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\(^[1,2,3]\). 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)\(^[4]\). 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).
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**

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

<sup>a</sup> CHCl<sub>3</sub>/MeOH (9:1), <sup>b</sup> n-butanol/acetic acid/water (4:1:5) upper layer, <sup>c</sup> UV (254 nm), <sup>d</sup> anisaldehyde, <sup>e</sup> 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 obtained 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.
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$_2$O) and 194.0931 (C$_{11}$H$_{14}$O$_3$, calcd. 194.0943, M$^+$ - 2H$_2$O). The IR absorption bands at 3430 and 1761 cm$^{-1}$ indicates the presence of hydroxyl groups and an $\gamma$-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.

<table>
<thead>
<tr>
<th>Position</th>
<th>(1) (^{[a]})</th>
<th>(2) (^{[a]})</th>
<th>(3) (^{[a]})</th>
<th>(4) (^{[a]})</th>
<th>(5) (^{[b]})</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4.60 (d, 8.1)</td>
<td>2.74 (ddd, 8.0, 5.7, 4.5, 4.3)</td>
<td>2.74 (ddd, 7.6, 5.7, 4.9, 4.3)</td>
<td>2.65 (ddd, 7.7, 5.7, 4.0)</td>
<td>2.98 (dddtd, 8.1, 7.6, 5.6, 3.9)</td>
</tr>
<tr>
<td>3</td>
<td>2.74 (ddd, 8.0, 5.7, 4.5, 4.3)</td>
<td>2.74 (ddd, 8.0, 5.7, 4.5, 4.3)</td>
<td>2.65 (ddd, 7.7, 5.7, 4.0)</td>
<td>2.65 (ddd, 7.7, 5.7, 4.0)</td>
<td>2.98 (dddtd, 8.1, 7.6, 5.6, 3.9)</td>
</tr>
<tr>
<td>4a</td>
<td>4.43 (dd, 9.2, 8.0)</td>
<td>4.39 (dd, 9.3, 7.6)</td>
<td>4.38 (dd, 9.3, 7.7)</td>
<td>4.40 (dd, 9.7, 7.6)</td>
<td>4.31 (dd, 9.0, 8.3)</td>
</tr>
<tr>
<td>4b</td>
<td>4.25 (dd, 9.2, 4.5)</td>
<td>4.21 (dd, 9.3, 4.9)</td>
<td>4.23 (dd, 9.3, 4.0)</td>
<td>4.36 (dd, 9.7, 5.6)</td>
<td>4.25 (dd, 9.0, 3.9)</td>
</tr>
<tr>
<td>5a</td>
<td>3.84 (dd, 11.5, 4.3)</td>
<td>3.83 (dd, 11.5, 4.3)</td>
<td>3.85 (d, 5.7)</td>
<td>4.47 (dd, 11.5, 3.9)</td>
<td>3.74 (dd, 10.9, 3.8)</td>
</tr>
<tr>
<td>5b</td>
<td>3.78 (dd, 11.5, 5.7)</td>
<td>3.81 (dd, 11.5, 5.7)</td>
<td>3.85 (d, 5.7)</td>
<td>4.30 (dd, 11.5, 5.6)</td>
<td>3.61 (dd, 10.9, 7.1)</td>
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<tr>
<td>6</td>
<td>3.74 (br.d, 10.3)</td>
<td>4.12 (t, 6.7)</td>
<td>4.21 (dd, 7.2, 5.8)</td>
<td>3.74 (br.d, 10.3)</td>
<td>4.12 (t, 6.7)</td>
</tr>
<tr>
<td>7</td>
<td>1.74 (m) / 1.78 (m)</td>
<td>2.27 (m) / 2.00 (m)</td>
<td>2.18 (m) / 1.95 (m)</td>
<td>2.65 (dd, 15.7, 0.8)</td>
<td>6.05 (dd, 15.7, 0.8)</td>
</tr>
<tr>
<td>8</td>
<td>2.24 (m) / 2.13 (m)</td>
<td>2.10 (m) / 1.89 (m)</td>
<td>1.78 (m) / 1.95 (m)</td>
<td>6.79 (dd, 15.7, 6.5)</td>
<td>2.07 (m) / 2.14 (m)</td>
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<tr>
<td>9</td>
<td>5.46 (m)</td>
<td>3.96 (td, 6.8, 2.6)</td>
<td>3.99 (m)</td>
<td>3.48 (dddd, 6.6, 4.4, 0.8)</td>
<td>4.40 (td, 7.1, 4.6)</td>
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<td>10</td>
<td>5.48 (dq, 9.5, 5.9)</td>
<td>4.10 (qd, 5.1, 2.6)</td>
<td>3.97 (qd, 6.4, 2.8)</td>
<td>3.30 (qd, 5.3, 4.4)</td>
<td>3.90 (qd, 6.5, 4.6)</td>
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<tr>
<td>11</td>
<td>1.64 (dd, 5.9, 1.0)</td>
<td>1.13 (d, 5.1)</td>
<td>1.11 (d, 6.4)</td>
<td>1.29 (d, 5.3)</td>
<td>1.19 (d, 6.5)</td>
</tr>
</tbody>
</table>

\[^{[a]}\]: in CDCl\(_3\) (500 MHz); \[^{[b]}\]: in CD\(_3\)OD (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\(^{-1}\)).
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 ($\delta$ 81.0) and H-9 ($\delta$ 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 ($\delta$ 2.74) and H-6 ($\delta$ 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 ($\delta$ 4.12) and H-9 ($\delta$ 3.96). 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.](image)

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 1H-NMR spectrum (CDCl$_3$, 500 MHz) of 4 exhibits 14 protons signals which indicate two conjugated olefinic protons at $\delta$ 6.79 (dd, $J$ = 15.7, 6.5 Hz) and 6.05 (dd, $J$ = 15.7, 0.8 Hz), four methine groups ($\delta$ = 4.60, 3.48, 3.30 and 2.98 ppm), two methylene groups ($\delta$  = 4.40/4.36 and 4.47/4.30 ppm) as well as a methyl group at $\delta$ 1.29. This is agreement with the 13C-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 ($\delta$  176.7) and a conjugated carbonyl ($\delta$ 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-.
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 $J_{8,9}$ (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-Hydroxy-4-methyl-hexyl)-4-hydroxymethyl-butanolide (7): The molecular formula, C$_14$H$_{22}$O$_5$, was determined from the HREI-MS spectrum of 5 (m/z = 228.1020, calcd. 228.0998) 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, 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,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-furan-2-one depicted in Scheme 1.

Table 4. $^1$H-NMR data of the compounds 7 to 10 and related butanolide 17.

![Scheme 2. Structures of isolated 2,4-disustituted butanolides 7 to 10 and related butanolide 17](image)

4-Hydroxymethyl-3-isobutyryl-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 only one molecular weight, 186 g/mol, for both compounds. Detailed investigation of 2D ($^1$H-1H COSY, HSQC and HMBC) NMR data led to the structures of both compounds. However, an observable NOE effect between H-6 (d $= 4.00$) and H-11 (d $= 6.5$) in the NOESY spectrum of 6 indicates a trans relative orientation of the substituted groups at C-2 and C-3 in the $\gamma$-lactone moiety, while a cis-2,4-disubstituted pattern in 17.

[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 {^1}H-NMR, and {^{13}}C-NMR spectra of 8 and 9 (Table 4 and Table 3) indicate that these compounds possess the same structural skeleton as 2-methylisoborneol [9]. The difference is that 8 possesses the same structural skeleton as 2-methylisoborneol [9]. The difference is that 8 possesses the same structural skeleton as 2-methylisoborneol [9].

Indoles and Miscellaneous Compounds

Threo-1-(1H-indol-3-yl)-butane-2,3-diol (12) and Ethreo-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 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 spectra of 12 exhibit the signals of a secondary methyl group (d 1.30, d 17.5), a methylene group (d 3.03 / 2.88, d 27.5), and two methine groups (d 3.94, d 74.5). The IR spectrum shows the presence of hydroxyl group (3400 cm^{-1}). The {^1}H-NMR (500 MHz, CDCl3) of 11 exhibits the signals of three tertiary methyl groups (d 1.14,1.12 and 0.86), a tertiary methylene group (d 1.74, s, d 78.6), a tertiary methine group (d 1.37 and 3.74, the latter bearing oxygen). The compounds 12 and 13 exhibit 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 11.

Fermentation:

Streptomyces sp. was tested in a number of biological tests [7] (antibacterial, antifungal, antiviral, cytotoxic and enzyme assays) and was found to be inactive except that both 12 and 13 were determined as 2-Methyl-furan-3-carboxylic acid (14). The HREI-MS spectrum shows the presence of a carbonylic carbon group (a broad and strong peak at 3258 cm^{-1}, and 1635 cm^{-1}). The IR spectrum shows the presence of a carbonylic carbon group (a broad and strong peak at 3258 cm^{-1}, and 1635 cm^{-1}). The {^1}H-NMR (300 MHz, CDCl3) of 14 exhibits the signals of five protons: a tertiary methyl group (d 2.36), and two conjugated olefinic protons (d 7.70, d 5.5 Hz and 6.41, d 5.5 Hz). The {^{13}}C-NMR (75.0 MHz, CDCl3) 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.

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 CombiFlashTM 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 bulk 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 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 Into parallel chromatography generates bulk 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 Into parallel chromatography generates bulk 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

Biological Activities

Ten compounds (1 to 4, 8, 10 to 12, 15 and 16) were tested in a number of biological tests [7] (antibacterial, antiinflugal, 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.

Experimental Section

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

Culture media: Medium A: Soluble starch 10 g/l, (NH4)2SO4 2 g/l, K2HPO4 (1 g/l), NaCl (1 g/l), MgSO4 x 7 H2O (1 g/l), CaCO3 (2 g/l), trace element solution (5 ml/l) of 3 g/l of CaCl2 x H2O, 1 g/l of Fe(II) citrate, 0.2 g/l of MnSO4, 0.1 g/l of ZnCl2, 0.025 g/l of CuSO4 x 5 H2O, 0.02 g/l of Na2B4O7 x 10 H2O, 0.004 g/l of CuCl2, 0.01 g/l of Na2MoO4 x 2 H2O, 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
which was chromatographed 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, MeOH), 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/mL of 1, 0.15 mg/mL of 2, 0.36 mg/mL of 3, 0.03 mg/mL of 5, 0.06 mg/mL of 9, 0.10 mg/mL of 10 (Fig. 1). After drying in vacuum at 30 °C, the second fraction (elution from 25% to 50% MeOH, gradient from 100% to 90%:10) was extracted with CHCl₃ and MeOH (10:1) to obtain 0.03 mg/mL of 4. 0.05 mg/mL of 6a and 0.04 mg/mL of 7, 0.13 mg/mL of 11, 0.15 mg/mL of 12, 0.08 mg/mL of 13, 0.05 mg/mL of 14, 0.30 mg/mL of 15, 0.10 mg/mL 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 to give 0.30 g of brown oil. This material was chromatographed 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, MeOH), 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/mL of 1, 0.19 mg/mL of 4, 0.05 mg/mL of 6a and 0.04 mg/mL of 7, 0.30 mg/mL of 11, 0.15 mg/mL of 12, 0.08 mg/mL of 13, 0.05 mg/mL of 14, 0.30 mg/mL of 15, 0.10 mg/mL of 16 (Fig. 2).

2-Methyl-2,5-bornandiol (11): Colorless oil. [α]D = + 46.3 (c = 0.53, methanol). - IR (film): n

3-(3-Methyl-oxiranyl)-acrylic acid 4-hydroxy-5-oxo-tetrahydro-furan-3-ylmethyl ester (4): Colorless oil. [α]D = -43.1 (c = 0.70, methanol). - IR (film): n


5-(1-Hydroxyethyl)-4´-hydroxymethyl-tetrahydro-[2,3´]bifuranyl-2´-one (5):

[16x721]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, MeOH), and parallel chromatography on silica gel (1.1 × 30 cm, n-hexane/EtOAc, gradient from 4:1 to 2:1) to obtain 0.32 mg/mL of 1, 0.19 mg/mL of 4, 0.05 mg/mL of 6a and 0.04 mg/mL of 7, 0.30 mg/mL of 11, 0.15 mg/mL of 12, 0.08 mg/mL of 13, 0.05 mg/mL of 14, 0.30 mg/mL of 15, 0.10 mg/mL of 16 (Fig. 2).

This material was chromatographed on silica gel (2.5 × 60 cm, CHCl₃/MeOH, gradient from 100% to 90%:10) and sequentially purified by parallel gel permeation chromatography on Sephadex LH-20 (2.5 × 50 cm, MeOH), 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/mL of 1, 0.19 mg/mL of 4, 0.05 mg/mL of 6a and 0.04 mg/mL of 7, 0.30 mg/mL of 11, 0.15 mg/mL of 12, 0.08 mg/mL of 13, 0.05 mg/mL of 14, 0.30 mg/mL of 15, 0.10 mg/mL of 16 (Fig. 2).
1.55 (1H, dd, J = 14.0, 4.3 Hz, H-6 b), 1.25 (1H, d, J = 14.6, 9.1 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, 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´), 118.9 (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).

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