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™ si1000s system (ISCO, Lincoln, USA) into purification process to speed up the isolation of secondary 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-disubstituted 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\cite{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™-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)\cite{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\cite{5-7}, a number of so-called “talented” strains\cite{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™ si1000s System**
The CombiFlash™ 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\textsuperscript{a} | Rf\textsuperscript{b} | Color reactions \textsuperscript{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                            |
| 13     | II      | 0.08         | 0.40                   | 0.93                  | Dark Purple                            |
| 14     | II      | 0.05         | 0.61                   | 0.85                  | Dark Light purple                      |
| 15     | II, III | 1.30         | 0.60                   | 0.97                  | Dark Blue gray                         |
| 16     | II, III | 0.10         | 0.34                   | 0.95                  | Dark Blue gray                         |

\textsuperscript{a} CHCl\textsubscript{3}/MeOH (9:1), \textsuperscript{b} n-butanol/acetic acid/water (4:1:5) upper layer, \textsuperscript{c} UV (254 nm), \textsuperscript{d} anisaldehyde, \textsuperscript{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™ 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 $\text{C}_{11}\text{H}_{18}\text{O}_{5}$ 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 $m/z = 212.1026$ ($\text{C}_{11}\text{H}_{16}\text{O}_4$, calcd. 212.1049, $M^+ - \text{H}_2\text{O}$) and 194.0931 ($\text{C}_{11}\text{H}_{14}\text{O}_3$, calcd. 194.0943, $M^+ - 2\text{H}_2\text{O}$). The IR absorption bands at 3430 and 1761 cm$^{-1}$ indicates the presence of hydroxyl groups and an $\alpha$-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: $	ext{-O-CH}_2\text{-CH-CH}_2\text{-O}$- and $	ext{-CH(O)-CH}_2\text{-CH-CH} = \text{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](image)

**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 | $^1$H-NMR data of the compounds 1 to 5. |
|----------|----------------------------------------|
| 2        | 4.60 (d, 8.1)                          |
| 3        | 2.74 (dd, 8.0, 5.7, 4.5, 4.3)           |
| 4a       | 4.43 (dd, 9.2, 8.0)                     |
| 4b       | 4.25 (dd, 9.2, 4.5)                     |
| 5a       | 3.84 (dd, 11.5, 4.3)                    |
| 5b       | 3.78 (dd, 11.5, 5.7)                    |
| 6        | 3.74 (br.d, 10.3)                       |
| 7        | 1.74 (m) / 1.78 (m)                     |
| 8        | 2.24 (m) / 2.10 (m)                     |
| 9        | 5.46 (m)                               |
| 10       | 5.48 (dq, 9.5, 5.9)                     |
| 11       | 1.64 (dd, 5.9, 1.0)                     |

[a]: in CDCl$_3$ (500 MHz); [b]: in CD$_3$OD (300 MHz).

$^3$-Hydroxy-5-(1-hydroxy-ethyl)-4'-hydroxyxymethyl-octahydro-[2,3']bifuranyl-2'-one (2) and *Epi*-3'-hydroxy-5-(1-hydroxy-ethyl)-4'-hydroxyxymethyl-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-$\gamma$-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 $^{3}$-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: $[\alpha]_D = +7.4$ (c = 3.51, methanol); 3: $[\alpha]_D = +15.5$ (c = 0.92, methanol)}. Therefore, $^{3}$ is $\text{epi}-3´$-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 $^{17}$.

|   | $^{1}$ [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) | 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) | 77.8 (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) | 17.8 (q) | 18.9 (q) | 18.0 (q) | 13.1 (q) | 19.3 (q) | 11.7 (q) | 22.9 (q) |

[a]: in CDCl$_3$ (125 MHz); [b]: in CD$_3$OD (75 MHz); [c]: in CD$_3$OD (125 MHz).

$\text{3-(3-Methyl-oxiranyl)-acrylic acid 4-hydroxy-5-oxo-tetrahydro-furan-3-ylmethyl ester (4)}$: 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 $\gamma$-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$_3$, 500 MHz) 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 ($d = 4.46, 3.48, 3.30$ 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 $\gamma$-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-.
The $J_{2,8}$ 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 MHz). 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$_{12}$H$_{22}$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,$\beta$-unsaturated carbonyl group (1721, 1659 cm$^{-1}$). The $^1$H- and $^{13}$C-NMR (300 MHz, CD$_3$OD) 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,$\beta$-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 shows the presence of hydroxyl group (3325 cm$^{-1}$) and a,$\beta$-unsaturated carbonyl group (1721, 1659 cm$^{-1}$). The $^1$H- and $^{13}$C-NMR (300 MHz, CD$_3$OD) spectra show signals of 14 protons and 11 carbons, respectively (Table 2 and Table 3). A comparison of NMR data 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) 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 related butanolide 17.

| H     | 7 [a] | 8 [a] | 9 [b] | 10 [b] | 17 [b] |
|-------|-------|-------|-------|--------|--------|
| 2     | 2.84  | 2.84  | 2.80  | 2.84   | 2.81   |
| 2a    |       |       |       |        |        |
| 3a    | 2.19  | 2.20  | 2.30  | 2.25   | 2.25   |
| 3b    | 2.04  | 2.05  | 2.23  | 2.20   | 2.17   |
| 4     | 4.49  | 4.50  | 4.50  | 4.57   | 4.57   |
| 5a    | 3.74  | 3.74  | 3.92  | 3.92   | 3.90   |
| 5b    | 3.62  | 3.62  | 3.70  | 3.70   | 3.70   |
| 6     | 4.00  | 4.00  | 4.15  | 4.19   | 4.16   |
| 7     | 1.50  | 1.45  | 1.64  | 1.65   | 1.48   |
| 8     | 1.48  | 1.45  | 1.70  | 1.50   | 1.48   |
| 9     | 1.33  | 1.60  | 1.70  | 1.50   |        |
| 10    | 1.36  | 0.90  | 1.25  | 1.70   | 1.34   |
| 11    | 0.88  | 0.91  | 1.27  | 0.90   | 0.86   |
| 12    | 0.89  | 1.23  |        |        | 0.87   |

[a]: in CD$_3$OD (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_{5}. The IR, 1H-NMR, and 13C-NMR spectra of 7 and 8 (Table 4 and Table 3) indicate that the two secondary ones (d 0.90, d, and 0.91, d) in 8. And one methane group (d 1.60, d, 36.3 in 8) is replaced by a quarternary 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-pentyl)-4-hydroxymethyl-butanolide (9): Combination of the results from HREI-MS and ESI-MS spectra of 9 revealed the molecular formula C_{12}H_{22}O_{5}, possessing one more oxygen atom in comparison to 8. The IR, 1H-NMR, and 13C-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 methane group (d 1.60, d, 36.3 in 8) is replaced by a quarternary 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.

Indoles and Miscellaneous Compounds

Three-1-(1H-indol-3-yl)-butane-2,3-diol (12) and Ethere-1-(1H-indol-3-yl)-butane-2,3-diol (13): Compounds 12 and 13 possess the same molecular formula C_{12}H_{22}O_{5} which were determined by HREI-MS and ESI-MS. The IR spectra of both compounds show the absorption peaks for indole skeleton (11: 1616, 1452 cm^{-1}; 12: 3410 cm^{-1} and hydroxyl group (11: 3405 cm^{-1}; 12: 3410 cm^{-1}). The 1H-NMR and 13C-NMR spectra of 12 exhibits the signals of a secondary methyl group (d 1.30, d, 17.5), a methane group (d 3.03 / 2.88, d, 27.5), and two methyne groups (d 3.94, d, 69.8, and d 3.92, d, 74.5) linkage to oxygen atom besides the 3-substituted indole moiety. The 1H-NMR COSY NMR data reveals a proton spin system: CH2-CH-CH(O)-CH2-. Details analysis of 2D-NMR data gives the structure of 12 as depicted in Scheme 1. The 1H-NMR and 13C-NMR (CDCl3) of 11 exhibits the signals of three tertiary methyl groups (d 1.14, 1.12, and 0.86), a tertiar methine (d 75.1) as well as a quaternary (d 78.6) bearing an oxygen, and two methyne groups (d 1.76 and 3.74, the latter bearing oxygen). The 13C-NMR (125.0 MHz, CDCl3) and DEPT spectra show 11 carbons: four methylenes, two methylenes, a methine, two quarternaries, and a methine (d 75.1) as well as a quaternary (d 78.6) bearing an oxygen. The 1H-NMR COSY NMR data reveals a proton spin system: CH2-CH-CH(O)-CH2-. Details analysis of 2D-NMR data gives the structure of 11 as depicted in Scheme 1. The 1H-NMR and 13C-NMR (CDCl3) of 9 exhibits the signals of three 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 methane group (d 1.60, d, 36.3 in 9) is replaced by a quarternary 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 9 is missing in 10. In comparison to 9, the analysis of the 2D-NMR spectra led to the constitution of 10 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 9 and 10.

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
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 50% to 60% methanol, 12) was dried in vacuum (56 g) and was extracted with methanol to give a oily crude material (15 g) which was chromatoographed on silica gel (5.0 × 35 cm, CHCl3/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 of I, 0.15 mg of J, 0.36 mg of K, 3.03 mg of L, 0.05 mg of N, 0.15 mg of M, 0.30 mg of O, 0.08 mg of P, 1.15 mg of Q, 0.30 mg of R, 1.0 mg of S (Table 1).

After drying in vacuum to yield 30 g of crude material, 61 and the first part of 100% methanol, 2.1) yield 30 g of crude material which was extracted with EtOAc to give 3.0 g of brown oil. This material was chromatoographed on silica gel (2.5 × 60 cm, CHCl3/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 of I, 0.19 mg of J, 0.04 mg of K, 6.04 mg of L, 0.13 mg of M, 0.15 mg of N, 0.10 mg of O (Fig.1).

The third fraction (elution of second part from 100% methanol, 10) 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 g of K (Fig.2).

Epi-3’-Hydroxy-5(1-hydroxy-ethyl)-4’-hydroxymethyl-octahydro-[2,3’]bifuranyl-2’-one (3): Colorless oil. [α] D = -43.1 (c = 0.70, methanol). - IR (film): n = 3405, 2972, 2915, 1761, 1633, 1426, 1206, 1069, 1032, 999 cm -1. – HREI MS: m/z 247 [M + H]+, 259 [M + Na]+, 499 [2M + Na]+. 13C-NMR and 1H-NMR: see Table 2 and 3.

2-Methyl-2,5-bornandiol (11): Colorless oil. - IR (film): n = 3340, 2970, 2915, 1761, 1633, 1426, 1206, 1069, 1032, 999 cm -1. – HREI MS: m/z 213 [M - H]-. 1H-NMR (500 MHz, CDCl 3) d 3.74 (1H, dd, J = 13.5, 5.0 Hz, H-3 b), 1.87 (1H, dd, J = 7.0, 5.0 Hz, H-4), 2.04 (1H, m, H-7), 1.07 (6H, d, J = 10.8, 5.7 Hz, H-3a), 3.17 (1H, m, H-5b), 1.20 (1H, d, J = 7.1 Hz, H-8), 1.14 (1H, d, J = 6.7 Hz, H-9), 6d: d 4.47 (1H, dd, J = 9.0, 2.5 Hz, H-6a), 4.25 (1H, dd, J = 9.0, 6.0 Hz, 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-9, 13C-NMR (75 MHz, CDCl3) d: 206.4 (c, C-6), 172.3 (c, C-7), 69.0 (c, C-4), 61.9 (c, C-5), 52.9 (c, C-2), 40.3 (d, C-7), 39.7 (d, C-3), 18.5 (q, C-8), 17.2 (q, C-9), 6d: b 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-9), 18.6 (q, C-7).

Epi-3’-Hydroxy-5(1-Hydroxy-ethyl)-4’-hydroxymethyl-octahydro-[2,3’]bifuranyl-2’-one (2): Colorless oil. - IR (film): n = 3425, 2972, 2915, 1761, 1633, 1426, 1206, 1069, 1032, 999 cm -1. – HREI MS: m/z 247 [M + H]+, 259 [M + Na]+, 479 [2M + Na]+. 13C-NMR and 1H-NMR: see Table 2 and 3.

2-[(1,4-Dihydroxy-4-methyl-pentyl)-4-hydroxymethyl-butanolide (9): Colorless oil. - IR (film): n = 2315, 2153, 2000, 1712, 1634, 1400, 1398, 1376, 1364, 1281, 1200, 1103 cm -1. – HREI MS: m/z 247 [M + H]+, 259 [M + Na]+, 479 [2M + Na]+. 13C-NMR and 1H-NMR: see Table 2 and 3.

2-[(1-Hydroxy-4-methyl-pentyl)-4-hydroxymethyl-butanolide (8): Colorless oil. - IR (film): n = 3430, 2970, 2915, 1761, 1633, 1426, 1206, 1069, 1032, 999 cm -1. – HREI MS: m/z 247 [M + H]+, 259 [M + Na]+, 479 [2M + Na]+. 13C-NMR and 1H-NMR: see Table 2 and 3.

2-[(1-Hydroxy-4-methyl-hexyl)-4-hydroxymethyl-butanolide (7): Colorless oil. - IR (film): n = 2470, 2315, 2122, 2000, 1712, 1634, 1400, 1398, 1376, 1364, 1281, 1200, 1103 cm -1. – HREI MS: m/z 247 [M + H]+, 259 [M + Na]+, 479 [2M + Na]+. 13C-NMR and 1H-NMR: see Table 2 and 3.

2-(1-Hydroxy-4-methyl-pentyl)-4-hydroxymethyl-butanolide (6): Colorless oil. - IR (film): m/z 247 [M+ + H], 264.1 [M+ + Na], 482.8 [2M + Na]+. 13C-NMR and 1H-NMR: see Table 2 and 3.

2-Methyl-2,5-bornandiol (11): Colorless oil. - IR (film): n = 3405, 2972, 2915, 1761, 1633, 1426, 1206, 1069, 1032, 999 cm -1. – HREI MS: m/z 247 [M + H]+, 259 [M + Na]+, 479 [2M + Na]+. 13C-NMR and 1H-NMR: see Table 2 and 3.

2-(1-Hydroxy-4-methyl-pentyl)-4-hydroxymethyl-butanolide (6): Colorless oil. - IR (film): m/z 247 [M+ + H], 264.1 [M+ + Na], 482.8 [2M + Na]+. 13C-NMR and 1H-NMR: see Table 2 and 3.

2-[(1-Hydroxy-4-methyl-6a)-4-hydroxymethyl-butanolide (5): Colorless oil. - IR (film): n = 3400, 2970, 2915, 1761, 1633, 1426, 1206, 1069, 1032, 999 cm -1. – HREI MS: m/z 247 [M + H]+, 259 [M + Na]+, 479 [2M + Na]+. 13C-NMR and 1H-NMR: see Table 2 and 3.
1.55 (1H, dd, J = 14.0, 4.3 Hz, H-6b), 1.25 (1H, d, J = 13.5 Hz, H-3a), 1.18 (1H, s, 2-CH3), 1.14 (3H, s, 7-CH3), 1.12 (3H, s, 1-CH3), 0.86 (3H, s, 1-CH3). 13C-NMR (125.0 MHz, CDCl3) 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 (s, C-3), 42.4 (t, C-6), 26.7 (q, 2-CH3), 22.4 (q, 7-CH3), 9.5 (q, 1-CH3).

**Threo-1-(1H-indol-3-yl)-butane-2,3-diol (12):** Colorless oil. [α]D = +38.6 (c = 0.39, methanol). - IR (film): ν = 3405, 2970, 1610, 1452, 1369, 1277, 1056, 981, 740 cm⁻¹. - HREI MS: calcd. for C12H15NO2 205.1103, found 205.1114 [M⁺] (90); calcd. for C10H9NO3Na 206.0448, found 206.0462 [M⁺ + Na⁺] (100). - ESI MS (positive ion); m/z: 206.1 [M⁺ + H⁺], 228.0 [M⁺ + Na⁺], 433.3 [2M⁺ + Na⁺]. 1H-NMR (500 MHz, CDCl3) d 8.10 (1H, br. s, NH), 7.70 (1H, d, J = 7.9 Hz, 4'-H), 7.38 (1H, dd, J = 0.9, 8.1 Hz, 7'-H), 7.22 (1H, d, J = 1.0, 8.0 Hz, 5'-H), 7.09 (1H, d, J = 2.3 Hz, 7'-H), 7.00 (1H, d, J = 7.9 Hz, 2'-H), 6.30 (3H, s, 3-CH3), 3.92 (1H, d, J = 9.1, 7.4, 3.7 Hz, 2-H), 3.03 (1H, dd, J = 14.6, 3.7, 0.8 Hz, 2-Ha), 2.88 (1H, dd, J = 14.6, 9.1 Hz, 1-Hb), 2.10 (1H, br. s, OH), 2.03 (3H, s, 2-H), 1.30 (3H, d, J = 6.3 Hz, 4-H). 13C-NMR (125 MHz, CDCl3) d 136.4 (s, C-7a), 127.5 (d, C-3a), 112.8 (s, C-4), 122.2 (d, C-6), 119.6 (d, C-5), 118.9 (d, C-4), 111.8 (s, C-3), 117.3 (d, C-7), 74.5 (d, C-2), 69.8 (q, 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. [α]D = +6.6 (c = 0.12, methanol). - IR (film): ν = 3410, 2910, 1600, 1452, 1369, 1277, 1045, 981, 740 cm⁻¹. - HREI MS: calcd. for C12H15NO2 204.1105, found 204.1114 [M⁺] (90); calcd. for C10H9NO3Na 205.0452, found 205.0462 [M⁺ + Na⁺] (100). - ESI MS (positive ion); m/z: 206.1 [M⁺ + H⁺], 228.0 [M⁺ + Na⁺]. 1H-NMR (500 MHz, CDCl3) 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, d, J = 0.9, 8.1 Hz, 6'-H), 7.14 (1H, d, J = 0.9, 7.9 Hz, 5'-H), 7.10 (1H, d, J = 2.2 Hz, 2'-H), 3.75 (1H, q, J = 5.9, 6.3Hz, 3-H), 3.71 (1H, d, J = 8.6, 5.9, 4.0 Hz, 2-H), 3.06 (1H, dd, 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). 13C-NMR (125 MHz, CDCl3) d 136.4 (s, C-7a), 127.5 (d, C-3a), 122.2 (d, C-6), 119.6 (d, C-5), 118.8 (d, C-4), 111.3 (s, C-3), 117.3 (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): ν = 3288, 3050, 2950, 1653, 1519, 1561, 1461, 1258, 1201, 1074, 921, 842 cm⁻¹. - HREI MS: calcd. for C6H7O2 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⁺]. 1H-NMR (300 MHz, CHCl3) 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-CH3). 13C-NMR (75.0 MHz, CHCl3) d 172.8 (s, 2-COOH), 154.3 (s, C-5), 148.5 (s, C-2), 143.1 (s, C-3), 112.8 (d, C-4), 142.1 (q, 1-CH3).

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