Adxanthromycins A and B, New Inhibitors of ICAM-1/LFA-1 Mediated Cell Adhesion Molecule from *Streptomyces* sp. NA-148

II. Physico-chemical Properties and Structure Elucidation

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Adxanthromycins A and B are new inhibitors of ICAM-1/LFA-1 mediated cell adhesion molecule isolated from the fermentation broth of *Streptomyces* sp. NA-148. The molecular formula of adxanthromycins A and B were determined as $C_{42}H_{40}O_{17}$ and $C_{48}H_{50}O_{22}$, respectively by FAB-MS and NMR spectral analyses, and the structures of both compounds were elucidated to be a dimeric anthrone peroxide skeleton containing α-D-galactose by various NMR spectral analyses and chemical degradation.

In the course of our screening program for new inhibitors of ICAM-1/LFA-1 mediated cell adhesion molecule from microorganisms, adxanthromycins A (**1**) and B (**2**) (Fig. 1) were isolated as new inhibitors from the fermentation broth of *Streptomyces* sp. NA-148 which was isolated from a soil sample collected in Kanuma City, Tochigi Prefecture, Japan. In the previous paper[1], we described the screening, the isolation procedure and the biological properties of compound **1**. During the purification of **1** from the fermentation broth of the strain *Streptomyces* sp. NA-148, we discovered an additional new inhibitor of ICAM-1/LFA-1 which was designated as adxanthromycin B (**2**)[2].

A search for inhibitors of ICAM-1/LFA-1 system from microbial metabolites was carried out on the basis of two methodologies: an aggregation assay using JY cells[3] and an adhesion assay using soluble ICAM-1 and LFA-1 positive SKW-3 T cell leukemia cells[4]. Both compounds inhibited the formation of the JY cell aggregates from 1.5 μg/ml in a dose dependent manner. Compounds **1** and **2** inhibited SKW-3 adhesion to soluble ICAM-1 in a dose dependent manner with IC_{50} values of 18.8 μg/ml and 25.0 μg/ml, respectively. The structures of **1** and **2** are unique in that a dimeric anthrone peroxide skeleton is connected with one or two molecules of α-D-galactose. In this paper, we describe the physico-chemical properties and structural elucidation of **1** and **2**.

Results and Discussion

Physico-chemical Properties

The physico-chemical properties of **1** and **2** are summarized in Table 1. Compounds **1** and **2** were obtained as pale yellow powders. The molecular formulas of **1** and **2** were established as $C_{42}H_{40}O_{17}$ [found *m/z* 815.2252 $(M-H)^-$, calcd. 815.2187 for $C_{42}H_{39}O_{17}$] and $C_{48}H_{50}O_{22}$ [found *m/z* 979.2934 $(M+H)^+$, calcd. 979.2872 for $C_{48}H_{51}O_{22}$], respectively on the basis of HRFAB-MS and NMR spectral analyses. Both compounds showed similar UV absorption maxima in MeOH at 272 nm (ε 5,770), 315 nm (ε 6,730) and 358 nm (ε 4,390) for **1** and 272 nm (ε 6,000), 312 nm (ε 6,500) and 356 nm (ε 4,400) for **2**, suggesting the presence

Table 1. Physico-chemical properties of adxanthromycins A (**1**) and B (**2**).

	1	**2**
Appearance	Pale yellow powder	Pale yellow powder
MP	233~235°C (dec.)	218°C < (dec.)
$[\alpha]_D$	+120.5° (*c* 0.2, 26°C, DMSO)	+105.4° (*c* 0.1, 25°C, DMSO)
Molecular formula	$C_{42}H_{40}O_{17}$	$C_{48}H_{50}O_{22}$
Molecular weight	816	978
FAB-MS (*m/z*)	817 $(M+H)^+$, 815 $(M-H)^-$, 839 $(M+Na)^+$	979 $(M+H)^+$, 977 $(M-H)^-$, 1001 $(M+Na)^+$
HRFAB-MS (*m/z*)		
Found :	815.2252 $(M-H)^-$	979.2934 $(M+H)^+$
Calcd :	815.2187 for $C_{42}H_{39}O_{17}$	979.2872 for $C_{48}H_{51}O_{22}$
UV λ_{max}^{MeOH} nm (ε)	272 (5,770), 315 (6,730), 358 (4,390)	272 (6,000), 312 (6,500), 356 (4,400)
$\lambda_{max}^{MeOH\text{-}0.1N\ HCl}$ nm (ε)	272 (5,830), 309 (6,900), 359 (3,590)	272 (5,050), 308 (7,430), 357 (3,360)
$\lambda_{max}^{MeOH\text{-}0.1N\ NaOH}$ nm (ε)	254 (9,310), 315 (6,400), 377 (8,170)	254 (6,000), 314 (4,340), 378 (5,630)
IR ν_{max}^{KBr} (cm^{-1})	3400, 2928, 1713, 1630, 1593, 1580, 1422, 1333, 1291, 1256, 1190, 1156, 1142, 1078, 1047, 885, 828	3405, 2928, 1715, 1628, 1595, 1578, 1422, 1335, 1291, 1256, 1192, 1156, 1142, 1082, 887, 828
TLC (Rf value)[a]	0.20	0.16

[a] Silica gel TLC (Merck No. 5715) : $CHCl_3$-MeOH-H_2O (10 : 6 : 1)

of the same chromophore. The IR spectra (KBr) of **1** and **2** indicated the presence of hydroxy (3400 cm^{-1} and 3405 cm^{-1}), carbonyl (1713 cm^{-1} and 1715 cm^{-1}) and hydrogen bonded carbonyl groups (1630 cm^{-1} and 1628 cm^{-1}). Both compounds were soluble in DMSO, DMF and alkaline H_2O, slightly soluble in MeOH and EtOAc and insoluble in $CHCl_3$, ether, *n*-hexane and H_2O. Compounds **1** and **2** gave positive color reactions to molybdophosphoric acid, sulfuric acid, iodine vapor and negative to ninhydrin reagent. The Rf values of **1** and **2** on Silica gel 60 F_{254} precoated glass plate using solvent system of $CHCl_3$ - MeOH - H_2O (10 : 6 : 1) were 0.20 and 0.16, respectively. Compounds **1** and **2** eluted with a retention time at 13.0 minutes and 10.6 minutes, respectively by HPLC analyses on SHISEIDO CAPCELL PAK C_{18} (SG120) with 35% acetonitrile in 10 mM phosphate buffer (pH 6.0) as a mobile phase.

Structure Elucidation of Adxanthromycin A (1)

The structural studies were first carried out for **1**, the major active component, and the structure of **2** was subsequently determined by comparing the NMR spectral data with those of **1**. The structures of compounds **1** and **2** were mainly deduced by various NMR spectral analyses including PFG (pulsed field gradient)-DQFCOSY, PFG-HMQC, PFG-HMBC, NOE difference and NOESY experiments.

Some of the ^{1}H and ^{13}C NMR signals of **1** in DMSO-d_6 were observed as broad signals. However, an addition of HCl gas sharpened these signals. The ^{13}C NMR spectrum of **1** (Fig. 2) accounts for the presence of 42 carbon signals. These are six methyl carbons (δ_C 15.3×2, 20.4, 20.7, 32.9 and 33.2), one oxygenated methylene carbon (δ_C 59.8), five oxygenated methine carbons (δ_C 67.9, 68.2, 69.3, 72.5 and 96.8), two quarternary carbons (δ_C 79.7 and 80.0), six sp^2 methine carbons (δ_C 110.7, 112.3, 117.0×2, 136.1 and 136.3), eighteen sp^2 quarternary carbons (δ_C 114.4, 114.6, 120.0, 122.3, 124.9, 125.1, 127.5, 129.1, 138.7, 139.5, 141.2, 141.6, 148.9×2, 155.8, 158.1 and 159.6×2) and four carbonyl carbons (δ_C 168.2, 168.9, 189.5 and 189.6) (Table 2). On the other hand, the ^{1}H NMR spectrum of **1** (Table 2) revealed the presence of six methyl protons [δ_H

Fig. 1. Structures of adxanthromycins A (**1**), B (**2**) and oxanthromicin (**3**).

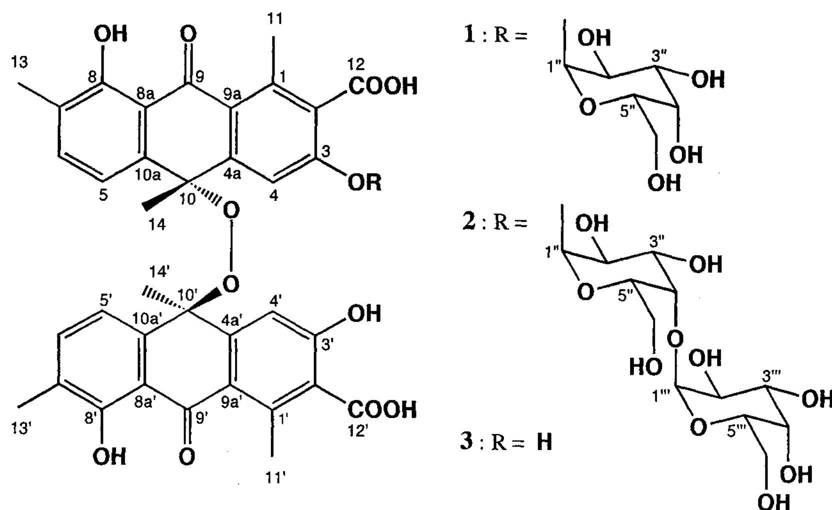


Fig. 2. ^{13}C NMR spectra of adxanthromycins A (**1**) and B (**2**) (125 MHz, in DMSO-d_6+HCl gas).

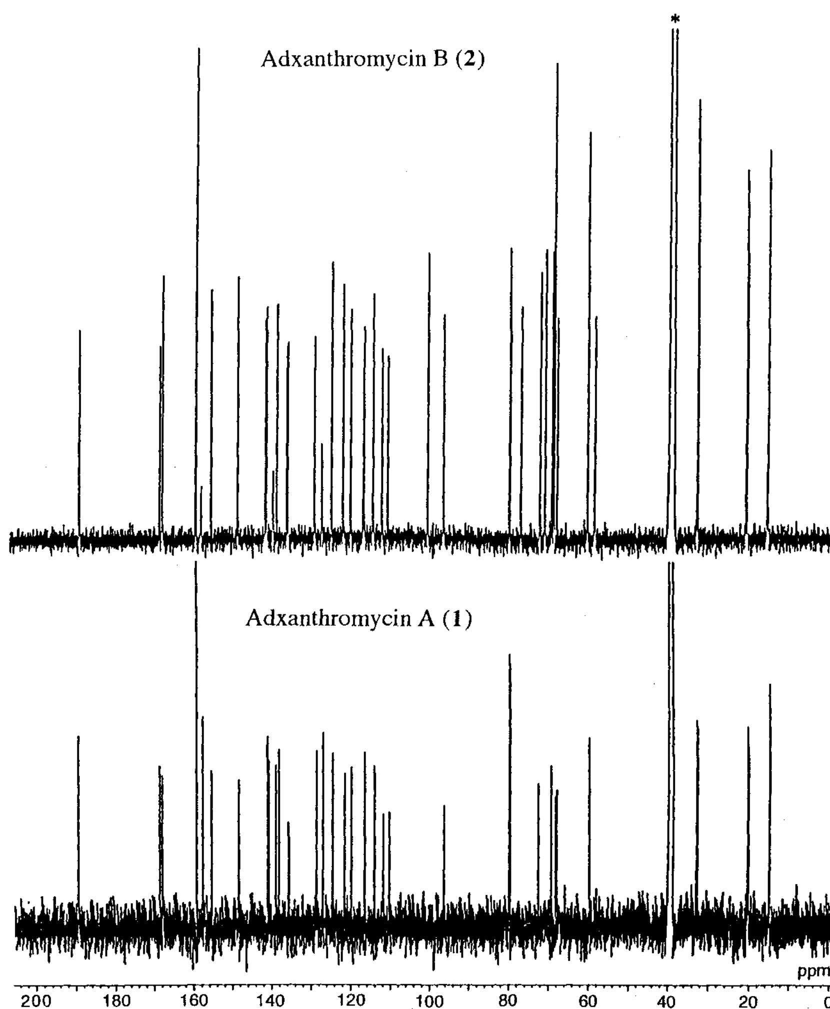
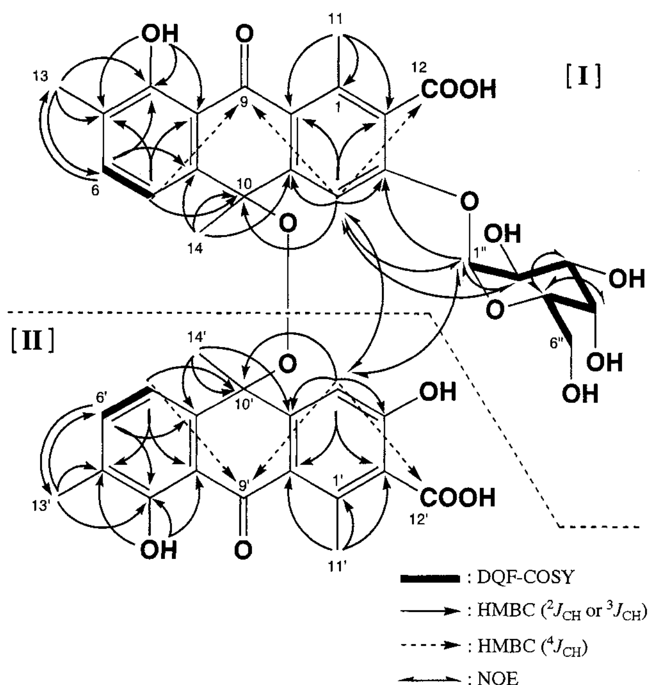


Table 2. ^{1}H (600 MHz) and ^{13}C (125 MHz) NMR chemical shift assignments of adxanthromycins A (**1**) and B (**2**) (in DMSO-d_6+HCl gas).

	1		**2**	
Position	^{13}C(δ)	^{1}H(δ)	^{13}C(δ)	^{1}H(δ)
C-1	138.7		138.6	
C-2	129.1		129.1	
C-3	155.8		155.5	
C-4	110.7	7.27 (1H, s)	110.6	7.29 (1H, s)
C-4a	148.9		148.6	
C-5	117.0	6.29 (1H, d, J = 8.3Hz)	116.8	6.31 (1H, d, J = 7.7Hz)
C-6	136.3	7.07 (1H, d, J = 8.3Hz)	136.1	7.06 (1H, d, J = 7.7Hz)
C-7	114.4		114.3	
C-8	159.6		159.5	
C-8a	124.9		124.8	
C-9	189.6		189.4	
C-9a	122.0		122.0	
C-10	80.0		79.9	
C-10a	141.6		141.5	
C-11	20.4	2.69 (3H, s)	20.4	2.71 (3H, s)
C-12	168.2		168.1	
C-13	15.3	2.19 (3H, s)	15.2	2.19 (3H, s)
C-14	33.2	1.39 (3H, s)	33.0	1.41 (3H, s)
C-8-OH		13.36 (1H, s)		13.35 (1H, s)
C-1'	139.5		139.5	
C-2'	127.5		127.3	
C-3'	158.1		158.1	
C-4'	112.3	7.20 (1H, s)	112.1	7.23 (1H, s)
C-4a'	148.9		148.7	
C-5'	117.0	6.28 (1H, d, J = 8.3Hz)	116.7	6.23 (1H, d, J = 7.7Hz)
C-6'	136.1	7.06 (1H, d, J = 8.3Hz)	135.8	7.02 (1H, d, J = 7.7Hz)
C-7'	114.6		114.4	
C-8'	159.6		159.5	
C-8a'	125.1		124.8	
C-9'	189.5		189.3	
C-9a'	120.3		120.0	
C-10'	79.7		79.7	
C-10a'	141.2		141.2	
C-11'	20.7	2.68 (3H, s)	20.6	2.69 (3H, s)
C-12'	168.9		168.7	
C-13'	15.3	2.19 (3H, s)	15.2	2.19 (3H, s)
C-14'	32.9	1.40 (3H, s)	33.1	1.40 (3H, s)
C-8'-OH		13.48 (1H, s)		13.48 (1H, s)
C-1"	96.8	5.73 (1H, d, J = 3.5Hz)	96.5	5.83 (1H, d, J = 3.3Hz)
C-2"	67.9	3.95 (1H, dd, J = 9.8, 3.5Hz)	67.9	3.95 (1H, dd, J = 10.3, 3.3Hz)
C-3"	69.3	3.74 (1H, m)	68.8	3.84 (1H, dd, J = 10.3, 2.9Hz)
C-4"	68.2	3.75 (1H, m)	77.0	3.91 (1H, d, J = 2.9Hz)
C-5"	72.5	3.55 (1H, dd, J = 7.0, 6.2Hz)	72.2	3.65 (1H, m)
C-6"	59.8	3.45 (1H, dd, J = 11.0, 7.0Hz) 3.24 (1H, dd, J = 11.0, 6.2Hz)	58.6	3.66 (1H, m) 3.25 (1H, m)
C-1"'			100.5	4.84 (1H, d, J = 3.7Hz)
C-2"'			68.7	3.65 (1H, dd, J = 10.3, 3.7Hz)
C-3"'			69.3	3.59 (1H, dd, J = 10.3, 2.9Hz)
C-4"'			68.8	3.78 (1H, d, J = 2.9Hz)
C-5"'			71.0	4.17 (1H, dd, J = 6.6, 6.6Hz)
C-6"'			60.4	3.53 (1H, dd, J = 10.6, 6.6Hz) 3.48 (1H, dd, J = 10.6, 6.6Hz)

1.39 (s), 1.40 (s), 2.19×2 (s), 2.68 (s) and 2.69 (s)], a set of oxygenated methylene protons [δ_H 3.45 (dd) and 3.24 (dd)], five oxygenated methine protons [δ_H 3.55 (dd), 3.74 (m), 3.75 (m), 3.95 (dd) and 5.73 (d)] and six aromatic protons [δ_H 6.28 (d), 6.29 (d), 7.06 (d), 7.07 (d), 7.20 (s) and 7.27 (s)]. The proton signals at δ_H 13.36 (s) and 13.48 (s) were deduced to be the hydrogen bonded phenolic hydroxy protons from their chemical shifts and the bathochromic shift in alkaline solution in the UV spectrum of **1**. The ^{1}H and ^{13}C NMR spectral pattern of **1** indicated that the compound was a symmetrical dimer skeleton with a sugar unit. The proton-proton connectivities were determined by PFG double quantum filtered COSY (PFG-DQFCOSY) and the proton sequences of two aromatic moiety [5-H (δ_H 6.29) to 6-H (δ_H 7.07) and 5′-H (δ_H 6.28) to 6′-H (δ_H 7.06)] and sugar moiety [1″-H (δ_H 5.73) to 6″-H (δ_H 3.24 and 3.45)] were established. In the PFG-HMBC spectra (duration time; 60 msec and 120 msec) of **1**, each proton signal showed $^{2}J_{CH}$, $^{3}J_{CH}$ and $^{4}J_{CH}$ correlations with the carbon signals. Thus, the methyl proton signals at δ_H 2.69 (11-CH_3), 2.19 (13 and 13′-CH_3), 1.39 (14-CH_3), 2.68 (11′-CH_3) and 1.40 (14′-CH_3) showed correlations with the carbon signals at δ_C 138.7 (1-C), 129.1 (2-C) and 122.0 (9a-C); δ_C 136.3 (6-C), 136.1 (6′-C), 114.4 (7-C), 114.6 (7′-C) and 159.6 (8-C and 8′-C); δ_C 148.9 (4a-C), 80.0 (10-C) and 141.6 (10a-C); δ_C 139.5 (1′-C), 127.5 (2′-C) and 120.3 (9a′-C); δ_C 148.9 (4a′-C), 79.7 (10′-C) and 141.2 (10a′-C), respectively. Moreover, the aromatic proton signals at δ_H 7.27 (4-H), 6.29 (5-H), 7.07 (6-H), 7.20 (4′-H), 6.28 (5′-H) and 7.06 (6′-H) correlated with the carbon signals at δ_C 129.1 (2-C), 155.8 (3-C), 148.9 (4a-C), 189.6 (9-CO), 122.0 (9a-C), 80.0 (10-C) and 168.2 (12-CO); δ_C 114.4 (7-C), 124.9 (8a-C), 189.6 (9-CO) and 80.0 (10-C); δ_C 159.6 (8-C), 141.6 (10a-C) and 15.3 (13-CH_3); δ_C 127.5 (2′-C), 158.1 (3′-C), 148.9 (4a′-C), 189.5 (9′-CO), 120.3 (9a′-C), 79.7 (10′-C) and 168.9 (12′-CO); δ_C 114.6 (7′-C), 125.1 (8a′-C), 189.5 (9′-CO) and 79.7 (10′-C); δ_C 159.6 (8′-C), 141.2 (10a′-C) and 15.3 (13′-CH_3), respectively. The location of two hydrogen bonded hydroxy proton signals at δ_H 13.36 (8-OH) and 13.48 (8′-OH) were assigned by the correlations with the carbon signals at δ_C 114.4 (7-C), 159.6 (8-C) and 124.9 (8a-C); δ_C 114.6 (7′-C), 159.6 (8′-C) and 125.1 (8a′-C). The remaining proton signals at δ_H 5.73 (1″-H), 3.95 (2″-H), 3.74 (3″-H), 3.75 (4″-H), 3.55 (5″-H) and 3.24/3.45 (6″-H_2) of the sugar moiety showed correlations with the carbon signals at δ_C 69.3 (3″-C) and 72.5 (5″-C); δ_C 69.3 (3″-C); δ_C 67.9 (2″-C); δ_C 69.3 (3″-C); δ_C 68.2 (4″-C) and 59.7 (6″-C); δ_C 68.2 (4″-C) and 72.5 (5″-C), respectively. The chemical shift pattern and the coupling constant of the sugar moiety ($C_6H_{11}O_5$) were characteristic of those of a galactose. The coupling constant (J=3.5 Hz) of the anomeric proton (δ_H 5.73) suggested that the sugar is attached to the aglycone by α-glycoside bond. It was estimated that the anomeric carbon was linked to 3-C of the aromatic portion, because the correlation between 1″-H (δ_H 5.73) and 3-C (δ_C 155.8) was observed in the PFG-HMBC spectrum. The observation of NOE between 4-H (δ_H 7.27) and 1″-H, and 4-H and 2″-H (δ_H 3.95) supported the estimation described above.

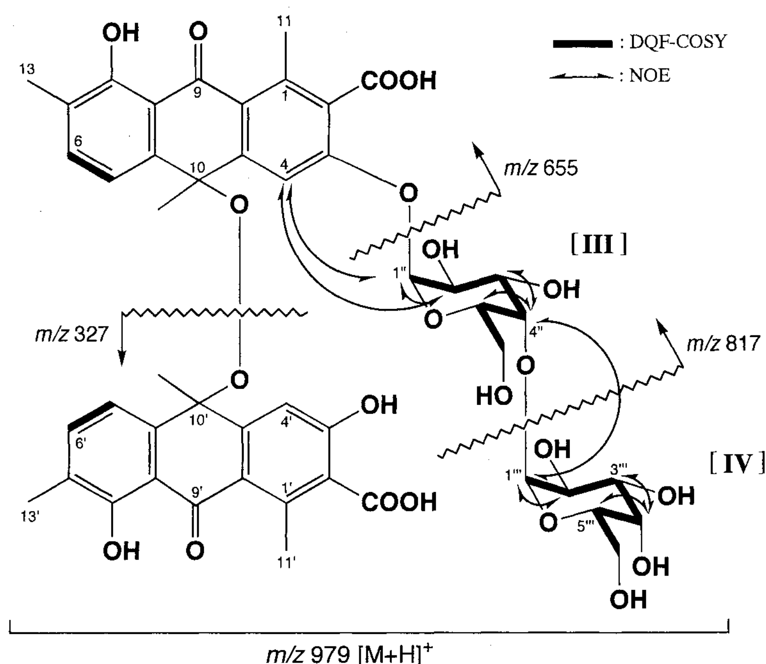
Fig. 3. DQF-COSY, HMBC and NOE experiments of adxanthromycin A (**1**).



The linkage between partial structures I and II was determined by the existence of correlations between the proton signals at δ_H 7.27 (4-H) and δ_H 7.20 (4′-H), and δ_H 5.73 (1″-H) and δ_H 7.20 (4′-H) in the NOE difference and the NOESY spectra, as shown in Fig. 3. Taking into consideration of the above NOE data, molecular formula and the characteristic FAB-MS fragment ion peak at *m/z* 327, an oxygenated carbon 10-C of the partial structure I should be connected to an oxygenated carbon 10′-C of the partial structure II *via* two oxygen atoms. Thus, the correlation of all protons and carbons were recognized, except for three hydroxy proton signals of 12-OH, 3′-OH and 12′-OH. From all of the observations described above, the structure of **1** was established as shown in Fig. 1.

Stereochemistry of the sugar moiety and the aglycone of **1** was determined by a degradation experiment. Treatment

Fig. 4. DQF-COSY, NOE and mass data of adxanthromycin B (**2**).



of **1** with 1 N HCl in MeOH at 60°C for 18 hours gave aglycone **3** and a galactose. The plane chemical structure of **3** was determined to be the identical with that of oxanthromicin[5,6] by a comparison of the NMR spectral data. On the other hand, optical rotation value of **3** ($[\alpha]_D^{25}$ −174.9°) indicated that this compound possessed the identical configurations at both of the C-10 and C-10′ positions with those of oxanthromicin. Optical rotation value of the galactose obtained by the degradation study of **1** was $[\alpha]_D^{25}$ +153.7° (*c* 0.1, H_2O), which was in agreement with that of D-galactose[7]. Therefore, the galactose moiety of compound **1** was determined to be the D-form.

Structure Elucidation of Adxanthromycin B (2)

NMR spectral data (Table 2 and Fig. 2) of compound **2** suggested that its structure was similar to that of **1**. Compound **2** possesses a molecular weight of 978 [*m/z* 979 $(M+H)^+$]; the difference in molecular weight between **1** and **2** is 162 mass units and it corresponds to $C_6H_{10}O_5$. Furthermore, the FAB-MS spectrum of **2** showed two fragment ion peaks at *m/z* 817 and 655 corresponding to the successive loss of sugar moieties from the parent ion, as shown in Fig. 4 (III and IV). The ^1H and ^{13}C NMR spectral data of **2** corresponded to those of **1** except for the signals of sugar moiety IV, as shown in Table 2. The sugar moiety IV of compound **2** was assigned to be a galactose by PFG-DQFCOSY, PFG-HMBC and NOE difference spectra. In the PFG-HMBC spectra, the proton signals at δ_H 4.84 (1‴-H), 3.59 (3‴-H), 3.78 (4‴-H), 4.17 (5‴-H), and 3.48 and 3.53 (6‴-H_2) of the sugar moiety IV showed correlations with the carbon signals at δ_C 69.3 (3‴-C) and 71.0 (5‴-C); δ_C 68.7 (2‴-C); δ_C 68.7 (2‴-C) and 69.3 (3‴-C); δ_C 100.5 (1‴-C), 68.8 (4‴-C) and 60.4 (6‴-C); δ_C 68.8 (4‴-C) and 71.0 (5‴-C), respectively. The coupling constants (J=3.3 and 3.7 Hz) of the two anomeric protons [δ_H 5.83 (1″-H) and δ_H 4.84 (1‴-H)] indicated that both of the anomeric stereochemistry are α. The connection of the two galactose moieties was elucidated by the correlation from 1‴-H to 4″-C, and from 4″-H to 1‴-C in the HMBC spectrum of **2**. The above mentioned partial structure was also supported by the existence of NOE between the proton signals at δ_H 4.84 (1‴-H) and δ_H 3.91 (4″-H). Thus, the structure of compound **2** was assigned to be a dimeric anthrone peroxide skeleton glycosidated with two molecules of α-D-galactose. Adxanthromycins A (**1**) and B (**2**) are the galactosides of oxanthromicin[5,6], which has been isolated as an antifungal antibiotic from *Actinomadura* sp. SCC 1646. On the other hand, as known inhibitors of ICAM-1/LFA-1 mediated cell adhesion molecule, seco-limonoids[8]

and cucurbitacin E[9] have been isolated from plant extracts.

Experimental

General

NMR spectra were measured on a JEOL JNM A-600 spectrometer with ^{1}H NMR at 600 MHz and ^{13}C NMR at 150 MHz, JEOL JNM ECP-500 spectrometer with ^{1}H NMR at 500 MHz and ^{13}C NMR at 125 MHz, and JEOL JNM EX-400 spectrometer with ^{1}H NMR at 400 MHz and ^{13}C NMR at 100 MHz in DMSO-d_6+HCl gas, respectively. Chemical shifts are expressed in δ values (ppm) with DMSO-d_6 (δ_C 39.5) as internal reference for ^{13}C NMR spectra and DMSO-d_6 (δ_H 2.49) as internal reference for ^{1}H NMR spectra. FAB-MS spectra were measured on a JEOL JMS AX-505 HA mass spectrometer with a matrix of glycerol, thioglycerol, 3-nitrobenzyl alcohol or polyethylenglycol. Infrared spectra (KBr) and Ultraviolet spectra were taken on a Shimadzu FT IR-4200 spectrophotometer and a Shimadzu UV-3100 spectrophotometer, respectively. Optical rotation was measured on a JASCO DIP-370 digital polarimeter. Melting points were recorded on a Gallenkamp melting point apparatus and are uncorrected. Thin layer chromatography was performed using Silica gel 60 F_{254} precoated glass plates [Merck, No. 5715 (0.25 mm)].

Analysis of Products

HPLC analyses were carried out using a Shimadzu LC-9A system consisting two LC-9A pumps, SPD-6A detector and Chromatopak C-R4A integrator with a reverse-phase column, SHISEIDO CAPCELL PAK C_{18}, SG120 (4.6×150 mm i.d., SHISEIDO CO., LTD.). Acetonitrile - 10 mM phosphate buffer (pH 6.0) (35 : 65) was used as a mobile phase (flow rate; 1.0 ml/minute) and absorbance was monitored at 272 nm. Sample was dissolved in 100 μl of the mobile phase and a 10 μl aliquot of the mobile phase solution was injected into the column.

Acid Hydrolysis of **1**

Acid hydrolysis of **1** (58.8 mg, 0.072 mmol) was carried out with 1 N HCl (3 ml) in MeOH (17 ml) at 60°C for 18 hours. After the evaporation of MeOH, the resultant aqueous solution was diluted with water (10 ml) and extracted twice with 10 ml of EtOAc at pH 3.0. The EtOAc layer was washed with water, dried over Na_2SO_4, and evaporated to dryness to give **3** as a yellow powder: mp 208~212°C (dec.) [lit[6]. mp 211~213°C (dec.)]; $[\alpha]_D^{25}$ $-174.9°$ (c 0.1, EtOH) [lit[6]. $[\alpha]_D^{26}$ $-172.1°$ (c 0.3, EtOH)]; $C_{36}H_{30}O_{12}$; ^{1}H NMR (400 MHz, DMSO-d_6+HCl gas) δ_H 13.49 (2H, s, 8- and 8′-OH), 7.37 (2H, s, 4- and 4′-H), 7.00 (2H, d, J=7.8 Hz, 6- and 6′-H), 6.24 (2H, d, J=7.8 Hz, 5- and 5′-H), 2.70 (6H, s, 11- and 11′-CH_3), 2.18 (6H, s, 13- and 13′-CH_3), 1.38 (6H, s, 14- and 14′-CH_3); ^{13}C NMR (100 MHz, DMSO-d_6+HCl gas) δ_C 189.3 (9- and 9′-C), 168.7 (12- and 12′-C), 159.5 (8- and 8′-C), 158.6 (3- and 3′-C), 148.8 (4a- and 4a′-C), 141.4 (10a- and 10a′-C), 139.6 (1- and 1′-C), 135.8 (6- and 6′-C), 127.0 (2- and 2′-C), 124.6 (8a- and 8a′-C), 119.8 (9a- and 9a′-C), 116.5 (5- and 5′-C), 114.4 (7- and 7′-C), 112.3 (4- and 4′-C), 79.8 (10- and 10′-C), 33.2 (14- and 14′-C), 20.5 (11- and 11′-C), 15.1 (13- and 13′-C).

The acidic aqueous layer was neutralized with Dowex 1×4 (1 ml, 100~200 mesh, OH type) and the solution was evaporated to dryness to give D-galactose (5.4 mg, 46.3% yield). TLC, Rf=0.33 ($CHCl_3$ - MeOH - H_2O, 10 : 10 : 1; *p*-anisidine-phthalic acid reagent). The ^{1}H NHR spectrum and the optical rotation ($[\alpha]_D^{25}$ +153.7° (c 0.1, H_2O)) of this compound were in good accordance with those reported for D-galactose.

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