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Byssochlamysol, a New Antitumor Steroid against IGF-1-dependent Cells from *Byssochlamys nivea*

II. Physico-chemical Properties and Structure Elucidation

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The structure of byssochlamysol, a new antitumor metabolite against IGF-1-dependent cancer cells from *Byssochlamys nivea* M#5187, was determined to be a highly oxidized ergostane steroid as shown in Fig. 1 by NMR studies.

In the preceding paper[1], we have described the fermentation, isolation and biological activity of byssochlamysol, a new antitumor metabolite against IGF-1-dependent cancer cells as well as the taxonomy of the producing organism, *Byssochlamys nivea* M#5187. We report herein the physico-chemical properties and structure elucidation of byssochlamysol.

Physico-chemical Properties

The physico-chemical properties of byssochlamysol are summarized in Table 1. The molecular formula was established to be $C_{32}H_{50}O_7$ by high-resolution FAB-MS. The IR spectrum indicated the presence of hydroxyl groups (3450 cm^{-1}) and carbonyl groups (1700 cm^{-1} and 1680 cm^{-1}).

Structure Elucidation

The ^{13}C NMR spectrum of byssochlamysol confirmed the presence of 32 carbons. A heteronuclear multiple-quantum coherency (HMQC)[2] experiment established all one-bond ^{1}H-^{13}C connectivities as shown in Table 2. A COSY experiment revealed four spin networks to generate partial structures A to D (Fig. 2), although further assignments were disturbed by severe overlap of 9-H and 14-H. The heteronuclear multiple-bond correlation (HMBC)[3] spectrum exhibited long-range correlations from 18-H_3 to C-12, C-13, C-14 and C-17, from 16-H to C-14, and from 15-H to C-8, indicating the connections of partial structures C and D as shown in Fig. 2. Long-range couplings from 19-H_3 to C-1, C-5, C-9 and C-10, and from 12-H to C-9 established the connection of partial structures A and C. A ketone carbonyl (C-6) coupled with 5-H and

Table 1. Physico-chemical properties of byssochlamysol.

Appearance	colorless powder
MP	265~268°C
$[\alpha]_D^{25}$	+53° (*c* 0.69, $CHCl_3$)
Molecular formula	$C_{32}H_{50}O_7$
HRFAB-MS (m/z)	
Found:	569.3456 $(M+Na)^+$
Calcd.:	569.3454
UV λ_{max} nm (ε) in MeOH	277 (120)
IR ν_{max} (KBr) cm^{-1}	3480, 1730

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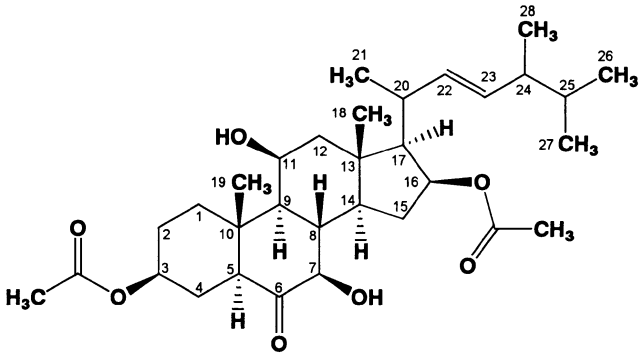
Table 2. ^{13}C and ^{1}H NMR data summary for byssochlamysol in $CDCl_3$.

No.	δ_C	δ_H (multiplicity, J = Hz)	No.	δ_C	δ_H (multiplicity, J = Hz)
1	35.7	1.98 (m), 1.40 (m)	18	15.4	1.12 (3H, s)
2	26.4	1.85 (m), 1.52 (m)	19	15.5	0.95 (3H, s)
3	72.2	4.66 (m)	20	34.3	2.51 (m)
4	25.5	1.86 (m), 1.74 (m)	21	21.1	1.05 (3H, d, 7.0)
5	54.4	2.30 (dd, 13.0, 3.0)	22	134.9	5.16 (m, 15.0)
6	209.3		23	133.0	5.13 (m, 15.0)
7	78.8	3.69 (dd, 9.0, 3.0)	24	43.2	1.76 (m)
8	42.4	2.12 (ddd, 12.0, 11.0. 9.0)	25	33.0	1.40 (m)
9	55.2	1.34 (m)	26	19.6	0.80 (3H, d, 7.0)
10	40.6		27	20.1	0.78 (3H, d, 7.0)
11	68.3	4.36 (dt, 7.0, 3.0)	28	18.0	0.85 (3H, d, 7.0)
12	48.8	2.15 (dd, 14.0, 3.0), 1.49 (m)	3-OAc	170.6	
13	42.9			21.3	2.02 (3H, s)
14	56.0	1.34 (m)	6-OAc	170.0	
15	36.4	2.60 (ddd, 15.0, 8.0, 8.0), 1.52 (m)		21.5	1.95 (3H, s)
16	74.6	5.08 (ddd 8.0, 8.0, 4.0)	7-OH		3.64 (d, 3.0)
17	60.1	1.21 (dd, 11.0, 8.0)			

7-H showed the connection of partial structures A and B *via* C-6. Long-range correlations between 7-H and C-9, and between 15-H and C-8 revealed the connectivities of partial structures B, C and D to form a tetracyclic structure. ^{1}H-^{13}C long-range couplings from two oxymethines (3-H and 16-H) and two methyls (δ 2.02, 1.95) to the relevant ester carbonyls (δ 170.6, 170.0) established the presence of two acetoxyl groups at C-3 and C-16. The geometrical configuration of C-22 was determined to be *E* based on a large coupling constant ($J_{22\sim23}$=15.0 Hz). From these results, the planar structure of byssochlamysol was determined to be a new ergostane steroid as shown in Fig. 1.

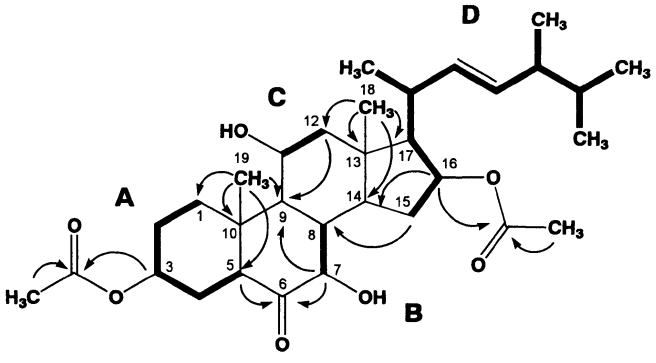
The relative stereochemistry of byssochlamysol was analyzed by a NOESY experiment. As shown in Fig. 3, NOEs among 2-H_{ax}, 4-H_{ax}, 8-H, 15-H_β, 18-H_3 and 19-H_3 indicated these protons to exist on the β side of the tetracyclic ring system. On the other hand, 1-H_{ax}, 3-H, 5-H, 7-H, 9-H, 12-H_{ax}, 14-H, 15-H_α, 16-H and 17-H were required to be on the α side from NOEs between couples of them. An NOE between 1-H_{eq} and 11-H assigned these protons to equatorial orientation, indicating a β configuration for the 11-hydroxyl group. Byssochlamysol

Fig. 1. Structure of byssochlamysol.



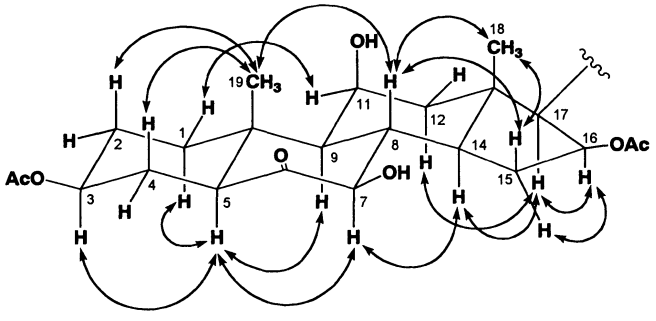
seems to be a 3-*O*-acetyl derivative of aniceqoul[4], an inhibitor for anchorage-independent growth of tumor cells from *Penicillium aurantiogriseum*, although the stereochemistry of the side chain of our compound is unclear. Further stereochemical studies on byssochlamysol are now underway.

Fig. 2. COSY and HMBC data summary for byssochlamysol.



Bold lines show proton spin networks, arrows indicate ^{1}H-^{13}C long-range correlations.

Fig. 3. NOESY data summary for byssochlamysol.



Experimental

UV and IR spectra were measured on Hitachi U-3210 and JASCO A-102 spectrometers, respectively. Mass spectra were obtained on a JEOL HX-110 spectrometer in the FAB mode using *m*-nitrobenzyl alcohol as matrix and polyethylene glycol as internal standard. Optical rotations were recorded on a JASCO DIP-1000 spectropolarimeter. ^{1}H and ^{13}C NMR spectra were measured on a JEOL JNM-A500 spectrometer with ^{1}H NMR at 500 MHz and ^{13}C NMR at 125 MHz. Chemical shifts are given in ppm using TMS as internal standard.

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