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NOTE

Establishment of the absolute configuration of the 34-membered polyol macrolide compound JBIR-129

Teppei Kawahara[1], Shuichi Ohira[2], Miho Izumikawa[1], Hiroshi Tanaka[2] and Kazuo Shin-ya[3]

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JBIR-129, isolated from the culture of *Streptomyces* sp. RK74, was discovered by the screening program first implemented for the search for bioactive substances in our extensive natural product extract library (>300 000 samples). The compound exhibits strong cytotoxicity against human ovarian adenocarcinoma SKOV-3 cells ($IC_{50} = 0.3\,\mu M$). Its planar structure, consisting of a 34-membered polyol macrolide skeleton with five deoxysugars, has been elucidated by MS and NMR spectroscopic data analyses.[1] The relative configurations of the deoxysugar units have been elucidated based on $J_{H,H}$ values and ROESY data.[1] In addition, two partial relative configurations of the aglycone moieties have been independently determined by using *J*-based configuration analysis.[2] Herein, we report the determination of the absolute configuration of JBIR-129 by further investigating the stereochemistry of the deoxysugar moieties.[3]

The absolute configurations of the sugar moieties of JBIR-129 were established by HPLC analysis using an optical rotation detector. As the JBIR-129-producing microorganism also produces many congeners consisting of the common aglycone and a variety of sugar units, the purification of JBIR-129 proved to be challenging.[1] According to previous analyses, the main sugar units of these congeners were identical to those of JBIR-129. Therefore, we attempted to obtain the main sugar moieties from a semi-purified fraction of JBIR-129.

A 1.0 g portion of the *n*-BuOH extract obtained from the fermentation broth of RK74[1] was subjected to silica gel medium-pressure liquid chromatography (MPLC; Purif-Pack SI-30, Shoko Scientific Co., Yokohama, Japan), eluting with a $CHCl_3$–MeOH gradient increasing in strength in 10% stepwise increments of MeOH to give a crude material containing the glycosidic macrolide (100% MeOH eluate, 239.5 mg). The material was added to a 5% HCl–MeOH solution (1 ml) and stirred (10 min, rt); the resulting material was applied to a silica gel MPLC column (Purif-Pack SI-30) and developed with a stepwise gradient system of $CHCl_3$–MeOH (0, 2, 5, 10, 20, 30 and 100% MeOH). The 20% MeOH elute fraction (15.4 mg), containing methyl α-olivoside as a major component and methyl β-olivoside as a minor component, was purified by reversed phase-HPLC-MS (CAPCELL PAK C_{18} MGII column, 4.6 i.d. × 150 mm, Shiseido Co., Ltd., Tokyo, Japan) eluted with 5% MeOH (aq) containing 0.1% formic acid (isocratic; flow: 1.0 ml min^{-1}; detected at *m/z* 185 $[M+Na]^+$) to afford methyl α-D-olivoside (3.0 mg, Rt = 11.7 min) as a colorless oil: $[\alpha]_D^{26} + 156$ (*c* 0.23, acetone) lit.[4] $[\alpha]_D^{23} + 149$ (*c* 0.54, acetone); ^1H NMR (600 MHz, acetone-d_6) δ 4.64 (d, $J = 3.6$ Hz, H-1), 3.71 (ddd, $J = 4.8$, 9.6, 12.6 Hz, H-3), 3.50 (dq, $J = 6.0$, 9.6 Hz, H-5), 3.24 (s, OMe-1), 2.92 (dd, $J = 9.6$, 9.6 Hz, H-4), 1.97 (dd, $J = 4.8$, 12.6 Hz, H_{eq}-2), 1.53 (ddd, $J = 3.6$, 12.6, 12.6 Hz, H_{ax}-2), 1.18 (d, $J = 6.0$ Hz, H_3-6). The combined fraction (183.5 mg) of the 30 and 100% MeOH eluates was then subjected to acid hydrolysis with 5% HCl–MeOH at 50 °C for 4 h; the resulting material was further fractionated by silica gel MPLC (Purif-Pack SI-30) using the same gradient $CHCl_3$–MeOH elution conditions as described above. The 10% MeOH fraction (31.8 mg) containing α-quinovoside (major) and β-quinovoside (minor) was purified by preparative reversed phase-HPLC-MS using a CAPCELL PAK C_{18} MGII column (20 i.d. × 150 mm) eluted with 3% MeOH (aq) containing 0.1% formic acid (isocratic; flow: 10.0 ml min^{-1}; detected at *m/z* 201 $[M+Na]^+$) to give methyl α-D-quinovoside (2.3 mg, Rt = 12.0 min) as a colorless oil: $[\alpha]_D^{26} + 122$ (*c* 0.11, $CHCl_3$) lit.[5] $[\alpha]_D^{22} + 147$ (*c* 1.00, $CHCl_3$); ^1H NMR (500 MHz, MeOH-d_4) δ 4.59 (d, $J = 3.5$ Hz, H-1), 3.58 (dq, $J = 6.0$, 10.0 Hz, H-5), 3.55 (dd, $J = 10.0$, 10.0 Hz, H-3), 3.39 (dd, $J = 3.5$, 10.0 Hz, H-2) 3.37 (s, OMe-1), 2.97 (dd, $J = 10.0$, 10.0 Hz, H-4), 1.24 (d, $J = 6.0$ Hz, H_3-6). In contrast to methyl α-D-olivoside and methyl α-D-quinovoside, the remaining sugar unit, methyl α-amicetoside could not be obtained by purification due to its volatile nature. Therefore, methyl α-D-amicetoside was prepared by organic synthesis according to the method reported previously.[6] These sugars were employed as authentic samples for optical rotation measurements.

The absolute configurations of the sugar units of JBIR-129 were determined as described below. JBIR-129 (20.1 mg) was added to a

[1]Japan Biological Informatics Consortium (JBIC), Tokyo, Japan; [2]Department of Applied Chemistry, Graduate School of Science and Engineering, Tokyo Institute of Technology, Tokyo, Japan and [3]National Institute of Advanced Industrial Science and Technology (AIST), Tokyo, Japan
Correspondence: Dr K Shin-ya, National Institute of Advanced Industrial Science and Technology (AIST), 2-4-7 Aomi, Koto-ku, Tokyo 135-0064, Japan.
E-mail: k-shinya@aist.go.jp
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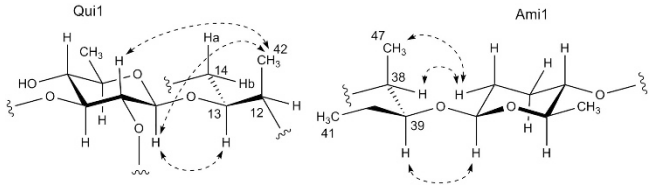

Figure 1 Configurational analysis of Qui1 and Ami1 glycosidic fragments of JBIR-129 (dashed arrow: ROESY correlation).

solution of 5% HCl–MeOH (1 ml) and heated at 40 °C for 20 h. After cooling, the mixture was analyzed by HPLC using a YMC-Pack Hydrosphere C18 (4.6 i.d. × 250 mm, YMC Co., Ltd., Kyoto, Japan; eluent: MeOH/H_2O 20:80, isocratic) in conjunction with a OR-2090 Plus chiral detector (JASCO Co., Tokyo, Japan). The sugar components of JBIR-129 were identified as D-olivoside, D-quinovoside and D-amicetoside by comparison with the retention times of authentic methyl α-D-olivoside, methyl α-D-quinovoside and methyl α-D-amicetoside (Rt = 4.67, 6.19 and 9.06 min, respectively) and the signs (all positive) of optical rotation.

In a previous study, we have established the relative configuration of two fragments of JBIR-129, from C-7 to C-27 and from C-33 to C-39. Therefore, determination of the absolute configuration at C-13 and C-39 would firmly establish the absolute configuration of JBIR-129 itself. The absolute configurations of a β-quinovopyranoside (Qui1) and a β-amicetopyranoside (Ami1), which are substituents at the C-13 and C-39 positions, respectively, offered key insights into the determination of the macrolactone absolute configuration as follows: the ROESY spectrum exhibited a strong correlation between H-13 and H-Qui1-1; furthermore, ROESY correlations from methyl proton H_3-42 to H-Qui1-1 and H-Qui1-2 were observed, whereas the correlations from H_2-14 to H-Qui1-2 were not observed. These results suggested the spatial location of C-13 in MeOH-d_4 as depicted in Figure 1; consequently, the absolute configuration at C-13 was deduced as *S*. In the same manner, the absolute configuration at C-39 was concluded to be *S* based on the presence of ROESY correlations from H-39 to H-Ami1-1 and from H-38 and H_3-47 to H_{eq}-Ami1-2, and the absence of a correlation from H-40b and H_3-41 to H_{eq}-Ami1-2. Hence, the absolute configuration of macrolactone moiety was determined as 7*S*, 9*R*, 12*S*, 13*S*, 15*S*, 16*R*, 17*S*, 19*S*, 20*R*, 21*S*, 23*S*, 24*R*, 25*S*, 27*S*, 33*S*, 34*S*, 35*S*, 36*R*, 37*R*, 38*R* and 39*S* (Figure 2).

JBIR-129 and its congener JBIR-139 showed strong cytotoxicities against human ovarian adenocarcinoma SKOV-3 cells with nearly identical IC_{50} values (0.3 and 0.4 μM, respectively). The structural difference between JBIR-129 and -139 is an additional *N*-acetylgalactosamine (GalNAc) moiety found at C-21 in JBIR-139. Therefore, the aglycone of JBR-129 is considered to make a significant contribution to the cytotoxicities of these compounds. As previously mentioned, the producing strain *Streptomyces* sp. RK74 produces many congeners of JBIR-129, making it difficult to study detailed

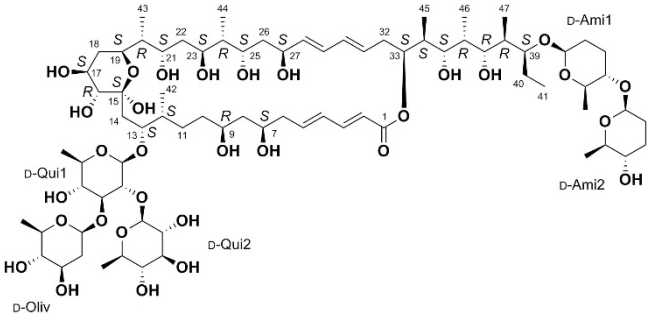

Figure 2 Absolute configuration of JBIR-129.

biological activities specific to the compound such as antitumor activities in mice models. It should be possible to generate the aglycone of JBIR-129 by heterologous expression; in this regard, we have employed a BAC (Bacterial Artificial Chromosome) vector to clone the complete relevant biosynthetic gene cluster (126.5 kbp). Heterologous production using a deletion mutant of the glycosylation enzyme found in the biosynthetic gene cluster could provide the aglycone moiety of JBIR-129 solely. Attempts are currently underway to accomplish the heterologous production of JBIR-129 in a SUKA (Special Use of Kitasato Actinobacteria) host strain.[7,8]

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