2.88, d_C 27.5), and two methine groups (d_H 3.94, d_C 69.8, and d_H 3.92, d_C 74.5) linkage to oxygen atom besides the 3-subsituted indol moiety. The ^{1}H-^{1}H COSY NMR data reveals a proton spin system: CH_3-CH(O)-CH(O)-CH_2- besides the aromatic system which is agreement with the coupling patterns. The $J_{2,3}$ coupling constant of 7.4 Hz corresponds to the *threo*-form of the two hydroxyl groups. Thus, **12** is *threo*-1-(1H-indol-3-yl)-butane-2,3-diol, which is conformed by the 2D NMR data. Analysis of the NMR data of **13** led to the identical constitution with **12**. However, the The $J_{2,3}$ coupling constant of 5.9 Hz indicates the *erythro*-form of the two hydroxyl groups in **13**. Therefore, **13** is *erythro*-1-(1H-indol-3-yl)-butane-2,3-diol.



11 **12** **13** **14** **15**: R = H **16**: R=OH

Scheme 3. Structures of indoles **12** and **13** and miscellaneous compounds **11**, **14** to **16**.

2-Methyl-2,5-bornandiol (11): The HREI-MS of **11** gives a molecular ion at *m/z* 184.2465, corresponding to the molecular formula $C_{11}H_{20}O_2$ (calcd. 184.2462). The IR spectrum shows the presence of hydroxyl group (3400 cm^{-1}). The ^{1}H-NMR (500 MHz, $CDCl_3$) of **11** exhibits the signals of three tertiary methyl groups (d 1.14, 1.12 and 0.86), a tertiary methyl group (d_H 1.18, s, d_C 78.6, s) bearing an oxygen, and two methine groups (d 1.76 and 3.74, the latter bearing oxygen). The ^{13}C-NMR (125.0 MHz, $CDCl_3$) and DEPT spectra show 11 carbons: four methyls, two methylenes, a methine, two quaternary, and a methine (d 75.1) as well as a quaternary (d 78.6) bearing oxygen. The ^{1}H-^{1}H COSY NMR data reveals a proton spin system: -CH_2-CH-CH(O)-CH_2-. Detail analysis of 2D NMR data gives the structure of **11** as depicted in Scheme 1. **11** possesses the same structural skeleton as 2-methylisoborneol[9]. The difference is that **11** bears an additional hydroxyl group at C-5. Both the observable NOE effect between 2-CH_3 (d 1.18) and H-6a (d 1.87), and lack of NOE effects between 2-CH_3 (d 1.18) and 7-CH_3 (d 1.14, 1.12) in the NOESY spectrum of **11** result the identical stereochemistry of C-2 to 2-methylisoborneol. The correlative signals between H-5 (d 3.74) and 2-CH_3 (d 1.18), H-3a (d 1.25) indicate the 5b-OH.

2-Methyl-furan-3-carboxylic acid (14): The HREI-MS of compound **14** gives a molecular ion at *m/z* 126.0317, corresponding to the molecular formula $C_6H_6O_3$ (calcd. 126.0317). The IR spectrum shows the presence of a carbonic acid group (a broad and strong peak at 3258 cm^{-1}, and 1653 cm^{-1}). The ^{1}H-NMR (300 MHz, $CDCl_3$) of **14** exhibits the signals of five protons: a tertiary methyl group (d 2.36), and two conjugated olefinic protons (d 7.70 d, J = 5.5 Hz and 6.41 d, J = 5.5 Hz). The ^{13}C-NMR (75.0 MHz, $CDCl_3$) shows six carbons. Besides the proton-attached carbon atoms, with the signals of three quaternary carbon atoms (d = 148.5, 143.1 and 172.8 ppm) the ^{13}C-NMR spectrum points to an a,b-unsaturated carbonyl group. To agree with the molecular formula, a furan ring is formed and led to the structure of **14** as 2-Methyl-furan-3-carboxylic acid.

6,8-Dihydroxy-3-methylisocoumarin (15) and 6,8-Dihydroxy-3-hydroxy-methylisocoumarin (16): The major isolated compound **15** and **16** were determined as 6,8-Dihydroxy-3-methylisocoumarin (**15**) and 6,8-Dihydroxy-3-hydroxymethylisocoumarin (**16**) according to the spectra data compared with literature[10].

Biological Activities

Ten compounds (**1** to **4**, **8**, **10** to **12**, **15** and **16**) were tested in a number of biological tests[7] (antibacterial, antifungal, antiviral, cytotoxic and enzyme assays) and was found to be inactive except that both **15** and **16** showed medium cytotoxic activity and strong inhibiting activity on horse radish peroxidase. **15** also exhibited antiviral activity as well as a distinct inhibiting activity on 3a-hydroxysteroid dehydrogenase (3a-HSD). Three 2,3-disubstituted butanolides (**1**, **2** and **4**) and two 2,4-disubstituted butanolides (**8** and **9**) were also tested in an A-factor assay[11], but have been found inactive.

Discussion

Parallel chromatography allows to separate several samples simultaneously and rapidly shortens the time of isolation procedure. It will play vital role in High-Throughput-Isolation of natural products and improve competitiveness of natural products with synthetic and combinatorial libraries in drug discovery process. Integration of parallel chromatography on both, gel permeation and silica gel chromatography by making use of the CombiFlash^{TM} si1000s system (ISCO, Lincoln, USA) into purification procedure allowed us rapidly to isolate sixteen secondary metabolites belong to different classes compounds from *Streptomyces* sp. (GT 061089). It shows that parallel chromatography is efficient approach for speeding up the process of purification natural products.

However, there are still some problems which has to be solved. Parallel chromatography generates hulk fractions which have to be examined by TLC, because on-line detection system is not possible to integrate into parallel chromatography at present. Automatic TLC spot system will partially solve this problem. The CombiFlash^{TM} si1000s system (ISCO) requires the isolated samples possessing similar polarity because of one solvent system for all columns. A new parallel system called Biotage Quad3^{TM} (Biotage UK Limited, Hertford, UK) can run up to 12 pre-parked cartridges, in parallel. The advantage of the system is that each cartridge has its own pump head, which allows to use different solvent system for different sample. The number of fractions of each column per run of both parallel chromatography systems are limited, typically 20 to 40 fractions per column. This might make parallel chromatography to be not suitable for the first isolation step because the extracts of microorganisms or plants are usually complex mixtures.

Experimental Section

General method. See ref.[6,7]. Parallel chromatography: CombiFlash-10 (ISCO).

Culture media: Medium A: Soluble starch 10 g/l, $(NH_4)_2SO_4$ 2 g/l, K_2HPO_4 (1 g/l), NaCl (1 g/l), Mg_2SO_4 x 7 H_2O (1 g/l), $CaCO_3$ (2 g/l), trace element solution (5 ml/l) of 3 g/l of $CaCl_2$ x H_2O, 1 g/l of Fe(III) citrate, 0.2 g/l of $MnSO_4$, 0.1 g/l of $ZnCl_2$, 0.025 g/l of $CuSO_4$ x 5 H_2O, 0.02 g/l of $Na_2B_4O_7$ x 10 H_2O, 0.004 g/l of $CoCl_2$, 0.01 g/l of Na_2MoO_4 x 2 H_2O, pH = 7.0 prior to sterilization. Medium B: Soybean meal 2 %, mannitol 2 %, pH = 7.5 prior to sterilization.

Fermentation: A 1 cm^2 slant of agar from 7 d old cultures of GT 061089 grown on medium A was used to inoculate a 300-ml Erlenmeyer flask containing 100 ml of medium B. The flask was cultivated for 6 d at

28 ° C on a rotary shaker (180 rpm). The shaking culture was used for both, TLC analysis in the screening routine and for inoculation of eight inoculation flasks (400 ml) which were used for inoculation of 200-l fermentor containing medium B (duration of fermentation: 5 d, at 28 ° C, 500 rpm, aeration 10 l/min).

Isolation and purification: After harvesting, the culture broth was filtered and the culture filtrate was passed through a Amberlite-XAD 16 column and eluted with water/methanol (gradient from 20% to 70% methanol, then 100% methanol) to yield three fractions. The first fraction (elution from 20% to 50% methanol, 12 l) was dried in vacuum (56 g) and was extracted with methanol to give a oily crude material (15 g) which was chromatographed on silica gel (5.0 × 35 cm, $CHCl_3$/MeOH, gradient from 100:0 to 85:15) and sequentially purified by parallel gel permeation chromatography on Sephadex LH-20 (2.5 × 50 cm, Methanol), RP-C18 HPLC (2.5 × 25 cm, 7 mm, MeOH/H2O) and parallel chromatography on silica gel (1.1 × 30 cm, *n*-hexane/EtOAc, gradient from 4:1 to 2:1) to obtain 0.05 mg/l of **1**, 0.15 mg/l of **2**, 0.36 mg/l of **3**, 0.03 mg/l of **5**, 0.06 mg/l of **9**, 0.10 mg/l of **10** (Fig.1).

After dried in vacuum the second fraction (elution from 50% to 70% methanol, 6 l and the first part of 100% methanol, 2 l) yield 30 g of crude material which was extracted with EtOAc to give 3.0 g of brown oil. This material was chromatographed on silica gel (2.5 × 60 cm, $CHCl_3$/MeOH, gradient from 100:0 to 90:10) and sequentially purified by parallel gel permeation chromatography on Sephadex LH-20 (2.5 × 50 cm, Methanol), and parallel chromatography on silica gel (1.1 × 30 cm, *n*-hexane/EtOAc, gradient from 4:1 to 2:1) and RP-C18 HPLC (2.5 × 25 cm, 7 mm, MeOH/H2O) to obtain 0.32 mg/l of **1**, 0.19 mg/l of **4**, 0.05 mg/l of **6a** and **6b**, 0.04 mg/l of **7**, 0.13 mg/l of **8**, 0.30 mg/l of **11**, 0.15 mg/l of **12**, 0.08 mg/l of **13**, 0.05 mg/l of **14**, 0.30 mg/l of **15**, 0.10 mg/l of **16** (Fig.2).

The third fraction (elution of second part from 100% methanol, 10 l) was dried (30 g) and was extracted with EtOAc (5 l) to give 10 g of viscous material after evaporated the solvent. To this material 200 ml of EtOAc was added and filtered. The residue was re-crystallized in methanol to yield 1.0 g of **15**.

(E)-3-hydroxy-3-(1-hydroxy-hex-4-enyl)-4-hydroxymethyl-dihydro-furan-2-one (1): Colorless oil. $[a]_D$ = + 46.3 (c = 0.53, methanol). - IR (film): n = 3405, 2915, 1753, 1439, 1399, 1213, 1143, 1070, 966 cm^{-1}. – HREI MS: calcd. for $C_{11}H_{16}O_4$ 212.1049, found 212.1026 [M^+ - H_2O] (6); calcd. for $C_{11}H_{14}O_3$ 194.0943, found 194.0931 [M^+ - $2H_2O$] (6); calcd. for $C_{10}H_{13}O_3$ 181.0865, found 181.0860 [M^+ - H_2O - CH_2O] (5); calcd. for $C_6H_9O_4$ 145.0501, found 145.0497 [M^+ - C_5H_9O] (85); calcd. for $C_5H_8O_4$ 132.0422, found 132.0417 [M^+ - $C_6H_{10}O$] (95); calcd. for $C_4H_5O_3$ 101.0239, found 132.0232 [M^+ - $C_7H_{13}O_2$] (100). – ESI MS (positive ion); *m/z*: 230.9 [M^+ + H], 247.8 [M^+ + NH_4], 253.0 [M^+ + Na], 482.6 [2M + Na]$^+$. ^{13}C-NMR and ^{1}H-NMR: see Table 2 and 3.

3´-Hydroxy-5-(1-hydroxy-ethyl)-4´-hydroxymethyl-octahydro-[2,3´]bifuranyl-2´-one (2): Colorless oil. $[a]_D$ = + 7.4 (c = 3.51, methanol). - IR (film): n = 3430, 2970, 2915, 1761, 1633, 1462, 1346, 1206, 1069, 1032, 999 cm^{-1}. – HREI MS: calcd. for $C_{11}H_{19}O_6$ 247.1182, found 247.1178 [M^+ +1] (2); calcd. for $C_9H_{13}O_5$ 201.0763, found 201.0772 [M^+ - C_2H_5O] (55); 183 (52); calcd. for $C_7H_9O_4$ 157.0501, found 157.0496 [M^+ - $C_4H_9O_2$] (90); calcd. for $C_5H_8O_4$ 132.0422, found 132.0417 [M^+ - $C_6H_{10}O_2$] (80); calcd. for $C_6H_{11}O_2$ 115.0759, found 115.0754 [M^+ - $C_5H_7O_4$] (100). – ESI MS (positive ion); *m/z*: 247.0 [M^+ + H], 264.1 [M^+ + NH_4], 268.8 [M^+ + Na], 515.2 [M^+ + 2Na]. ^{13}C-NMR and ^{1}H-NMR: see Table 2 and 3.

***Epi*-3´-hydroxy-5-(1-hydroxy-ethyl)-4´-hydroxymethyl-octahydro-[2,3´]bifuranyl-2´-one (3):** Colorless oil. $[a]_D$ = + 15.5 (c = 0.92, methanol). - IR (film): n = 3425, 2970, 2915, 1761, 1635, 1376, 1216, 1067, 1015 cm^{-1}. – HREI MS: calcd. for $C_{11}H_{19}O_6$ 247.1182, found 247.1164 [M^+ +1] (5); calcd. for $C_{11}H_{17}O_5$ 229.1076, found 229.1091 [M^+ - OH] (4); calcd. for $C_9H_{13}O_5$ 201.0763, found 201.0760 [M^+ - C_2H_5O] (90); calcd. for $C_9H_{11}O_4$ 183.0656, found 183.0651 [M^+ - $C_2H_7O_2$] (85); calcd. for $C_7H_9O_4$ 157.0501, found 157.0493 [M^+ - $C_4H_9O_2$] (90); calcd. for $C_5H_8O_4$ 132.0422, found 132.0425 [M^+ - $C_6H_{10}O_2$] (70); calcd. for $C_6H_{11}O_2$ 115.0759, found 115.0754 [M^+ - $C_5H_7O_4$] (100). – ESI MS (positive ion); *m/z*: 247.0 [M^+ + H], 264.1 [M^+ + NH_4], 269.1 [M^+ + Na]. ^{13}C-NMR and ^{1}H-NMR: see Table 2 and 3.

3-(3-Methyl-oxiranyl)-acrylic acid 4-hydroxy-5-oxo-tetrahydro-furan-3-ylmethyl este (4): Colorless oil. $[a]_D$ = -43.1 (c = 0.70, methanol). - IR (film): n = 3370, 2960, 1766, 1722, 1640, 1451, 1359, 1303, 1266, 1194, 1009, 980 cm^{-1}. – HREI MS: calcd. for $C_{11}H_{14}O_6$ 242.0791, found 242.0790 [M]$^+$ (10); calcd. for $C_9H_{10}O_5$ 198.0528, found 198.0529 [M^+ - C_2H_4O] (50); calcd. for $C_5H_7O_3$ 115.0395, found 115.0393 [M^+ - $C_6H_7O_3$] (100); calcd. for $C_6H_6O_2$ 110.0368, found 110.0364 [M^+ - $C_5H_8O_4$] (90). – ESI MS (positive ion), *m/z*: 243.0 [M^+ + H], 260.0 [M^+ + NH_4]. ^{13}C-NMR and ^{1}H-NMR: see Table 2 and 3.

5-(1-Hydroxyethyl)-4´-hydroxymethyl-tetrahydro-[2,3´]bifuranyl-2´-one(5): Colorless oil. - IR (film): n = 3325, 2974, 1721, 1659, 1572, 1378, 1260, 1200, 1033 cm^{-1}. – HREI MS: calcd. for $C_{11}H_{16}O_5$ 228.0998, found 228.1020 [M]$^+$ (12); calcd. for $C_{10}H_{13}O_4$ 197.0915, found 197.0919 [M^+ - CH_3O] (100); calcd. for $C_{10}H_{11}O_3$ 179.0708, found 179.0716 [M^+ - H_2O – CH_3O] (85). – ESI MS (positive ion), *m/z*: 229.0 [M + H]$^+$, 250.9 [M + Na]$^+$, 479.2 [2M + Na]$^+$. ^{13}C-NMR and ^{1}H-NMR: see Table 2 and 3.

4-Hydroxymethyl-3-isobutryl-dihydro-furan-2-one / 6-hydroxy-6-isopropyl-tetrahydro-furo[3.4-c]furan-1-one (6): EI-MS; *m/z*:169 [M^+ - HO]. – ESI MS (positive ion); *m/z*: 208.9 [M^+ + Na]; (negative ion); *m/z*: 185.0 [M - H]$^-$. ^{1}H-NMR (300 MHz, $CDCl_3$) **6a**: d 4.44 (1H, dd, *J* = 9.0, 2.5 Hz, H-4a), 4.14 (1H, dd, *J* = 9.0, 6.6 Hz, H-4b), 3.85 (1H, d, *J* = 7.0 Hz, H-2), 3.71 (1H, dd, *J* = 10.8, 5.6 Hz, H-5a), 3.69 (1H, dd, *J* = 10.8, 5.7 Hz, H-5b), 3.21 (1H, m, H-3), 3.17 (1H, m, H-7), 1.20 (3H, d, *J* = 7.1 Hz, H-8), 1.14 (3H, d, *J* = 6.7 Hz, H-9). **6b**: d 4.47 (1H, dd, *J* = 9.0, 2.5 Hz, H-4a), 4.25 (1H, dd, *J* = 9.0, 6.0 Hz, H-4b), 4.20 (1H, dd, *J* = 8.9, 5.6 Hz, H-5a), 4.01 (1H, dd, *J* = 8.9, 5.6 Hz, H-5b), 3.40 (1H, m, H-3), 3.20 (1H, d, *J* = 7.0 Hz, H-2), 2.04 (1H, m, H-7), 1.07 (6H, d, *J* = 6.8 Hz, H-8, H-9). ^{13}C-NMR (75 MHz, $CDCl_3$) **6a**: d 206.4 (s, C-6), 172.3 (s, C-1), 69.0 (t, C-4), 61.9 (t, C-5), 52.9 (d, C-2), 40.3 (d, C-7), 39.7 (d, C-3), 18.5 (q, C-8), 17.2 (q, C-9). **6b**: d 174.5 (s, C-1), 109.1 (s, C-6), 71.7 (t, C-5), 71.4 (t, C-4), 51.9 (d, C-2), 41.9 (d, C-3), 36.7 (d, C-7), 17.2 (q, C-8), 16.7 (q, C-9).

2-(1-Hydroxy-4-methyl-hexyl)-4-hydroxymethyl-butanolide (7): Colorless oil. $[a]_D$ = + 47.4 (c = 0.92, methanol). - IR (film): n = 3415, 2950, 1753, 1460, 1362, 1194, 1054 cm^{-1}. – HREI MS: calcd. for $C_{11}H_{21}O_4$ 217.1440, found 217.1445 [M^+ + 1] (5); calcd. for $C_{11}H_{18}O_3$ 198.1256, found 198.1256 [M^+ - H_2O] (2); calcd. for $C_{10}H_{17}O_3$ 185.1178, found 185.11680 [M^+ - CH_2OH] (12); calcd. for $C_6H_9O_4$ 145.0501, found 145.0497 [M^+ - C_5H_{11}] (90); calcd. for $C_6H_7O_3$ 127.0395, found 127.0389 [M^+ - $C_5H_{13}O$] (70); calcd. for $C_5H_8O_3$ 116.0473, found 116.0465 [M^+ - $C_6H_{12}O$] (100). – ESI MS (^{1}H positive ion); *m/z*: 217.0 [M^+ + H], 239.0 [M^+ + Na], 455.3 [2M + Na]$^+$. ^{13}C-NMR and -NMR: see Table 4 and 5.

2-(1-Hydroxy-4-methyl-pentyl)-4-hydroxymethyl-butanolide (8): Colorless oil. - IR (film): n = 3415, 2925, 1752, 1454, 1371, 1194, 1054 cm^{-1}. – HREI MS: calcd. for $C_{12}H_{23}O_4$ 231.1596, found 213.1596 [M^+ + 1] (3); calcd. for $C_{12}H_{20}O_3$ 212.1412, found 212.1401 [M^+ - H_2O] (5); calcd. for $C_{11}H_{19}O_3$ 199.1334, found 199.1335 [M^+ - CH_2OH] (10); calcd. for $C_{11}H_{17}O_2$ 181.1229, found 181.1228 [M^+ - H_2O - CH_2OH] (50); calcd. for $C_6H_9O_4$ 145.0501, found 145.0501 [M^+ - C_5H_{11}] (90); calcd. for $C_6H_7O_3$ 127.0395, found 127.0404 [M^+ - $C_5H_{13}O$] (70); calcd. for $C_5H_8O_3$ 116.0473, found 116.0469 [M^+ - $C_6H_{12}O$] (100). – ESI MS (^{1}H positive ion); *m/z*: 231.0 [M^+ + H], 253.1 [M^+ + Na], 483.3 [2M + Na]$^+$. ^{13}C-NMR and -NMR: see Table 4 and 5.

2-(1,4-Dihydroxy-4-methyl-pentyl)-4-hydroxymethyl-butanolide (9): Colorless oil. - IR (film): n = 3395, 2965, 2960, 1751, 1634, 1450, 1363, 1197, 1056, 907 cm^{-1}. – HREI MS: calcd. for $C_{11}H_{19}O_4$ 215.1283, found 215.1270 [M^+ - OH] (2); calcd. for $C_{10}H_{15}O_4$ 199.0970, found 199.0971 [M^+ - CH_3 - H_2O] (85); calcd. for $C_{10}H_{13}O_3$ 181.0865, found 181.0866 [M^+ - $2H_2O$ – CH_3] (18); calcd. for $C_6H_9O_4$ 145.0501, found 145.0501 [M^+ - $C_5H_{11}O$] (35); calcd. for $C_7H_9O_2$ 125.0603, found 125.0600 [M^+ - $C_4H_{11}O_3$] (55); calcd. for $C_6H_{11}O$ 99.0810, found 99.0799 [M^+ - $C_5H_9O_4$] (100). – ESI MS (positive ion); *m/z*: 233.0 [M^+ + H], 250.0 [M^+ + NH_4], 255.3 [M^+ + Na], 487.2 [2M + Na]$^+$. – ESI MS (negative ion); *m/z*: 231.1 [M - H]$^-$. ^{13}C-NMR and ^{1}H-NMR: see Table 4 and 5.

2-(1,4-Dihydroxy-4-methyl-hexyl)-4-hydroxymethyl-butanolide (10): Colorless oil. - IR (film): n = 3385, 2965, 2960, 1752, 1453, 1366, 1195, 1056, 949 cm^{-1}. – HREI MS: calcd. for $C_{12}H_{21}O_4$ 229.1439, found 229.1430 [M^+ - OH] (2); calcd. for $C_{11}H_{17}O_4$ 213.1127, found 213.1136 [M^+ - CH_3 - H_2O] (30); calcd. for $C_{10}H_{15}O_4$ 199.0970, found 199.0971 [M^+ - C_2H_5 - H_2O] (18); calcd. for $C_{10}H_{16}O_3$ 184.1089, found 184.1094 [M^+ - $C_2H_6O_2$] (32); calcd. for $C_6H_9O_4$ 145.0501, found 145.0497 [M^+ - C_6H_3O] (55); calcd. for $C_7H_{13}O$ 113.0966, found 113.0964 [M^+ - $C_5H_9O_4$] (100). – ESI MS (positive ion); *m/z*: 269.1 [M^+ + Na], 515.3 [$2M^+$ + Na], 761.0 [3M + Na]$^+$. – ESI MS (negative ion); *m/z*: 245.1 [M - H]$^-$. ^{13}C-NMR and ^{1}H-NMR: see Table 4 and 5.

2-Methyl-2,5-bornandiol (11): White powder. $[a]_D$ = - 23.5 (c = 0.72, methanol). - IR (KBr): n = 3400, 2970, 2870, 1485, 1449, 1355, 1281, 1199, 1103, 1028 cm^{-1}. – HREI MS: calcd. for $C_{11}H_{20}O_2$ 184.2462, found 184.2465 [M^+] (2); calcd. for $C_{10}H_{17}O_2$ 169.1229, found 169.1228 [M^+ - CH_3] (10); calcd. for $C_{11}H_{18}O$ 166.1358, found 166.1348 [M^+ - H_2O] (25); calcd. for $C_{10}H_{15}O$ 151.1123, found 151.1125 [M^+ - CH_3 – H_2O] (90); calcd. for $C_8H_{11}O$ 123.0810, found 123.0819 [M^+ - C_3H_9O] (80); calcd. for $C_8H_{13}O$ 109.1017, found 109.1009 [M^+ - C_3H_9O] (100). – ESI MS (negative ion); *m/z*: 183.1 [M - H]$^-$. ^{1}H-NMR (500 MHz, $CDCl_3$) d 3.74 (1H, dd, *J* = 7.9, 4.3Hz, H-5), 2.07 (1H, dd, *J* = 13.5, 5.0 Hz, H-3b), 1.87 (1H, dd, *J* = 14.0, 7.9 Hz, H-6a), 1.76 (1H, d, *J* = 5.0 Hz, H-4), 1.60 (2H, brs, 2 x OH)

1.55 (1H, dd, *J* = 14.0, 4.3 Hz, H-6b), 1.25 (1H, d, *J* = 13.5 Hz, H-3a), 1.18 (3H, s, 2-CH_3), 1.14 (3H, s, 7-CH_3), 1.12 (3H, s, 7-CH_3), 0.86 (3H, s, 1-CH_3). ^{13}C-NMR (125.0 MHz, $CDCl_3$) d 78.6 (s, C-2), 75.1 (d, C-5), 54.0 (d, C-4), 53.2 (s, C-1), 48.5 (s, C-7), 43.8 (t, C-3), 42.4 (t, C-6), 26.7 (q, 2-CH_3), 22.4 (q, 7-CH_3), 22.0 (q, 7-CH_3), 9.5 (q, 1-CH_3).

***Threo*-1-(1H-indol-3-yl)-butane-2,3-diol (12):** Colorless oil. $[a]_D$ = + 38.6 (c = 0.39, methanol). - IR (film): n = 3405, 2970, 2910, 1616, 1452, 1349, 1227, 1056, 981, 740 cm^{-1}. – HREI MS: calcd. for $C_{12}H_{15}NO_2$ 205.1103, found 205.1116 $[M]^+$ (90); calcd. for $C_{10}H_{10}NO$ 160.0762, found 160.0768 $[M^+ - CH_5O]$ (8); calcd. for C_9H_8O 130.0657, found 130.0660 $[M^+ - C_3H_7O_2]$ (100). – ESI MS (positive ion); *m/z*: 206.1 $[M^+ + H]$, 228.0 $[M^+ + Na]$, 433.3 $[2M + Na]^+$. ^{1}H-NMR (500 MHz, $CDCl_3$) d 8.10 (1H, br. s, NH), 7.63 (1H, d, *J* = 7.9 Hz, 4´-H), 7.38 (1H, dd, *J* = 0.9, 8.1 Hz, 7´-H), 7.22 (1H, dt, *J* = 1.0, 7.9 Hz, 6´-H), 7.14 (1H, dt, *J* = 1.0, 8.0 Hz, 5´-H), 7.09 (1H, d, *J* = 2.3 Hz, 2´-H), 3.94 (1H, dq, *J* = 7.4, 6.3Hz, 3-H), 3.92 (1H, ddd, *J* = 9.1, 7.4, 3.7 Hz, 2-H), 3.03 (1H, ddd, *J* = 14.6, 3.7, 0.8 Hz, 1-Ha), 2.88 (1H, dd, *J* = 14.6, 9.1 Hz, 1-Hb), 2.10 (1H, br. s, OH), 2.03 (1H, br. s, OH), 1.30 (3H, d, *J* = 6.3 Hz, 4-H). ^{13}C-NMR (125 MHz, $CDCl_3$) d 136.3 (s, C-7´a), 127.5 (s, C-3´a), 122.8 (d, C-2´), 122.4 (d, C-6´), 119.6 (d, C-5´), 118.9 (d, C-4´), 111.8 (s, C-3´), 111.3 (d, C-7´), 74.5 (d, C-2), 69.8 (d, C-3), 27.5 (t, C-1), 17.5 (q, C-4).

***Ethro*-1-(1H-indol-3-yl)-butane-2,3-diol (13):** Colorless oil. $[a]_D$ = + 6.6 (c = 0.12, methanol). - IR (film): n = 3410, 2910, 1600, 1452, 1351, 1228, 1045, 981, 740 cm^{-1}. – HREI MS: calcd. for $C_{12}H_{15}NO_2$ 205.1103, found 205.1114 $[M]^+$ (50); calcd. for $C_{10}H_{10}NO$ 160.0762, found 160.0768 $[M^+ - CH_5O]$ (8); calcd. for C_9H_8O 130.0657, found 130.0656 $[M^+ - C_3H_7O_2]$ (100). – ESI MS (positive ion); *m/z*: 206.1 $[M^+ + H]$, 228.0 $[M^+ + Na]$, 433.3 $[2M + Na]^+$. – ESI MS (negative ion); *m/z*: 204.1 $[M - H]^-$. ^{1}H-NMR (500 MHz, $CDCl_3$) d 8.10 (1H, br. s, NH), 7.63 (1H, d, *J* = 7.9 Hz, 4´-H), 7.37 (1H, d, *J* = 8.1 Hz, 7´-H), 7.22 (1H, dt, *J* = 0.9, 8.1 Hz, 6´-H), 7.14 (1H, dt, *J* = 0.9, 7.9 Hz, 5´-H), 7.10 (1H, d, J = 2.2 Hz, 2´-H), 3.75 (1H, dq, *J* = 5.9, 6.3Hz, 3-H), 3.71 (1H, ddd, *J* = 8.6, 5.9, 4.0 Hz, 2-H), 3.06 (1H, ddd, J = 14.6, 4.0, 0.7 Hz, 1-Ha), 2.85 (1H, dd, J = 14.6, 8.6 Hz, 1-Hb), 2.20 (2H, br. s, 2 × OH), 1.31 (3H, d, J = 6.3 Hz, 4-H). ^{13}C-NMR (125 MHz, $CDCl_3$) d 136.4 (s, C-7´a), 127.5 (s, C-3´a), 122.9 (d, C-2´), 122.3 (d, C-6´), 119.6 (d, C-5´), 118.8 (d, C-4´), 111.3 (s, C-3´), 111.3 (d, C-7´), 75.5 (d, C-2), 70.2 (d, C-3), 29.6 (t, C-1), 19.5 (q, C-4).

2-Methyl-furan-3-carboxylic acid (14): Colorless crystal. - IR (film): n = 3258, 3050, 2950, 1653, 1619, 1561, 1461, 1258, 1201, 1074, 921, 842 cm^{-1}. – HREI MS: calcd. for $C_6H_6O_3$ 126.0317, found 126.0317 $[M]^+$ (100). – ESI MS (positive ion); *m/z*: 126.8 $[M^+ + H]$, 148.7 $[M^+ + Na]$, 274.8 $[2M + Na]^+$. ^{1}H-NMR (300 MHz, $CHCl_3$) d 7.70 (1H, d, *J* = 5.5 Hz, H-5), 6.41 (1H, d, *J* = 5.5 Hz, H-4), 2.36 (3H, s, 1-CH_3). ^{13}C-NMR NMR (75.0 MHz, $CHCl_3$) d 172.8 (s, 2-COOH), 154.3 (d, C-5), 148.5 (s, C-2), 143.1 (s, C-3), 112.8 (d, C-4), 14.2 (q, 1-CH_3).

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