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Structural Revision of Isovalertatins D03 and D23 Isolated from the Culture Filtrate of *Streptomyces luteogriseus*

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Abstract: Two aminooligosaccharides, isovalertatins D03 (**1**) and D23 (**2**), were isolated from the culture filtrate of *Streptomyces luteogriseus*. Their structures were reinvestigated and revised by spectroscopic evidences including ESI multistage mass spectrometry and 2-dimensional NMR techniques.

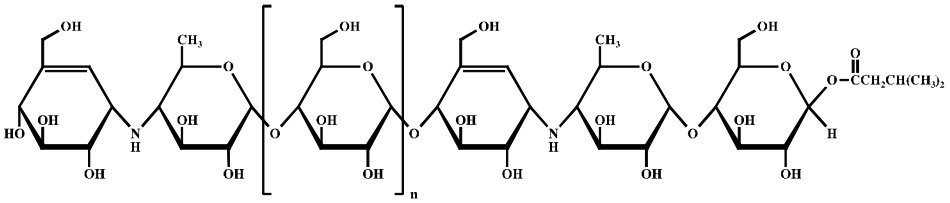
Keywords: *Streptomyces luteogriseus*, aminooligosaccharide, isovalertatin D03, isovalertatin D23.

In a program of sequentially investigating the chemical constituents of the complex, WC670, extracted from the culture filtrate of *Streptomyces luteogriseus*, we found it contains the reported aminooligosaccharides, *yiwutadings* B-F. But, when verifying their structures, there were several inconsistencies against the corresponding ESI ion trap multistage mass spectra. Our previous paper[1] reported the structural revision of *yiwutadings* F, D and C, and these monomers were re-designated as isovalertatins M03, M13 and M23, respectively. Here, this paper describes the structural revision of *yiwutadings* E and B, and these compounds are re-designated as isovalertatins D03 and D23, respectively.

The aminooligosaccharide-containing complex, WC670, was dissolved in water and used for separation by means of semi-preparative reversed-phase HPLC on a Spherisorb C_8 column (300 × 8.0 mm i.d., 10 μm) where the mobile phase was MeCN:1.5 mmol/L aqueous ammonia with UV detection at 206 nm, which yielded two more monomers isovalertatins D03 (**1**) and D23 (**2**).

Isovalertatin D03 (**1**), white amorphous powder, gave positive reactions with silver nitrate-sodium hydroxide and with anthrone. Its IR spectrum displayed absorption bands for the ester carbonyl group (1719 cm^{-1}), C=C bond (1653 cm^{-1}), and the oligosaccharide nature (3389, 1025 cm^{-1}). The molecular formula was determined as $C_{61}H_{102}N_2O_{41}$ by combined positive HRESIMS (found $[M+H]^+$ 1519.6050, calcd. 1519.6036) and the NMR data. An ESI/MS/MS spectrum of its protonated molecular ion at *m/z* 1519 showed main fragment ions at *m/z* 1501, 1361, 1339, 1216, 1177, 1054, 1015, 896, 769, 624 and 466. Most of the above fragment ions including the most abundant ions at *m/z*

Figure 1 Previously reported structures of isovalertatins D03 (**1**, n=4) and D23 (**2**, n=6)



1216 and 769 could not be accommodated by its reported structure (**Figure 1**)[2]. Consulting the fragmentation pathways for N-containing oligosaccharides[1,3], the outlined structure for **1** could be recommended as shown in **Figure 2**. But the linkage positions and their configurations among an isovaleryl group, two moieties of acarviosine[4], and five glucose units remained undetermined.

The ^{1}H and ^{13}C NMR spectra of **1** showed the characteristic signals for an isovaleryl group at δ_H 0.94 (6H, d, J 6.7 Hz, CH_3), 2.05 (m, CH), 2.32 (2H, d, J 7.0 Hz, CH_2), and δ_C 22.3 (q, CH_3), 26.0 (d, CH), 43.5 (t, CH_2), 176.7 (s, C=O), for an isovalerylated hydroxyl group at position 6 of a glucose unit at δ_H 4.45 (dd, J 11.6, 6.5 Hz, D6a), 4.20 (m, D6b), and δ_C 64.0 (t, D6), as well as the typical signals at δ_H 5.21 (0.6 H, d, J 3.5 Hz, A1α), 4.63 (0.4 H, d, J 8.0 Hz, A1β), 3.25 (0.4 H, t, J 8.7 Hz, A2β), and δ_C 92.6 (d, A1α), 96.4 (d, A1β), 74.7 (d, A2β) for the existence of the reducing glucose terminus. In the TOCSY and HMQC spectra of **1**, resonances for protons and carbon atoms in the inner unit of acarviosine appeared at δ_H 5.36 (d, J 3.9 Hz, E1), 2.57 (t, J 9.6 Hz, E4), 1.30 (3H, d, J 6.5 Hz, E6), 3.52 (t, J 6.5 Hz, F1), 4.20 (overlapped, F4), 5.96 (d, J 4.5 Hz, F7), and δ_C 98.3 (d, E1), 64.7 (d, E4), 18.0 (q, E6), 55.8 (d, F1), 76.8 (d, F4), 138.4 (s, F5), 125.4 (d, F7), whereas for those in the terminal unit at δ_H 5.31 (d, J 3.1 Hz, H1), 2.65 (t, J 9.9 Hz, H4), 1.34 (3H, d, J 6.2 Hz, H6), 3.54 (t, J 6.5 Hz, I1), 4.05 (overlapped, I4), 5.88 (d, J 4.8 Hz, I7), and δ_C 101.1 (d, H1), 65.0 (d, H4), 18.0 (q, H6), 56.7 (d, I1), 71.7 (d, I4), 141.4(s, I5), 122.3 (d, I7). Compared with the NMR data for the terminal unit, the differences of chemical shifts of the corresponding protons and carbon atoms in the inner unit matched the glycosylated pattern to the hydroxyl group at position F4[1,5]. In the HMQC spectrum of **1**, signals of five methines at position 4 of five glucose units exhibited at about δ_H 3.62-3.66 (overlapped) and δ_C 78.1-78.4 (d), with no correlation at about δ_H 3.40/δ_C 70.2, revealed that all of the five hydroxyl groups at position 4 of five glucose units were glycosylated. As the chemical shifts for four anomeric protons at rings B, C, D and F wavered at δ5.38 (4H, overlapped), and the coupling constants at about 3.0 Hz, the configuration of the above glycosidic bonds was determined as α-1,4 mode[6]. Thus, the chemical structure of **1** was reestablished as shown in **Figure 3**.

Isovalertatin D23 (**2**) was acquired as white amorphous powder. Its molecular formula was deduced as $C_{73}H_{122}N_2O_{51}$ by combined positive HRESIMS (found $[M+H]^+$ 1843.7090, calcd. 1843.7093) and the NMR evidences. The color reactions, spectroscopic characteristics, and liquid chromatographic behaviors closely resembled that of **1**, suggesting **2** to be an analogue appended by two more glucose units. The

ESI/MS/MS spectrum of its pseudo-molecular ion at *m/z* 1843 gave main fragment ions

Figure 2 Diagnostic fragment ions and the structure of isovalertatin D03 (**1**)

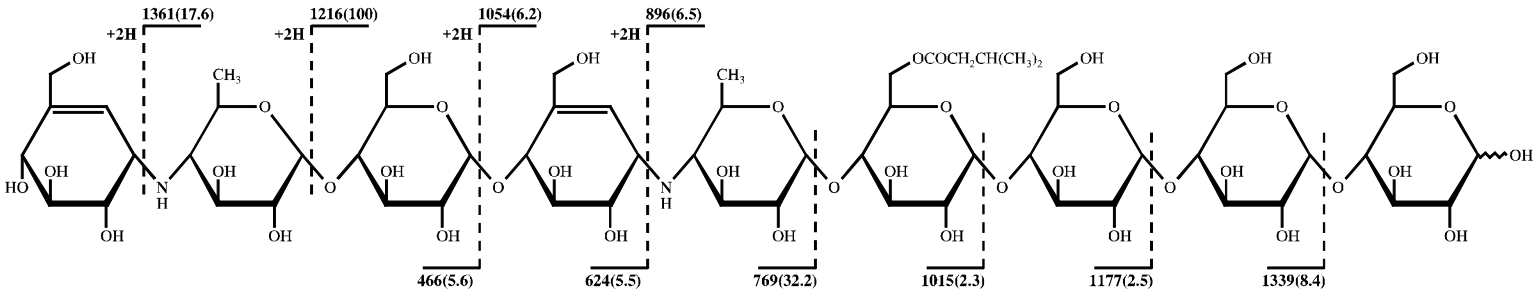
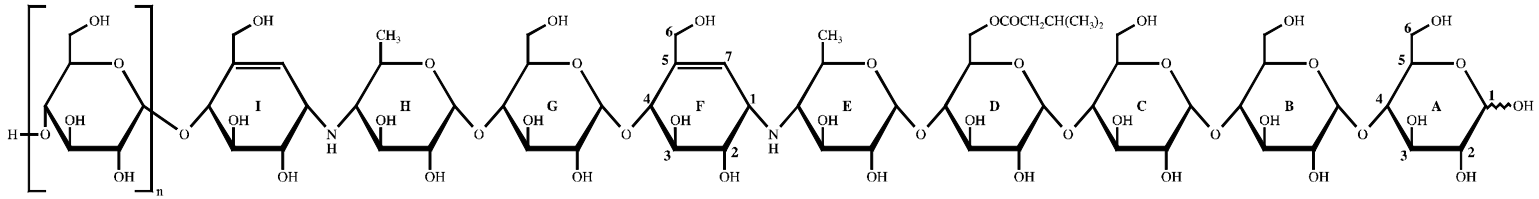


Figure 3 Revised structures of isovalertatins D03 (**1**, n=0) and D23 (**2**, n=2)



at *m/z* 1825, 1681, 1663, 1519, 1501, 1361, 1339, 1216, 1198, 1093, 1054, 1036, 896 and 628. In accordance with the fragmentation pathways shown in **Figure 2**, the appended two units of glucose should be attached to the left side of compound **1**.

The ^{1}H and ^{13}C NMR spectra of **2** appeared much alike to those of **1**, showing the diagnostic signals for an isovaleryl group at δ_H 0.94 (6H, d, J 6.5 Hz, CH_3), 2.05 (m, CH), 2.32 (2H, d, J 7.0 Hz, CH_2), and δ_C 22.3 (q, CH_3), 26.1 (d, CH), 43.5 (t, CH_2), 176.8 (s, C=O), for an esterified hydroxyl group at position 6 of a glucose unit at δ_H 4.45 (dd, J 11.5, 6.0 Hz, D6a), 4.20 (m, D6b), and δ_C 64.0 (t, D6), as well as for the reducing glucose terminus at δ_H 5.21 (0.6 H, d, J 3.5 Hz, A1α), 4.63 (0.4 H, d, J 8.0 Hz, A1β), 3.25 (0.4 H, t, J 8.0 Hz, A2β), and δ_C 92.6 (d, A1α), 96.5 (d, A1β), 74.7 (d, A2β). The configuration of the six glycosidic bonds in **2** should also be determined as α mode because of the classic signals for the anomeric protons at δ 5.38 (6H, overlapped) with the coupling constants at about 3.0 Hz. The marked differences were found in signals representing the olefinic protons and carbon atoms of the two moieties of acarviosine. There existed one doublet for the two protons at δ 5.95 (2H, d, J 3.0 Hz, F7 and I7) and two signals for the four carbon atoms at δ 137.2 (s, F5 and I5), 126.9 (d, F7 and I7). These alterations suggested a glycosylated hydroxyl group attached to position I4 in **2** instead of a free hydroxyl group in **1**[1,5]. Another difference occurred in a new correlation between signals at δ_H 3.40 (t, J 9.8 Hz, G4) and δ_C 70.2 (d, G4), in the HMQC spectrum of **2**, indicating the presence of a free hydroxyl group at position K4[6]. These evidences confirmed that the appending unit of maltose was linked to position I4 through an *O*-α-glycosidic bond. Thus, the chemical structure of **2** was revised as shown in **Figure 3**.

Acknowledgments

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