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Versipelostatin, a novel GRP78/Bip molecular chaperone down-regulator of microbial origin

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Abstract—We discovered a novel down-regulator of *grp*78 gene expression designated as versipelostatin from the culture broth of *Streptomyces versipellis* 4083-SVS6. Its structure was determined to be a macrocyclic compound consisting of an α-acyltetronic acid and sugar moieties. © 2002 Elsevier Science Ltd. All rights reserved.

GRP78 acts as a molecular chaperone in endoplasmic reticulum (ER) by associating transiently with incipient proteins as they traverse the ER and aiding in their folding and transport.[1–3] Furthermore, the GRP78 protein is also induced under various stress condition such as glucose starvation, inhibition of protein glycosylation by tunicamycin, perturbation of ER function and protein movement by brefeldin A, and suppression of ER-calcium-ATPase pump by thapsigargin.[4,5] The enhancement of ER stress response (also known as the unfolded protein response) takes part in the resistant mechanism against chemotherapy and hypoxic stress in solid tumor,[6] while the reduction of ER stress response involves in the pathology of central nervous diseases such as Alzheimer's and Parkinson's diseases.[7] The ER stress response causes an increase in gene expression of a number of ER chaperones such as GRP78/Bip and GRP94.[1] Thus, substances that directly down- and up-regulate *grp*78 transcription are expected to be useful drugs for the treatment of cancer and Alzheimer's disease, respectively.

In the course of our screening program for chaperone modulators, we employed the reporter gene assay system utilizing luciferase gene. HeLa cells, which are transformed with luciferase gene under the control of *grp*78 promoter designated as HeLa 78C6 cells,[2] respond sensitively to luciferase *grp*78 induction by ER

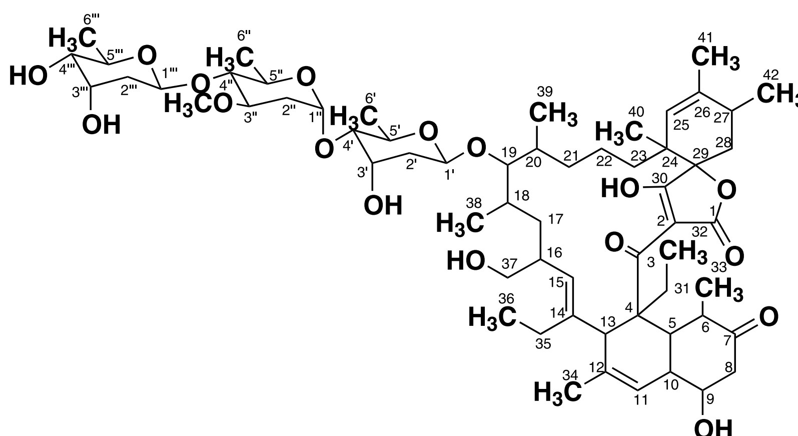

Figure 1. Structure of versipelostatin (**1**).

Keywords: versipelostatin; macrocyclic; α-acyltetronic acid; *grp*78; endoplasmic reticulum stress.

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stress such as the treatment of tunicamycin. By using this screening system, we isolated a several compounds such as pyrisulfoxin[8] and alternariol.[9] Further screening resulted in the isolation of a novel compound designated as versipelostatin (**1**)[10] from *Streptomyces versipellis* 4083-SVS6 as a down-regulator of the *grp*78 gene (Fig. 1). We report herein the fermentation, isolation and structure determination and brief biological activity of **1**.

The versipelostatin producing strain 4083-SVS6, identified as *S. versipellis*, was cultivated in a seed medium consisting of starch 1.0%, polypepton 1.0%, molasses 1.0%, meat extract 1.0% (pH 7.2) for 3 days at 27°C on a rotary shaker. The seed culture was innoculated into a production medium composed of starch 2.5%, soybean meal 1.5%, dry yeast 0.2%, $CaCO_3$ 0.4% (pH 7.0) and cultivated on a rotary shaker (200 rpm) for 5 days at 27°C. The active principle was extracted from the supernatant of cultural broth with EtOAc. The solvent layer was dried over Na_2SO_4, and concentrated to give an oily residue. The residue was subjected to a silica gel column chromatography using $CHCl_3$–MeOH (20:1) as a solvent system. The active fraction was concentrated under reduced pressure and purified by preparative HPLC using a PEGASIL ODS column (Senshu-Pak, 20 i.d.×250 mm) developed with 80% MeOH.

The molecular formula of **1** was established as $C_{61}H_{94}O_{17}$ by high-resolution FAB-MS spectrum [$(M+Na)^+$, m/z 1121.6398 (+0.9 mmu error)]. IR absorptions at 3400 and 1760 cm^{-1} implied the presence of hydroxyl groups and γ-lactone, respectively. The characteristic UV absorption maxima in MeOH at 250 and 270 nm indicated the presence of an α-acyltetronic acid chromophore, which was typical value for α-acyltetronic acids involved in kijanimicin and tetrocarcins.[11,12]

Three major substructures and three sugar moieties were revealed by the interpretation of the DQF-COSY and the constant time HMBC (CT-HMBC)[13] spectra. ^1H–^1H spin coupling systems were shown by bold line in Fig. 2. A spin coupling system from a methylene proton 8-H (δ_H 2.98) to an olefinic proton 11-H (δ_H 5.84), which was allylic coupled to a methyl proton 34-H (δ_H 1.63) was established as shown in Fig. 2. In addition, a spin coupling system among 33-H (δ_H 0.92), 6-H (δ_H 2.46) and 5-H (δ_H 2.42), which in turn spin coupled to 10-H (δ_H 2.29), established the existence of a 4-ethyl-2-heptenyl moiety as shown in Fig. 2. The long-range couplings from the methyl proton 33-H (δ_H 0.92), methylene protons 8-H (δ_H 2.98) and a methane proton 9-H (δ_H 3.78) to a carbonyl carbon C-7 (δ_C 210.0) revealed a cyclohexanone moiety. Long-range couplings from methylene protons 31-H (δ_H 2.12) to a quaternary carbon C-4 (δ_C 58.6), methine carbons C-5 (δ_C 39.3) and C-13 (δ_C 60.0), which was then long-range coupled to methyl proton 34-H established a decalin substructure as shown in Fig. 2. According to the ^{13}C chemical shift of C-9 (δ_C 71.3), an oxygen atom was substituted at the C-9 position. A long-range coupling from 31-H to a carbonyl carbon C-3 (δ_C 205.1) also confirmed the substituent at the C-4 position in the decalin substructure.

The sequence from an olefinic proton 15-H (δ_H 5.11) to methylene protons 23-H (δ_H 1.53, 1.26) through 16-H which was coupled to oxygenated methylene protons 37-H (δ_H 3.51, 3.41, δ_C 64.7), 17-H (δ_H 1.59, 0.59), 18-H (δ_H 1.94), an oxymethine proton 19-H (δ_H 3.21, δ_C 91.1), 20-H (δ_H 1.61), 21-H (δ_H 1.49, 1.11) and 22-H (δ_H 1.58, 1.33) was observed in the DQF-COSY spectrum of **1**. In addition, two spin couplings from methyl protons 38-H (δ_H 0.91) and 39-H (δ_H 0.88) to 18-H and 20-H, respectively, were also observed. From these

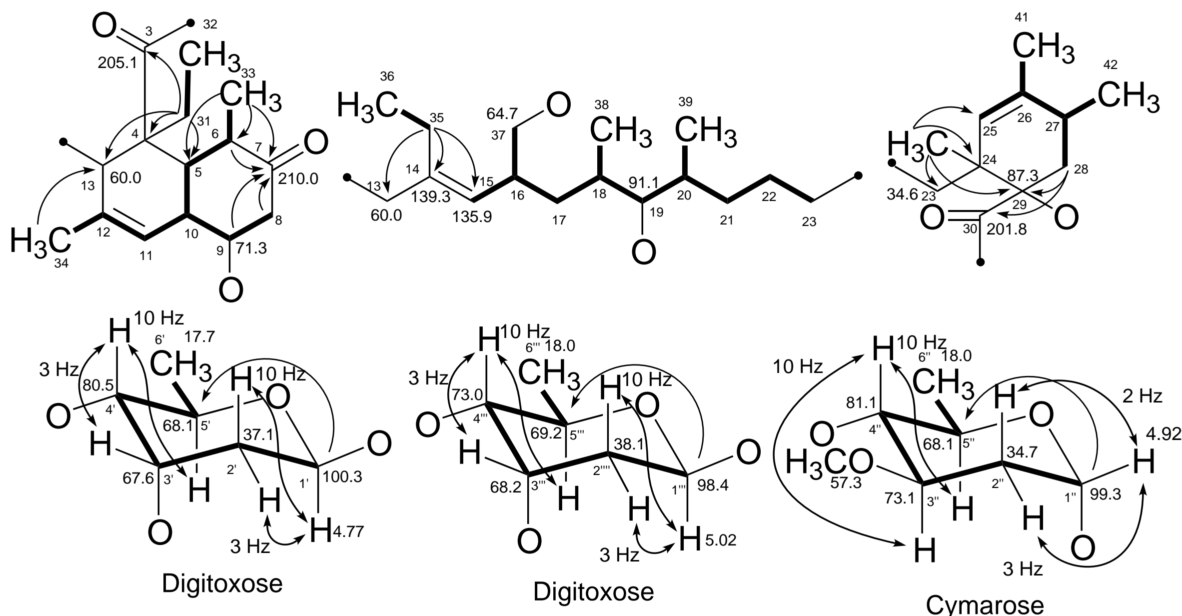

Figure 2. Partial structures of **1** revealed by the interpretation of DQF-COSY and HMBC spectral data. Bold line and arrows indicated DQF-COSY and ^1H–^{13}C long-range couplings in HMBC spectra, respectively.

results, a substituted nonyl residue was established as shown in Fig. 2. Additionally, long-range couplings from methylene protons 35-H (δ_H 1.92, 1.88) to olefinic carbons C-14 (δ_C 139.3), C-15 (δ_C 135.9) and the quaternary carbon C-13 (This carbon is common to that assigned to the member of decalin moiety and this relationship establish the connectivity between these substructures vide infra) established a substituted dodeca-3-enyl chain.

Both a methyl proton 41-H (δ_H 1.67) and a methine proton 27-H (δ_H 2.37), which was also vicinal coupled to a methyl proton 42-H (δ_H 1.03) and a methylene proton 28-H (δ_H 1.75), showed allylic couplings to an olefinic proton 25-H (δ_H 5.26). This connectivity was also confirmed by long-range couplings from 41-H to olefinic carbons C-25 (δ_C 126.6) and C-26 (δ_C 135.3), and the methine carbon C-27 (δ_C 31.1). Long-range couplings from a singlet methyl proton 40-H (δ_H 1.01) to a quaternary carbon C-24 (δ_C 41.4), an olefinic carbon C-25 and a quaternary carbon C-29 (δ_C 87.3), which was in turn long-range coupled to the methylene proton 28-H (δ_H 2.21) established a substituted cyclohexene moiety. A long-range coupling from 28-H to C-30 (δ_C 201.8) established the substituent at C-29.

In addition to these units, three sugar moieties were elucidated by analyses of the DQF-COSY and HMBC spectra as follows. The sequence from an anomeric proton 1′-H (δ_H 4.77) to 6′-H (δ_H 1.17) through methylene proton 2′-H (δ_H 2.11, 1.65), oxymethine protons 3′-H (δ_H 4.03), 4′-H (δ_H 3.21) and 5′-H (δ_H 3.76) was determined by analyses of the DQF-COSY spectrum of **1**. Furthermore, a long-range coupling from 1′-H to C-5′ (δ_C 17.7) proved the existence of a sugar moiety. According to the coupling constants of these protons, this sugar moiety was deduced to be a β-digitoxose moiety as shown in Fig. 2. Likewise, two sugar substructures were established by the spin coupling systems from 1″-H (δ_H 4.92) to 6″-H (δ_H 1.24) and 1‴-H (δ_H 4.92) to 6‴-H (δ_H 1.22), together with long-range couplings from 1″-H to C-5″ (δ_C 68.1) and 1‴-H to C-5‴ (δ_C 69.2), and coupling constants to be an α-cymarose and the additional β-digitoxose, respectively. Methanolysis of **1** produced two methyl β-digitoxosides and a methyl α-cymaroside. The optical rotations of these sugar proved that these sugar moieties were methyl β-D-digitoxosides ($[\alpha]_D = -51°$, c 0.08 in $CHCl_3$, lit. $[\alpha]_D = -36°$, c 1.0 in $CHCl_3$[14]) and a methyl α-D-cymaroside ($[\alpha]_D = +197°$, c 0.05 in MeOH, lit. $[\alpha]_D = +212°$, c 1.2 in MeOH[15])

The connectivity between the decalin substructure, the dodecyl residue and the cyclohexene moiety was confirmed by long-range couplings from the methylene proton 35-H (δ_H 1.92) to C-13 (δ_C 60.0), olefinic carbons C-14 (δ_C 139.3) and C-15 (δ_C 135.9), and from 40-H to the methylene carbon C-23 (δ_C 34.6), respectively. The three glycosidic linkages and the substituted position were confirmed by the long-range couplings between 1′-H and C-19 (δ_C 91.1), 1″-H and 4′-C (δ_C 80.5) and 1‴-H and 4″-C (δ_C 81.1) as shown in Fig. 3.

Remaining units which could not be assigned by NMR studies were C-1 (δ_C 166.3) and C-2 (δ_C 103.2). According to the characteristic UV absorption, these units must form an α-acyltetronic acid functional group. The ^{13}C chemical shifts of these units also supported the existence of α-acyltetronic acid moiety.[11,12,16–18] Thus, the carbon signals at C-1, C-2 and C-30 together with the carbonyl carbon C-3 were assigned to the α-acyltetronic acid moiety. This moiety was deduced to be attached to the cyclohexene ring at C-29 with a spiro-structure, by long-range couplings from 28-H (δ_H 2.21) to C-30 (δ_C 201.8) and C-29 vide supra. Thus, the aglycon of **1** was determined to be a 17-membered macrocyclic skeleton involving the α-acyltetronic acid moiety as shown in Fig. 2. The geostereochemistries at C-11, C-14 and C-25 were determined to be *Z*, *E* and *Z*, respectively, configurations according to the low field ^{13}C chemical shifts at C-34 (δ_C 22.8) and C-41 (δ_C 21.4) and NOEs between 13-H (δ_H 3.07) and 15-H (δ_H 5.11), 25-H and 41-H, and 11-H and 34-H. The relative stereochemistries of the decalin and the cyclohexene moieties deduced by NOE experiments were shown in Fig. 4. The NOEs between 8-H_a and 10-H, 5-H and 9-H, and 6-H and 10-H oriented a chair formation of this moiety. Furthermore, the NOE between 9-H and

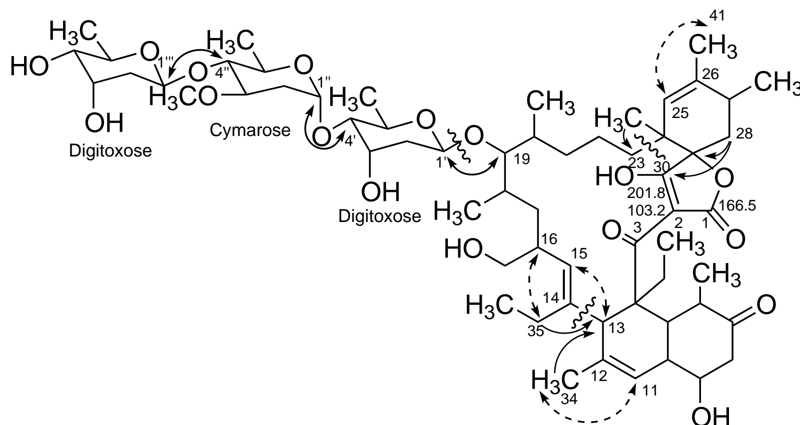

Figure 3. Connectivity of **1** elucidated by the analysis of ^1H–^{13}C long-range couplings in HMBC spectrum. Arrows and dashed double-arrows indicated ^1H–^{13}C long-range couplings in HMBC spectrum and NOE, respectively.

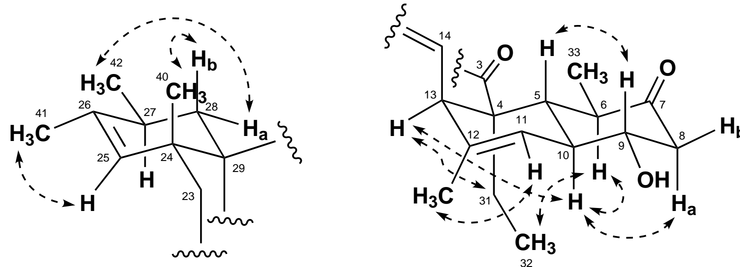

Figure 4. Relative stereochemistry of the decalin and the cyclohexene moieties in **1**. Dashed double-arrows indicated NOE.

32-H determined the relative stereochemistry of the decalin moiety as shown in Fig. 4. The relative stereochemistry of cyclohexene moiety was established by the NOE between 28-H_b and 40-H, and 28-H_a and 42-H. Studies of the absolute stereochemistry of **1** is now under way.

Although α-acyltetronic acid derivatives such as kijanimicin,[11] tetrocarcins,[12] and teronothiodin[18] were reported, the 17-membered macrocyclic skeleton involving the α-acyltetronic acid moiety in **1** was the first example so far. Studies on the absolute structure of **1** were now underway.

In the evaluation system we employed, the treatment of HeLa78C6 cells with 2 μg/ml of tunicamycin induced the expression of luciferase under the control of the ERSE promoter five times larger than that of control. **1** reduced this reporter gene expression at concentrations from 1 to 100 μM. **1** exhibited limited cytotoxic effects against various cancer cell lines. Compound **1** also inhibited the expression of GRP78 induced by a variety of ER stress such as the treatment of tunicamycin, A23187 and hypoglycemic insult. Thus, it is expected that **1** would be a promising cancer chemotherapeutic agent against solid tumor, and/or expected to exhibit unique biological activities when using in combination therapy with antitumor drugs. Since **1** is the first compound that altered the expression of GRP78, **1** is also expected to be a useful tool to study the molecular chaperone in mammalian cells. Detailed investigation on other biological activities of **1** such as a combination treatment with antitumor drugs is now under way.

Acknowledgements

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10. $[\alpha]_D = -52°$ (*c* 0.8, MeOH); UV absorption in methanol at λ_{max} nm (ε); 250 (8,000), 270 (7,700) in MeOH; 243 (11,400), 269 (8,900) in 0.01N NaOH–MeOH; 263 (8,500) in 0.01N HCl–MeOH; IR ν_{max} (KBr): 3400, 1760, 1210, 1060, 980 cm^{-1}. The NMR data for **1** are as follows: ^1H NMR (δ_H, $CDCl_3$ at 500 MHz): 5.84 (brs, 11-H), 5.26 (brs, 25-H), 5.11 (d, *J* = 11.0 Hz, 15-H), 5.02 (d, *J* = 10.0 Hz, 1‴-H), 4.92 (d, *J* = 2.0 Hz, 1″-H), 4.77 (d, *J* = 10.0 Hz, 1′-H), 4.04 (d, *J* = 3.0 Hz, 3‴-H), 4.03 (d, *J* = 3.0 Hz, 3′-H), 3.78 (m, 9-H), 3.76 (dq, *J* = 10.0, 6.5 Hz, 5′-H), 3.64 (dq, *J* = 10.6, 6.5 Hz, 5‴-H), 3.62 (dq, *J* = 10.0, 6.5 Hz, 5″-H), 3.51 (dd, *J* = 10.0, 4.0 Hz, 37-H_b), 3.46 (dd, *J* = 12.0, 10.0 Hz, 3″-H), 3.41 (dd, *J* = 10.0, 4.0 Hz, 37-H_a), 3.36 (s, 3″-OCH_3), 3.27 (t, *J* = 10.0 Hz, 4″-H), 3.22 (dd, *J* = 10.0, 3.0 Hz, 4‴-H), 3.21 (dd, *J* = 10.0, 3.0 Hz, 4′-H), 3.21 (m, 19-H), 3.07 (s, 13-H), 2.98 (m, 8-H_b), 2.52 (q, *J* = 8.0 Hz, 31-H_b), 2.46 (m, 6-H), 2.42 (m, 5-H), 2.41 (dd, *J* = 14.0, 7.0 Hz, 8-H_a), 2.37 (m, 27-H), 2.29 (m, 10-H), 2.28 (m, 16-H), 2.21 (dd, *J* = 14.0, 7.0 Hz, 28-H_b), 2.19 (dd, *J* = 12.0, 4.0 Hz, 2″-H_b), 2.12 (q, *J* = 8.0 Hz, 31-H_a), 2.11 (dd, *J* = 10.0, 3.0 Hz, 2′-H_b), 2.11 (dd, *J* = 10.0, 3.0 Hz, 2‴-H_b), 1.94 (m, 18-H), 1.92 (q, *J* = 7.0 Hz, 35-H_b), 1.88 (q, *J* = 7.0 Hz, 35-H_a), 1.75 (dd, *J* = 14.0, 7.0 Hz, 28-H_a), 1.67 (s, 41-H), 1.63 (s, 34-H), 1.65 (m, 2′-H_a), 1.61 (m, 2‴-H_a), 1.61 (m, 20-H), 1.59 (m, 17-H_b), 1.58 (m, 22-H_b), 1.53 (m, 23-H_b), 1.53 (m, 2″-H_a), 1.49 (m, 21-H_b), 1.33 (m, 22-H_a), 1.26 (m, 23-H_a), 1.24 (d, *J* = 6.5 Hz, 6″-H), 1.22 (d, *J* = 6.5 Hz, 6‴-H), 1.17 (d, *J* = 6.5 Hz, 6′-H), 1.11 (m, 21-H_a), 1.03 (d, *J* = 7.0 Hz, 42-H), 1.01 (s, 40-H), 0.92 (m, 33-H), 0.91 (m, 32-H), 0.91 (m, 38-H), 0.88 (m, 36-H), 0.88 (m, 39-H), 0.59 (t, *J* = 11.0 Hz, 17-H_a). ^{13}C NMR (δ_C, $CDCl_3$ at 125 MHz): 210.0 (C-7), 205.1 (C-3), 201.8 (C-30), 166.4 (C-1), 139.3 (C-14), 135.9 (C-15), 135.3 (C-26), 134.2 (C-12), 126.6 (C-25), 120.8

(C-11), 103.2 (C-2), 100.3 (C-1′), 99.3 (C-1″), 98.4 (C-1‴), 91.1 (C-19), 87.3 (C-29), 81.1 (C-4″), 80.5 (C-4′), 73.1 (C-3″), 73.0 (C-4‴), 71.3 (C-9), 69.2 (C-5‴), 68.2 (C-3‴), 68.1 (C-5′), 68.1 (C-5″), 67.6 (C-3′), 64.7 (C-37), 60.0 (C-13), 58.6 (C-4), 57.3 (3″-OCH_3), 50.1 (C-8), 49.8 (C-6), 47.2 (C-10), 41.4 (C-24), 39.3 (C-5), 38.1 (C-2‴), 37.9 (C-16), 37.3 (C-2′), 36.9 (C-28), 35.4 (C-18), 34.7 (C-2″), 34.6 (C-23), 32.2 (C-17), 32.2 (C-21), 31.1 (C-27), 29.2 (C-20), 23.4 (C-31), 22.8 (C-34), 22.3 (C-39), 21.8 (C-35), 21.4 (C-41), 20.8 (C-40), 20.0 (C-22), 19.4 (C-42), 18.0 (C-6″), 18.0 (C-6‴), 17.7 (C-6′), 17.1 (C-38), 16.3 (C-33), 14.6 (C-36), 12.4 (C-32).

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