ORGANIC LETTERS

2007
Vol. 9, No. 8
1457−1460

Relative and Absolute Configuration of Versipelostatin, a Down-Regulator of Molecular Chaperone GRP78 Expression

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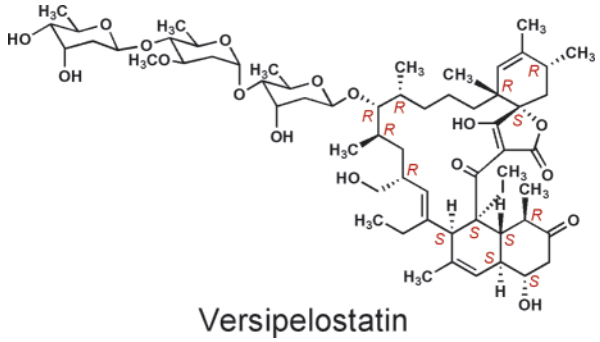
Received January 7, 2007

ABSTRACT



Versipelostatin is the first compound which specifically inhibits the expression of GRP78 and the resultant robust cell death under stress conditions, in contrast to the weak cytotoxicity under normal conditions. Versipelostatin consists of a macrocyclic aglycone with an α-acyltetronic acid and three sugar moieties. The relative and absolute configuration of the aglycone moiety was established to be 4*S*, 5*S*, 6*R*, 9*S*, 10*S*, 13*S*, 16*R*, 18*R*, 19*R*, 20*R*, 24*R*, 27*R*, and 29*S* utilizing NMR techniques.

GRP78, which is well-known as a molecular chaperone in the endoplasmic reticulum (ER), also plays an important role as a survival factor in solid tumors, due to its acquisition of a resistant mechanism against both chemotherapy and hypoglycemic stress.[1] Thus, specific down-regulators of GRP78 transcription can reasonably be expected to become promising drugs in cancer chemotherapy.[2] In the course of our screening program, we isolated a novel GRP78 down-regulator, which we designated as versipelostatin (**1**).[3,4] **1** consisted of an α-acyltetronic acid containing macrocycle as an aglycone and three sugar moieties, possessing 13 chiral centers in the aglycone moiety (Table 1). **1** is the first

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(1) Kaufman, R. J. *Genes Dev.* **1999**, *13*, 1211−1233.
(2) Katschinski, D. M.; Jacobson, E. L.; Wiedmann, G. J.; Robins, H. I. *J. Cancer Res. Clin. Oncol.* **2001**, *127*, 425−435.
(3) Park, H.-R.; Furihata, K.; Hayakawa, Y.; Shin-ya, K. *Tetrahedron Lett.* **2002**, *43*, 6941−6945.
(4) Chijiwa, S.; Park, H.-R.; Furihata, K.; Ogata, M.; Endo, T.; Kuzuyama, T.; Hayakawa, Y.; Shin-ya, K. *Tetrahedron Lett.* **2003**, *44*, 5897−5900.

10.1021/ol070042t CCC: $37.00
Published on Web 03/13/2007

Table 1. ^{13}C (125 MHz) and ^{1}H (500 MHz) NMR Data for Versipelostatin in $CDCl_3$

no.	δ_C		δ_H (multiplicity, J = Hz)	no.	δ_C	δ_H (multiplicity, J = Hz)
1	166.4			31	23.4	2.12 (q, J = 8)
2	103.2					2.52 (q, J = 8)
3	205.1			32	12.4	0.91
4	58.6			33	16.3	0.92
5	39.3		2.42 (m)	34	22.8	1.63 (s)
6	49.8		2.46 (m)	35	21.8	1.88 (q, J = 7)
7	210.0					1.92 (q, J = 7)
8	50.1	a	2.41 (m)	36	14.6	0.88
		b	2.98 (dd, J = 14, 7)	37	64.7	3.41 (dd, J = 10, 4)
9	71.3		3.78 (m)			3.51 (dd, J = 10, 4)
10	47.2		2.29 (m)	38	17.1	0.91
11	120.8		5.84 (br s)	39	22.3	0.88
12	134.2			40	20.8	1.01 (s)
13	60.0		3.07 (s)	41	21.4	1.67 (s)
14	139.3			42	19.4	1.03 (d, J = 7)
15	135.9		5.11 (d, J = 11)	1′	100.3	4.77 (d, J = 10)
16	37.9		2.28 (m)	2′	37.3	1.65 (m)
17	32.2	a	0.59 (t, J = 11)			2.11 (dd, J = 10, 3)
		b	1.59 (m)	3′	67.6	4.03 (d, J =3)
18	35.4		1.94 (m)	4′	80.5	3.21 (dd, J = 10, 3)
19	91.1		3.21 (m)	5′	68.1	3.76 (dq, J = 10, 6.5)
20	25.2		1.61 (m)	6′	17.7	1.17 (d, J = 6.5)
21	32.2	a	1.11 (m)	1″	99.3	4.92 (d, J = 2)
		b	1.52 (m)	2″	34.7	1.53 (m)
22	20.0	a	1.34 (m)			2.19 (dd, J = 12, 4)
		b	1.60 (m)	3″	78.1	3.46 (dd, J = 12, 10)
23	34.6	a	1.28 (m)	4″	81.1	3.27 (t, J = 10)
		b	1.56 (m)	5″	68.1	3.62 (dq, J = 10, 6.5)
24	41.4			6″	18.0	1.24 (d, J = 6.5)
25	126.6		5.26 (br s)	1‴	98.4	5.02 (d, J = 10)
26	135.3			2‴	38.1	1.61 (m)
27	31.1		2.37 (m)			2.11 (dd, J = 10, 3)
28	36.9	ax	1.75 (dd, J = 14, 7)	3‴	68.2	4.04 (d, J = 3)
		eq	2.21 (dd, J = 14, 7)	4‴	73.0	3.22 (dd, J = 10, 3)
29	87.3			5‴	69.2	3.64 (dq, J = 10, 6.5)
30	201.8			6‴	18.0	1.22 (d, J = 6.5)
				3″-OCH_3	57.3	3.36 (s)

example of an α-acyltetronic acid with a 17-membered macrocyclic skeleton. **1** is also the first compound which has been found to down-regulate the expression of the molecular chaperone, GRP78, which is induced by ER stress.[5] Thus, establishment of the relative and absolute configuration of **1** (Figure 1) is necessary, in order to determine whether the mechanism underlying its mode of action is predicated on its structure. We report herein our determination of the stereochemistry of **1** using NMR techniques, including ROESY spectrum and *J*-resolved HMBC,[6] to analyze the $^{3}J_{H-H}$, $^{2}J_{C-H}$, and $^{3}J_{C-H}$ establishing dihedral angles.[7]

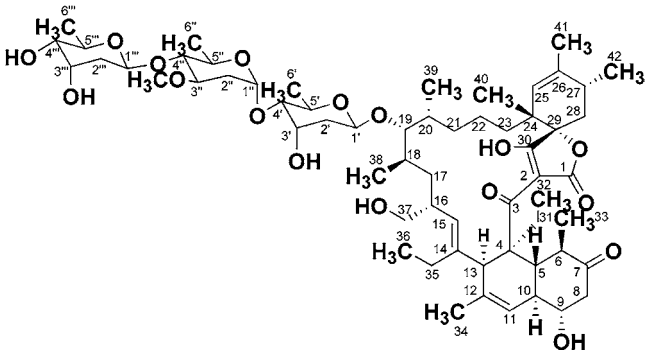

Figure 1. Absolute structure of **1**.

Relative Configuration between the Sugar and (C-19 and C-20 in) the Aglycone Moieties. The sugar moieties in **1** had already been established to be two β-D-digitoxoses and an α-D-cymarose.[3,8,9] One clue which helped us to resolve the absolute configuration of the aglycone moiety in **1** was found in the NOEs between protons in the β-D-digitoxose (1′-H and 2′-H) moieties and methine protons 19-H and 20-H in the aglycone moiety. A strong NOE, located between 1′-H and 19-H, suggested that these protons resided near one another. Furthermore, an NOE between 2′-H and the methyl proton 39-H confirmed the configuration of C-20. Thus, the absolute configuration at C-19 was designated as *R*. The large coupling constant between 19-H and C-39 ($^{3}J_{C-H}$ = 5 Hz) indicated that they were in the *anti* orientation. In the same way, the coupling constant between 19-H and 20-H ($^{3}J_{H-H}$ = 4 Hz) suggested that these protons were in *gauche* orientation. An NOE between 20-H and 1′-H established the configuration at C-20 to be *R* (Figure 2).

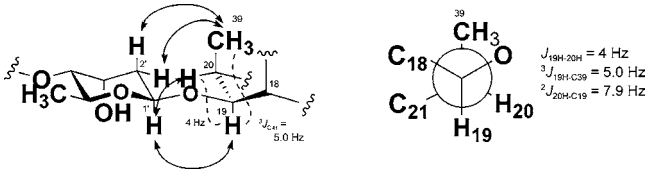

Figure 2. Configuration and conformation of the sugar and aglycone moieties derived from *J* and NOE data (arrows) of **1**.

Absolute Configuration of the Cyclohexene and α-Acyltetronic Acid Moieties. The absolute structure of the cyclohexene moiety was determined based on the absolute stereochemistry at C-20. Large $^{3}J_{H-H}$ coupling constants (~10 Hz) between 20-H, 21-H_a (δ_H 1.12), 22-H_b (δ_H 1.61), and 23-H_a (δ_H 1.29) suggested that these protons were in *anti* orientation. In the same manner, 21-H_b (δ_H 1.53), 22-H_a (δ_H 1.35), and 23-H_b (δ_H 1.56) were shown to be in *anti*

(5) Park, H.-R.; Tomida, A.; Sato, S.; Tsukumo, Y.; Yun, J.; Yamori, T.; Hayakawa, Y.; Tsuruo, T.; Shin-ya, K. *J. Natl. Cancer Inst.* **2004**, *96*, 1300−1310.

(6) Furihata, K.; Seto, H. *Tetrahedron Lett.* **1999**, *40*, 6271−6275.

(7) Murata, M.; Matsuoka, S.; Matsumori, N.; Paul, G. K.; Tachibana, K. *J. Am. Chem. Soc*. **1999**, *121*, 870−871.

(8) Mallams, A. K.; Puar, M. S.; Rossman, R. R. *J. Chem. Soc., Perkin Trans. 1* **1983**, 1497−1534.

(9) Roush, W. R.; Brown, R. J. *J. Org. Chem.* **1983**, *48*, 5093−5101.

orientations ($^3J_{H-H}$ = ~10 Hz). In addition to these coupling constants, an NOE between methyl protons 39-H and 22-H_a established that they were in the same orientation. Thus, this chain moiety was characterized by a zigzag conformation. A large $^3J_{C-H}$ coupling between 23-H_b and C-25 ($^3J_{C-H}$ = 5 Hz), and 23-H_a and C-40 ($^3J_{C-H}$ = 6 Hz) suggested that these units were in *anti* orientations. This suggests that the absolute stereochemistry at C-24 is the *R* configuration (Figure 3, top).

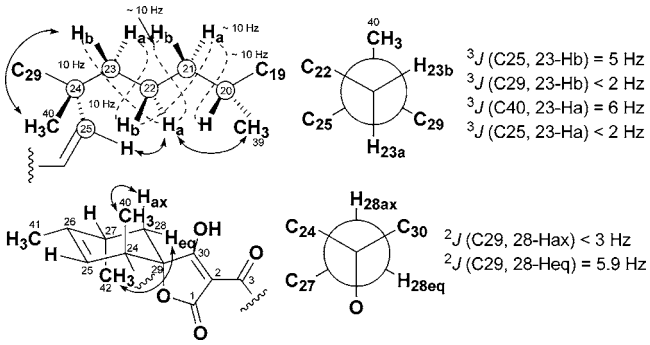

Figure 3. Configuration and conformation of the cyclohexene and α-acyltetronic acid moieties derived from *J* and NOE data (arrows) of **1**.

A strong NOE between 28-H_{ax} and the methyl proton 40-H proved that both 28-H_{ax} and C-40 are in axial orientations. A small coupling constant between 28-H_{ax} and C-29 ($^2J_{C-H}$ < 3 Hz) and the large coupling constant between 28-H_{eq} and C-29 ($^2J_{C-H}$ = 5.9 Hz) revealed that 28-H_{ax} and an oxygen atom located at C-29 were in *anti* orientation. Since these carbons are the members of the cyclohexene ring, the absolute stereochemistry at C-29 is clearly *S*. An NOE between the methyl protons 42-H and 28-H_{eq} assigned the remaining chiral carbon center, C-27, as being in *R* configuration (Figure 3, bottom).

Absolute Configuration at C-16 and C-18. The large coupling constant ($^2J_{C-H}$ < 2 Hz) which existed between 18-H and C-19 showed that 18-H and an oxygen atom substituted at C-19 were in *gauche* orientation. The relatively large coupling constant ($^3J_{C-H}$ = 4 Hz) between 19-H and C-17 and the small coupling constant ($^3J_{C-H}$ < 2 Hz) between 19-H and C-38 showed that they were almost completely in *anti* and *gauche* orientations, respectively. Thus, the absolute configuration at C-18 was established as *R*. The coupling constant ($^3J_{H-H}$ = 4 Hz) and the strong NOE between 18-H and 19-H supported the analysis of stereochemistry at C-18. The large coupling constants existing between 18-H and 17-H_a, and 17-H_b and 16-H ($^3J_{H-H}$ = 11 and 13 Hz, respectively) suggested that these protons were in *anti* orientation. The NOE between 38-H and 16-H confirmed that 16-H was located in the same direction as one of the methyl residues (C-38). The large coupling constant between 17-H_a and C-15 ($^3J_{C-H}$ = 7.5 Hz) proved that they were in *anti* orientation. Taking these results into consideration, the absolute configuration at C-16 was established to be *R* (Figure 4).

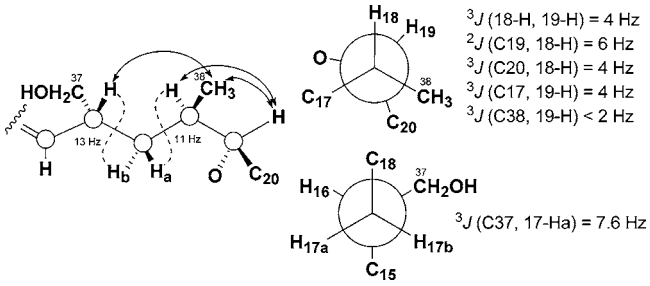

Figure 4. Configuration and conformation from C-16 to C-18. The conformations are derived from *J* and NOE data (arrows) of **1**.

Absolute Configuration of the Octalone Moiety. The large coupling constant between 15-H and 16-H, in addition to the strong NOE between 16-H and 35-H, showed that 15-H and 16-H are in the *anti* orientation. NOEs between 15-H, 13-H, and 31-H suggested that these protons were in the same orientation. In addition to these data, a critical NOE, located between the methylene proton 37-H and the methyl proton 34-H, proved that the absolute structure at C-13 was the *S* configuration. The large coupling constants between 5-H, 6-H, and 10-H ($^3J_{5H-10H}$ = 10 Hz, $^3J_{6H-10H}$ = 10 Hz), together with the NOEs which were found between 5-H and 9-H and between 6-H and 8-H_{ax}, established a transform octalone substructure. Moreover, the NOEs found between 6-H and 32-H, and 13-H and 31-H proved that these protons were in the same orientation, leading to the establishment of the relative stereochemistry of the transform octalone substructure. Thus, the remaining absolute stereochemistries on the octalone ring were deduced to be 4*S*, 5*S*, 6*R*, 9*S*, and 10*S*, respectively (Figure 5).

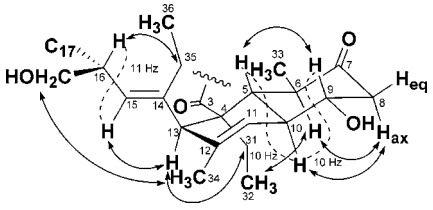

Figure 5. Configuration and conformation of the octalone moiety derived from NOE data (arrow) of **1**.

1 is the only compound consisting of a 17-membered macrocyclic structure with an α-acyltetronic acid moiety, which exhibited specific inhibitory activity on ER stress, thereby ameliorating the resultant induction of GRP78 expression.[3,5] Tetrocarcin A, which was recently reported to be a bcl-2 inhibitor, is the representative α-acyltetronic acid containing derivative.[10−12] Although tetrocarcin A and its hydrolysate derivative, known as tetronolide,[14] involve α-acyltetronic acid moieties, they are structurally characterized by a 13-membered macrocycle and hence did not exert the same ER stress inhibitory activity as did **1**. The absolute structure of the octalone and tetronic acid moieties in **1** faced

uniformly in the same direction, which was not the case in the 13-membered derivatives.[12,13] The truth of this statement was further confirmed by the NMR methodology we employed here. Thus, the characteristic activity of **1** may be due to either the 17-membered macrocyclic structure or the sugar moieties. GRP78 is now gaining widespread attention, not only in the cancer field but also in the field of neuroscience. ER stress is now also considered to play a significant role in the pathogenesis of central nervous diseases, most notably Alzheimer's and Parkinson's diseases.[14] Therefore, **1** is now strongly expected to provide a new hope for the improvement of cancer treatments and central nervous diseases. Detailed studies on the relevant structure−activity relationships are now underway.

(10) (a) Tomita, F.; Tamaoki, T.; Shirahata, K.; Kasai, M.; Morimoto, M.; Ohkubo, S.; Mineura, K.; Ishii, S. *J. Antibiot.* **1980**, *33*, 668−670. (b) Tamaoki, T.; Kasai, M.; Shirahata, K.; Ohkubo, S.; Morimoto, M.; Mineura, K.; Ishii, S.; Tomita, F. *J. Antibiot*. **1980**, *33*, 946−950. (c) Morimoto, M.; Fukui, M.; Ohkubo, S.; Tamaoki, T.; Tomita, F. *J. Antibiot*. **1982**, *35*, 1033−1037.

(11) (a) Nakashima, T.; Miura, M.; Hara, M. *Cancer Res.* **2000**, *60*, 1229−1235. (b) Anether, G.; Tinhofer, I.; Senfter, M.; Greil, R. *Blood* **2003**, *101*, 4561−4568.

(12) (a) Hirayama, N.; Kasai, M.; Shirahata, K.; Ohashi, Y.; Sasada, Y. *Tetrahedron Lett.* **1980**, *21*, 2559−2560. (b) Tamaoki, T.; Tomita, F. *J. Antibiot.* **1983**, *36*, 595−598.

(13) Mallams, A. K.; Puar, M. S.; Rossman, R. R.; McPhail, A. T.; Macfarlane, R. D. *J. Am. Chem. Soc*. **1981**, *103*, 3940−3943.

(14) Cahn, S. L.; Furukawa, K.; Mattson. M. P. *Neuromol. Med*. **2002**, *2*, 167−196.

Acknowledgment. This work was supported by Industrial Technology Research Grant Program in 01A04006b from New Energy and Industrial Technology Development Organization (NEDO) of Japan.

Supporting Information Available: Selected spectra used for configuration assignments of **1**; DQF-COSY, ROESY, and *J*-resolved HMBC for measurement of $^{2,3}J_{C-H}$ (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

OL070042T