S19159, a Modulator of Neurite Outgrowth Produced by the Ascomycete *Preussia aemulans*

II. Structure Elucidation

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In the preceding report[1], we presented the producing strain, fermentation, isolation and biological activity of S19159, a modulator of neurite outgrowth produced by *Preussia aemulans*. Herein we describe the structure determination of S19159.

High resolution FAB-MS data indicated the molecular formula $C_{30}H_{44}O_5$, which is consistent with the ^{13}C NMR data. The ^{13}C NMR spectrum exhibited thirty signals which were assigned as shown in Table 1 by analyses of DEPT and 2D PFG-HMQC[2] and HMBC[3] spectra. The ^{13}C NMR spectrum indicated the presence of one ketone group (C-1, 211.63 ppm), one carboxyl group (C-28, 181.15 ppm), a tri-substituted double bond at C-7 (117.82 and 142.48 ppm) and an exomethylene at C-24 (157.72 and 106.89 ppm) and also included five methyls, eight methylenes, seven aliphatic methines (including two oxygenated methines) and four sp^3 quaternary carbons. The molecular formula and hydrogen deficient index together with the ^{13}C NMR data suggested that S19159 consists of a pentacyclic structure. The presence of the partial structures shown in Fig. 2 as bold lines was revealed by analyses of DQF-COSY and spin-decoupling experiments. Assignments of ^1H NMR signals for the D ring and side chain portions, which were severely overlapped, were supported by 1D selective PFG-TOCSY[4] experiments, using well separated signals of H-14 at 3.04 ppm, Me-21 at 0.97 ppm, H-23 at 2.14 ppm and also H-7 at 5.24 ppm which had long range coupling with the H-14 methine proton. One remaining isolated methylene group at 1.38 and 1.51 ppm (Hs-19) had a small geminal coupling constant value of 4.2 Hz, indicating the presence of cyclopropane ring system[5~7].

The connectivities of the partial structures and quaternary carbons were determined by analyses of the HMBC spectrum. The connectivity surrounding C-24 at 157.72 ppm was established by the long range correlations from all protons of the isopropyl group (H-25~27) and allylic methylene protons (H-23). Positions of the two singlet methyl groups were easily determined to be C-4 and C-13 by HMBC data shown in Fig. 2. The position of the carboxyl group (C-28) was also determined to be C-4 by long range correlation from Me-29. Protons of the isolated methylene group (C-19) possessed long range correlations to quaternary carbons consisting of ketone carbon C-1, olefinic carbon C-8, and two methine carbons C-11 and C-14. The isolated methylene carbon C-19 also had correlations with methine protons H-5 and H-11. Based on the above mentioned NMR data the planar structure of S19159 was determined as shown in Fig. 2 with the triterpenoid skeleton of 30-norcycloartane having one additional carbon at C-24.

The stereochemistry of S19159 was determined by NOE differential experimental data and J values (Table 1). NOE data are summarized in Fig. 3. The axial orientation of H-3 was supported by the vicinal coupling constant ($J=9.3$ Hz) between axial H-2 and H-3 in addition to the observed NOE from H-5 to H-3. Thus, the beta orientation of the hydroxyl group at C-3 was establised. The orientation of Me-29 and the carboxyl group C-28 at C-4 was determined to be axial and equatorial respectively, by observed NOEs from H-19 at 1.51 ppm to Me-29 and from Me-29 to axial H-2 at 2.49 ppm. These data indicated that the C-19 methylene possesses the beta orientation. An NOE was observed between oxygenated methine H-11 and the upfield proton signal of the C-19 methylene group at 1.38 ppm, suggesting that the hydroxyl group at C-11 has the alpha orientation. The stereochemistry of C, D ring junction was determined to be normal trans by NOE data from H-17 to H-14 and from H-14 to the axial proton of H-12ax. The relative configuration at C-20 was deduced as shown in Fig. 1 by biogenetic consideration and by comparison of the 13-C chemical shifts of the side chain and the proton signal of H-17 (1.46 ppm, pseudo quartet,

Table 1. NMR data for S19159 in CD_3OD.

^{13}C (150 MHz)		^{1}H (600 MHz)		HMBC	NOE dif.
No.	ppm	No.	ppm (mult., J in Hz)		
1	211.63				
2	47.90	2eq	2.87 (dd, 6.8, 16.4)	C-1, 3	
		2ax	2.49 (dd, 9.3, 16.4)	C-1, 3, 4	
3	73.53	3	4.55 (dd, 6.8, 9.3)	C-29	
4	53.87				
5	38.94	5	2.37 (dd, 6.4, 9.3)	C-3, 6, 10, 19, 29	H-3
6	24.32	6eq	1.88 (m)		
		6ax	1.88 (m)		
7	117.82	7	5.24 (m)		
8	142.48				
9	36.97				
10	39.27				
11	70.13	11	4.34 (d, 5.9)	C-8, 9, 13, 19	H-12eq, 19a
12	46.86	12eq	1.98 (dd, 5.9, 14.7)	C-13, 14, 18	
		12ax	1.93 (d, 14.7)	C-11, 13, 17, 18	
13	43.00				
14	49.69	14	3.04 (m)		H-12ax
15	24.24	15a	1.42 (m)	C-13	
		15b	1.70 (m)	C-13	
16	29.75	16a	1.32 (m)		
		16b	1.98 (m)		
17	58.73	17	1.46 (q)	C-20	H-14
18	17.17	18	0.67 (s)	C-12, 13, 14, 17	
19	28.45	19a	1.38 (d, 4.2)	C-1, 8, 11, 14	H-11
		19b	1.51 (d, 4.2)	C-1, 8, 11	H-6, H-29
20	37.28	20	1.42 (m)	C-13	
21	18.90	21	0.97 (d, 4.2)	C-17, 20, 22	H-20
22	35.62	22a	1.18 (m)		
		22b	1.59 (m)		
23	32.05	23a	1.92 (m)	C-20, 22, 24, 31	
		23b	2.14 (m)		
24	157.72				
25	34.91	25	2.23 (m)	C-24, 26, 27	H-26, 27, 31b
26	22.45	26	1.02 (d, 6.8)	C-24, 25, 27	
27	22.47	27	1.03 (d, 6.8)	C-24, 25, 26	
28	181.15				
29	9.92	29	1.14 (d, 6.8)	C-3, 4, 5, 28	H-2ax
31	106.89	31a	4.66 (br. s)	C-23, 25	
		31b	4.72 (br. s)	C-23, 25	

$J=9.6$ Hz) with those of sterols that have a similar moiety[8~10]. Therefore the structure of S19159 including stereochemistry, was determined to be 3β,11∂-dihydroxy-24-methylene-1-oxo-30-norcycloart-7-en-28-oic acid as shown in Fig. 1. Cycloartane triterpenoids are well known as metabolites of some species of the plant kingdom[11] and 24-methylene-cycloartane compounds have been isolated[5,6]. Previously some aromatic antifungal metabolites, *e.g.* culpin[12] and preussomerins[13] have been isolated from *Preussia* spp. fungi[11]. How-

Fig. 1. Structure of S19159.

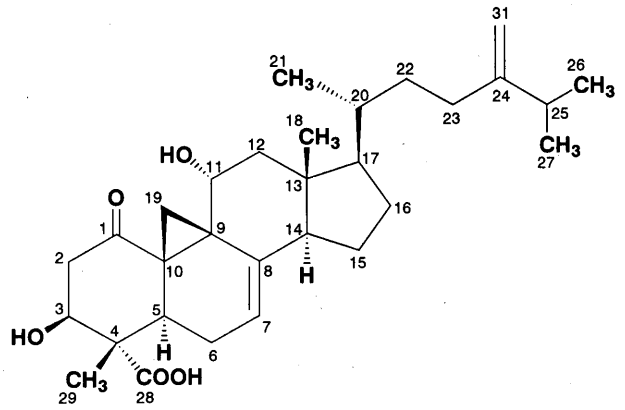
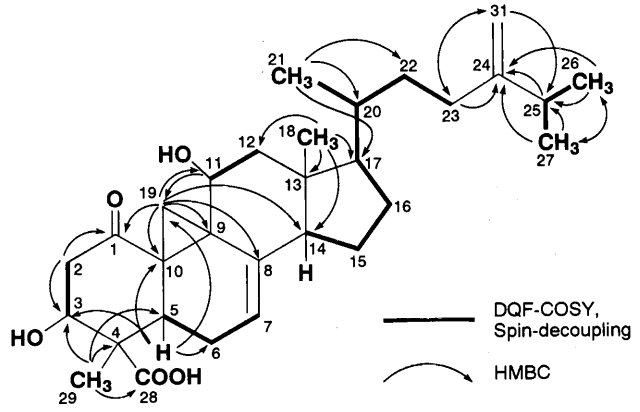


Fig. 2. Planar structure of S19159 elucidated by NMR analyses.



ever 30-norcycloartane such as S19159 is unique as a fungal metabolite.

Experimental

NMR spectra were obtained on a JEOL JNM A600 FT NMR spectrometer, and measured in CD_3OD solution. Chemical shifts are given in ppm, using the solvent signals for CD_2HOD at 3.30 ppm and CD_3OD at 49.0 ppm as internal standards for ^1H and ^{13}C NMR, respectively.

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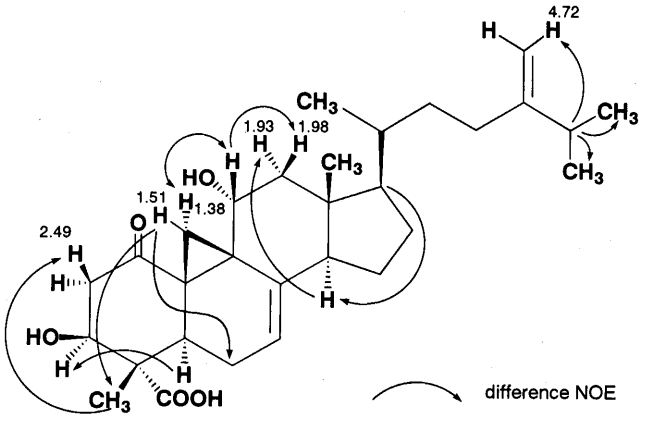
Fig. 3. Stereochemistry of S19159 with NOE differential experimental data.



References

1) SATO, T.; T. HANADA, M. ARIOKA, K. ANDO, J. SUGIYAMA, M. URAMOTO, M. YAMASAKI & K. KITAMOTO: S19159, a modulator of neurite outgrowth produced by the ascomycete *Preussia aemulans*. I. Producing strain, fermentation, isolation and biological activity. J. Antibiotics 51: 897~901, 1998
2) HURD, R. E. & B. K. JOHN: Gradient-enhanced proton-detected heteronuclear multiple-quantum coherence spectroscopy. J. Magn. Reson. 91: 648~653, 1991
3) WILLKER, W.; D. LEIBFRITZ, R. KERSSEBAUM & W. BERMELL: Gradient selection in inverse heteronuclear correlation spectroscopy. Magn. Reson. Chem. 31: 287~292, 1993
4) FÄCKE, T. & S. BERGER: Application of pulsed field gradients in an improved selective TOCSY experiments. J. Magn. Reson. A. 113: 257~259, 1995
5) OSBORNE, R. & K. H. PEGEL: Jessic acid and related acid triterpenoids from combretum elaeagnoides. Phytochemistry 23: 635~637, 1984
6) KIKUCHI, T.; S. KADOTA & K. TSUBONO: Studies on the constituents of orchidaceous plants. IV. Proton and carbon-13 signal assignments of cycloeucalenol-type triterpenes from Nervilia purpurea SCHLECHTER by two-dimensional nuclear magnetic resonance spectroscopy. Chem. Pharm. Bull. 34: 2479~2486, 1986
7) ROGERS, C. B.: Isolation of the 1a-hydroxycycloartenoid mollic acid a-L-arabinoside from combretum edwardsii leaves. Phytochemistry 28: 279~281, 1989
8) LEET, J. E.; S. HUANG, S. E. KLOHR & K. D. MCBRIEN: Ascosteroside, a new antifungal agent from *Ascotricha amphitricha*. II. Isolation and structure elucidation. J. Antibiotics 49: 553~559, 1996
9) KNIGHT, S. A.: Carbon-13 NMR spectra of some tetra- and pentacyclic triterpenoids. Org. Magn. Resonance 6: 603~611, 1974
10) KOSHINO, H.; T. YOSHIHARA, S. SAKAMURA, T. SHIMANUKI, T. SATO & A. TAJIMI: A ring B aromatic sterol from stromata of *Epichloe typhina*. Phytochemistry 28: 771~772, 1989

11) BUCKINGHAM, J.: Dictionary of natural products. Chapman & Hall, London, 1995
12) JOHNSON, J. H.; E. MEYERS, J. O'SULLIVAN, D. W. PHILLIPSON, G. ROBINSON, W. H. TREJO & J. SCOTT: Culpin, a novel hydroquinone antibiotic of fungal origin. J. Antibiotics 42: 1515~1517, 1989
13) WEBER, H. A. & J. B. GLOER: The preussomerins: Novel antifungal metabolites from the coprophilous fungus *Preussia isomera* Cain. J. Org. Chem. 56: 4355~4360, 1991