IB-00208, a New Cytotoxic Polycyclic Xanthone Produced by a Marine-derived *Actinomadura*

II. Isolation, Physico-chemical Properties and Structure Determination

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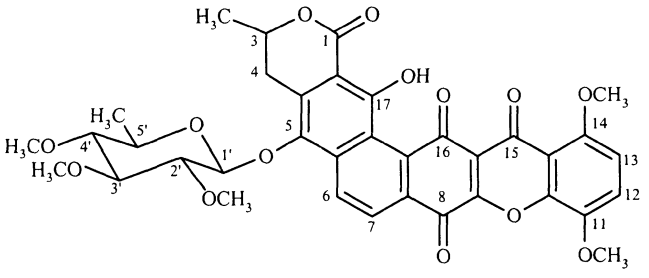
In the course of our screening program for new cytotoxic compounds produced by marine microorganisms, a new natural polycyclic xanthone IB-00208 has been isolated from the culture broth of *Actinomadura* sp. The structure IB-00208 (**1**) contains a xanthone unit and is structurally related to a small family of polycyclic antibiotics including cervinomycins[2~5], citreamicins[6,7], simaomycins[8~10] and actinoplanones[11,12], compounds isolated from different terrestrial strains of *Micromonospora*, *Streptomyces*, *Actinomadura* and *Actinoplanes*, respectively. In this paper the isolation, physico-chemical properties and structure elucidation of IB-00208 are described.

Isolation

The producing microorganism, *Actinomadura* sp., was cultivated as shown in previous paper[1]. After completion of the cultivation, whole harvested broth (4.5 liters) was filtered with diatomaceous earth. The mycelial cake was extracted twice with HCl_3 - MeOH - H_2O 2 : 1 : 1 (1.5 liters). After filtration, the organic layer was concentrated under reduced pressure giving a brownish oily residue (1.2 g). The extract was chromatographied on silica gel by a VFC (Vacum flash chromatography) system and eluted with a stepwise gradient of EtOAc-MeOH. The active fractions containing IB-00208 were eluted with EtOAc - MeOH 1 : 1. Further purification was achieved by column chromatography on silica gel using $CHCl_3$-MeOH as eluting solvent to yield 18 mg of pure IB-00208 eluted with $CHCl_3$ - MeOH 95 : 5. The purity of each preparation was confirmed by TLC visualized with vanillin in conc. H_2SO_4, by anlytical HPLC with photo-diode array detector and by monitoring the cytotoxic activity against P-388 cells. The isolation procedure of pure IB-00208 compound is summarized in Figure 2.

Fig. 1. Proposed structure of IB-00208 (**1**).



Results and Discussion

IB-00208 (**1**) was isolated as an orange powder, which was soluble in common organic solvent such as $CHCl_3$, MeOH and DMSO, but was insoluble in hexane and water. The physico-chemical properties of **1** are summarized in Table 1. UV absortions at 225, 255, 325, and 385 nm suggested the presence of a polycyclic xanthone moiety[13].

Fig. 2. Isolation procedure for IB-00208 (**1**).

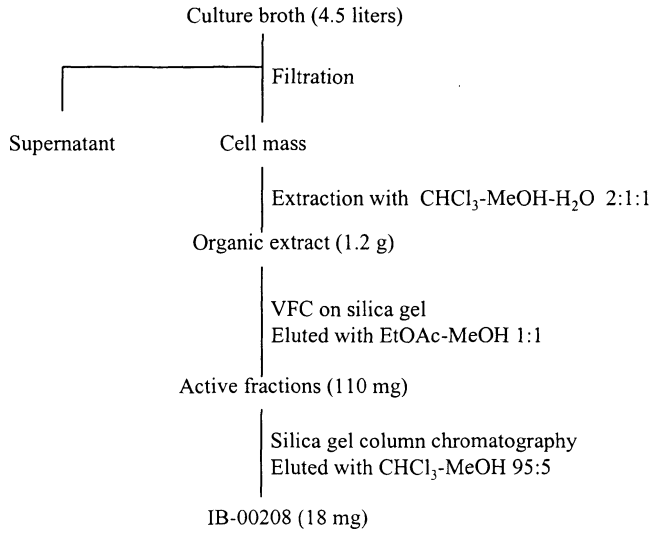


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Table 1. Physico-chemical properties of IB-00208 (**1**).

Appearance	Orange powder
Molecular formula	$C_{36}H_{34}O_{14}$
ESI-MS $(M+Na)^+$	713
ESI-MS $(M+H)^+$	691
HRFAB-MS $(M+3H)^+$	693.2194 (Calcd; 693.2183)
$[\alpha]_D^{25}$	- 65.0° (c 0.15, $CHCl_3$)
UV λ_{max} nm	225, 255, 325, 385
IR υ_{max}(KBr) cm^{-1}	3400, 1697, 1623, 1509, 1436
TLC[a] Rf value[b]	0.75
HPLC (Rt, minutes)[c]	4.53

a Silica gel 60 F_{254}, Merck
b Solvent : $CHCl_3$-MeOH (9:1)
c Symmetry C18 column (5μ); mobile phase: MeOH:H_2O+1% HOAc (99:1); flow rate: 0.3 ml/min.; detection: 325 nm

Table 2. ^{13}C and ^1H NMR assigments of IB-00208 (**1**) in $CDCl_3$.

Position C/H No.	^{13}C shifts[a] ($CDCl_3$)	^1H shifts[a,b,c] ($CDCl_3$)
1	170.4	
3	76.2	4.68 m, 1H
3-CH_3	21.1	1.56 d, 3H (J=6.4)
4	30.5	2.80 dd, 1H (J=11.2, 16.2)
		3.74 dd, 1H (J=3.0, 16.4)
4a	131.8	
5	139.9	
5a	138.6	
6	127.6	8.56 d, 1H (J=8.8)
7	124.8	8.26 d, 1H (J=8.8)
7a	129.8	
8	178.8	
8a	153.2	
10	149.8	
11	148.7	
11-OCH_3	62.3	4.03 s, 3H
12	119.7	7.38 d, 1H (J=9.2)
13	114.6	7.46 d, 1H (J=9.2)
14	151.5	
14-OCH_3	57.0	3.95 s, 3H
14a	121.1	
15	173.3	
15a	121.3	
16	181.8	
16a	136.9	
16b	119.9	
17	160.3	
17-OH		12.71 s, 1H
17a	104.8	
1'	105.2	4.49 d, 1H (J=8.0)
2'	84.7	3.34 dd, 1H ((J=8.0, 9.1)
2'-OCH_3	61.4	3.81 s, 3H
3'	86.7	3.17 t, 1H (J=9.0)
3'-OCH_3	61.3	3.69 s, 3H
4'	85.4	2.84 t, 1H (J=9.0)
4'-OCH_3	61.0	3.56 s, 3H
5'	71.6	3.02 dd, 1H (J=6.0, 9.2)
5'-CH_3	17.7	1.15 d, 3H (J=6.0)

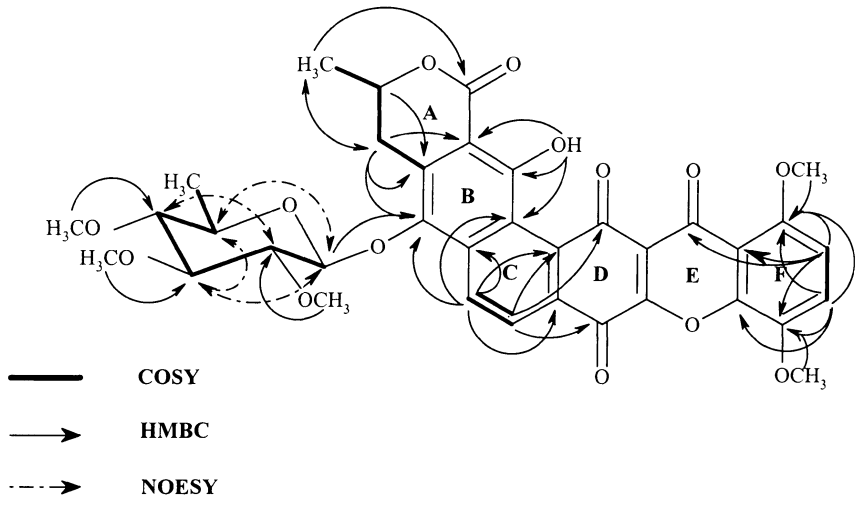
NMR spectra were acquired on a Varian Mercury NMR spectrometer (400 MHz for ^1H, 100 MHz for ^{13}C)
[a] Chemical shifts (δ) are in ppm
[b] Coupling constants (J) in hertz (Hz) are given in parentheses
[c] s: singlet; d: doublet; t: triplet; m: multiplet

The IR spectrum of **1** indicated the presence of hydroxyl (3400 cm^{-1}) and conjugated carbonyl (1694, 1623 cm^{-1}) funcionalities. Analysis by HPLC-ESI-MS revealed its molecular mass to be 690. The molecular formula of **1** was established as $C_{36}H_{34}O_{14}$ by HRFAB-MS. The accurate mass measurements were done on the $(M+3H)^+$ molecular adduct ion [$(M+3H)^+$: found *m/z* 693.2194, calcd for $C_{36}H_{37}O_{14}$ *m/z* 693.2183], since the antibiotic was converted to their hydroquinone analog in the mass spectrometer under most experimental conditions. This reduction phenomenon in FAB-MS is well documented for quinones[6,14].

The ^1H and ^{13}C NMR spectral data of **1** are shown in Table 2. The ^{13}C NMR spectrum demostrated 36 signals which were assigned to seven methyls, one methylene, ten methines and eighteen quaternary carbons by DEPT and PFG-HMQC experiments. ^1H and ^{13}C NMR spectra of **1** indicated the presence of a glycopyranosyl moiety in the structure, because the characteristic signals at δ 4.49 (1′-H) and δ 105.2 (C-1′) were assignable to the anomeric signals. The configuration of this glycosidic bond was determined to be β based on its large coupling constant ($J_{1',2'}$=8.0 Hz). As shown in Figure 3, ^1H-^1H COSY data showed the connectivity from 1′-H to 6′-H in the sugar moiety and revealed connectivity from 4-H (δ 2.80 and 3.74) to 3-H (δ 4.68), from 3-H to 3-CH_3 (δ 1.56), from 6-H (δ 8.56) to 7-H (δ 8.26) and from 12-H (δ 7.38) to 13-H (δ 7.46).

The precise connectivities of **1** were established by interpretation of PFG-HMBC data summarized in Figure 3. Correlations of methylene 4-H to C-3, 3-CH_3, C-4a, C-5 and C-17a, and the methyl 3-CH_3 to C-3, C-4 and C-1 indicated the presence of the lactone A ring in conjunction with the highly substituted aromatic B ring. The OH signal at δ 12.71 was coupled with C-17, C-17a and C-16b, this OH was located at C-17 on the B ring. The long range coupling of 1′-H (anomeric proton) to C-5 (δ 139.9) in the HMBC experiment showed the linkage of the sugar moety to the B ring at C-5. HMBC data showed that the 6-H

Fig. 3. Summary of ^{1}H-^{1}H COSY and HMBC and NOESY experiments of IB-00208 (**1**).



signal (δ 8.56) correlated to C-5, C-5a, C-7a, C-16a, C-16b, and C-17, and 7-H (δ 8.26) correlated to C-5a, C-8, C-16a and a four-bond coupling of 7-H to C-16 was also observed. These correlations revealed that the B ring had a fused-ring junction with the C ring through C-5a and C-16b and the C ring was connected to the D ring at C-7a and C-16a. Correlations of 12-H (δ 7.38) to C-10, C-11, and C-14, and 13-H (δ 7.46) to C-11, C-14 and C-14a supported the assigments of F ring and the connection to C-10 and C-14a on the E ring. In addition, a four-bond coupling of 13-H to C-15 gave evidence for the location of the carbonyl of γ-pyrone system.

The relative stereochemistry of the sugar was established by the analysis of spin-spin coupling constants. The four vecinal coupling constants in the sugar were all large (8.0~9.4 Hz), these values determined the diaxial configuration of the coupling protons. This result was confirmed by the NOESY experiment of **1**, cross peaks were observed between 1′-H and 3′-H, 1′-H and 5′-H, 3′-H and 5′-H, and 2′-H and 4′-H, respectively. These observations indicated that the conformation of sugar part is a chair-form with equatorial substituents.

Acknowledgments

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