ANTHRACYCLINE METABOLITES FROM *Streptomyces violaceus* A262

III. NEW ANTHRACYCLINE OBELMYCINS PRODUCED BY A VARIANT STRAIN SE2-2385

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(Received for publication February 6, 1991)

New anthracycline antibiotics, designated as obelmycins A, D, E, F and G, were isolated from the culture broth of a variant strain of β-rhodomycin-producing *Streptomyces violaceus* A262, identified as β-isorhodomycinone glycosides and γ-isorhodomycinone glycosides and assayed for their *in vitro* cytotoxicities against murine leukemic L1210 cell culture and the antimicrobial activities in comparison with some known anthracyclines.

During the course of our investigations of new anthracycline producers, we obtained several unique blocked mutants from *Streptomyces violaceus* A262 which is known to produce β-rhodomycinone glycosides, A262-1, A262-2 and A262-3[1]. One of the variant strains isolated was found to produce four β-isorhodomycinone glycosides (obelmycins A, B, C and D) and three γ-isorhodomycinone glycosides (obelmycins E, F and G)[2]. Anthracycline antibiotics belonging to the β-rhodomycin and γ-rhodomycin class have been isolated by several groups[3,4]. However, there are few reports concerning β-isorhodomycins and γ-isorhodomycins. BROCKMANN and PATT isolated β-isorhodomycin II in 1955[5] and β-isorhodomycin S-1a in 1975[6], and recently 1-hydroxycytorhodins were disclosed in 1986[7].

This paper describes new anthracycline antibiotic obelmycins which are produced by a variant strain of *S. violaceus* A262. Obelmycins B and C were identical to 1-hydroxycytorhodins A and N[7], respectively.

Materials and Methods

Microorganisms

The obelmycin-producing strain SE2-2385 was isolated from *S. violaceus* A262 as previously described[1], cultivated at 28°C on YS agar slant (yeast extract 0.3%, soluble starch 1.0% and agar 1.5%, pH 7.2) and then stored at 5°C. This strain was deposited as FERM P-8165 in Fermentation Research Institute, Agency of Industrial Science and Technology.

Fermentation

A seed culture was grown aerobically at 28°C for 48 hours in a 500-ml Erlenmeyer flasks containing

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100 ml of a following medium; soluble starch 0.5%, glucose 0.5%, soybean meal 1.0%, yeast extract 0.1%, NaCl 0.1%, K_2HPO_4 0.1%, $MgSO_4 \cdot 7H_2O$ 0.1%, pH 7.4. This culture was employed as inoculum (5%) to two 30-liter jar fermenters containing each 15 liters of the following medium; soluble starch 50 g, soybean meal 30 g, yeast extract 2 g, NaCl 2 g, $CaCO_3$ 2 g, $CuSO_4 \cdot 5H_2O$ 0.01 g, $FeSO_4 \cdot 7H_2O$ 0.0016 g, $ZnSO_4 \cdot 7H_2O$ 0.0032 g, $MnCl_2 \cdot 4H_2O$ 0.013 g per 1,000 ml of tap water, pH 7.0. Conditions used were 15 liters/minute for aeration and 300 rpm for agitation. Cultivation was carried out at 28°C for 130 hours at which time the mycelial purple pigments were produced at approximately maximum level. About 30 liters of the culture broth was used for the isolation of purified obelmycins.

Biological Activity

In vitro cytotoxicitiy and inhibition of DNA and RNA syntheses against a cell culture of murine leukemia L1210 were assayed according to the method as previously described[8]. Antimicrobial activity was assayed by the standard broth dilution method†.

Qualitative Determination of Aglycone and Sugars by TLC

Obelmycin in 0.1 N HCl (10 mg/ml) was heated at 85°C for 30 minutes in a water bath. The aglycone, thus obtained, was extracted with $CHCl_3$. The $CHCl_3$ layer was evaporated *in vacuo* to dryness and the purple pigment residue was then purified by preparative TLC on Silica gel plate PF_{254} (E. Merck) using a developing solvent of $CHCl_3$ - MeOH (20 : 1). Purified aglycone was subjected to TLC on Silica gel F_{254} (E. Merck) using a developing solvent of $CHCl_3$ - MeOH (20 : 1).

Alternatively, the aqueous layer containing sugar components was neutralized by addition of silver carbonate with a small amount of charcoal and centrifuged. The supernatant fluid was concentrated *in vacuo* and subjected to TLC on Silica gel plate F_{254} (E. Merck) using a developing solvent of BuOH - acetic acid - H_2O (4 : 1 : 1). Sugars were detected by spraying with *p*-anisaldehyde - H_2SO_4 (each 5%) in 90% EtOH and heating at 90°C. Aclarubicin and MA144-N1[9] were also hydrolyzed under the same conditions and the aqueous layers were used as a source of authentic sugars including L-rhodosamine (RN), 2-deoxy-L-fucose (dF), L-rhodinose (R) and L-cinerulose A (CinA); Rf values were 0.12, 0.56, 0.71 and 0.82, respectively.

Total Acid Hydrolysis of Obelmycin D

Obelmycin D (40 mg) was hydrolyzed under the same conditions described above. After extraction with $CHCl_3$, the hydrolysate was passed through a Dowex 50W ion exchange resin and eluted with 1 N HCl. The eluate containing rhodosamine (adjusted to pH 6.0 with Diaion WA-20) was evaporated to dryness *in vacuo* and chromatographed on a column of silica gel (Wakogel C-200) using a solvent mixture of $CHCl_3$ - MeOH (10 : 1 ~ 3 : 1). Alternatively, the effluent containing rhodinose and 2-deoxyfucose was treated similarly, however, it was chromatographed on a column of silica gel using a solvent mixture of $CHCl_3$ - MeOH (50 : 1 ~ 10 : 1).

Mild Acid Hydrolysis

Partial hydrolysis of obelmycin (1 mg/ml of 0.1 N HCl) was carried out as follows. Obelmycin in solution was held at room temperature for 1 hour. After adjusting the pH to 8.0 with 0.1 N NaOH, the hydrolysate was extracted with $CHCl_3$ and the organic layer was evaporated to dryness *in vacuo*. The purple pigment residue was then subjected to TLC on Silica gel plate F_{254} using a developing solvent of $CHCl_3$ - MeOH - aq NH_3 (80 : 10 : 1).

Hydrogenolysis

Obelmycin in MeOH (10 mg/ml) was hydrogenated over a double weight of 5% Pd - $BaSO_4$ at room temperature and atmospheric pressure for 2 hours. An equal volume of H_2O was added, the products were extracted with $CHCl_3$ and evaporated to dryness *in vacuo*. The purple pigment residue was subjected to TLC on Silica gel F_{254} using a developing solvent of $CHCl_3$ - MeOH (15 : 1).

† National Committee for Clinical Laboratory Standards: Standard methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved Standards, NCCLS, Villanova, 1983.

HPLC Analysis

Purity of the products was determined by HPLC using a Hitachi 655 liquid chromatographic apparatus with a reverse phase analytical column, A312 (ODS) (6×150 mm) (Yamamura Chemical Laboratories Co., Ltd.). Acetonitrile-water (35:65) (adjusted to pH 2.0 with H_3PO_4) was used as the mobile phase and was run at a flow rate of 1.0 ml/minute. Samples were dissolved in the mobile phase and 10 μl of samples were injected. Detection was performed at 254 nm using a UV detector (UVILOG-5 III A, Oyo-Bunko Kiki Co., Ltd.).

General

MP's were determined on a Kofler hotstage microscope. UV spectra were carried out on a Hitachi EPS 3T and IR spectra (KBr pellet) on a Hitachi EPI-GS spectrophotometer. ^1H and ^{13}C NMR were recorded with a Jeol GX-400 spectrometer at 400 MHz and 100 MHz, respectively. Chemical shifts are expressed in δ values (ppm) with TMS as an internal reference and coupling constants are given in J (Hz). Mass spectra were recorded with a Hitachi M-80H spectrometer. Specific rotations were determined on a Jasco DIP-181 Digital Polarimeter.

Results

Isolation and Purification of Obelmycins

The fermentation broth (30 liters) was filtered and the anthracycline products were extracted from the mycelial cake with a total of 10 liters of acetone. The acetone extract was evaporated to about 2 liters *in vacuo*. The concentrate (pH 8.0) was extracted with $CHCl_3$ and the $CHCl_3$ layer was evaporated to a small volume *in vacuo*. An excess of *n*-hexane was added to precipitate the crude mixture of anthracycline products. The yield was about 9.4 g of a purple crude powder which contained some seven violet components as observed on silica gel TLC ($CHCl_3$-MeOH-aq NH_3 (80:10:1)). The powder was chromatographed on a silica gel column which was developed successively with $CHCl_3$-MeOH (100:1, 100:2, 100:5 and 100:10 mixtures) to elute obelmycins E, F, B, C, D, G and A in that order. Each compound was further purified by preparative TLC using a mixture of $CHCl_3$-MeOH-aq NH_3 (100:10:1 for obelmycins B, C, D, E and F, and 100:18:2 for A and G). Each obelmycin band was scraped from the TLC plate and extracted with $CHCl_3$-MeOH (7:1). The extract was evaporated to dryness; the residue was dissolved in 0.1 M acetate buffer (pH 3.5) and washed with toluene. The aqueous layer was adjusted to pH 7.5 by addition of saturated aqueous $NaHCO_3$ and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with H_2O, dried over Na_2SO_4 and concentrated. An excess of *n*-hexane was added to precipitate the respective obelmycin. The purified obelmycins A, B, C, D, E, F and G yielded 18 mg, 126 mg, 73 mg, 82 mg, 18 mg, 12 mg and 10 mg, respectively. The purity of these compounds was determined to be greater than 95% as determined by HPLC. Obelmycins A, B, C, D, E, F and G had Rt's of 3.8, 3.6, 2.9, 2.3, 18.1, 8.5 and 6.2 minutes, respectively. Obelmycins are soluble in acetone, methanol, ethanol, chloroform, ethyl acetate and acidic water, but are insoluble or only slightly soluble in ether, *n*-hexane and water.

Structural Determination of Obelmycins

The structural determination of obelmycins was established on the basis of 1) their physico-chemical properties, 2) analysis of hydrogenated products, 3) TLC analysis of the sugar components obtained from acid hydrolysis, and 4) FD-MS and NMR analyses. The physico-chemical properties of obelmycins are shown in Table 1. The UV and visible light absorption spectra of all obelmycins in 90% MeOH solution exhibited a maximum peak at 242 nm and some characteristic peaks at 522~525, 549~553 and 561~564 nm due to their purple color. The IR absorption spectra (KBr) of obelmycins indicated

Table 1. Physico-chemical properties of obelmycins.

	Obelmycin A	Obelmycin B	Obelmycin C	Obelmycin D	Obelmycin E	Obelmycin F	Obelmycin G
Molecular formula	$C_{28}H_{33}NO_{11}$	$C_{60}H_{88}N_2O_{21}$	$C_{60}H_{88}N_2O_{22}$	$C_{60}H_{88}N_2O_{23}$	$C_{40}H_{53}NO_{14}$	$C_{40}H_{53}NO_{15}$	$C_{28}H_{33}NO_{10}$
FD-MS (m/z)	559 M^+	1,173 M^+	1,189 M^+	1,205 M^+	772 $(M+H)^+$	787 M^+	543 M^+
MP (°C, dec)	191~194	175~178	182~185	184~187	148~151	160~163	194~197
$[\alpha]_D^{23}$ ($CHCl_3$)	+682°	+528°	+788°	+815°	+595°	+523°	+773°
	(c 0.004)	(c 0.004)	(c 0.004)	(c 0.004)	(c 0.008)	(c 0.008)	(c 0.008)
UV $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{cm}^{1\%}$)	206 (233),	205 (233),	207 (295),	205 (124),	204 (239),	204 (210),	207 (sh, 253),
	241 (387),	242 (354),	242 (422),	242 (319),	242 (673),	242 (592),	242 (765),
	298 (116),	306 (76),	326 (139),	301 (51),	300 (111),	300 (98),	300 (125),
	492 (sh, 181),	364 (149),	496 (sh, 92),	497 (sh, 87),	490 (180),	491 (sh, 157),	490 (203),
	523 (274),	496 (sh, 92),	525 (156),	525 (147),	522 (320),	522 (280),	522 (365),
	550 (263),	525 (157),	533 (153),	552 (144),	549 (305),	549 (267),	549 (351),
	562 (275)	553 (156),	564 (158)	564 (149)	561 (336)	561 (293)	561 (385)
		564 (162)					
IR ν_{max} (KBr) cm^{-1}	3400, 2920, 1590,	3450, 2920, 1580,	3400, 2920, 1580,	3400, 2920, 1580,	3400, 2920, 1580,	3400, 2920, 1580,	3400, 2920, 1580,
	1450, 1300, 1190,	1450, 1300, 1190,	1450, 1300, 1190,	1450, 1300, 1190,	1450, 1305, 1190,	1450, 1305, 1190,	1450, 1305, 1190,
	1010, 980, 790	1000, 795	990, 790	1000, 800	1000, 800	1000, 800	1020, 990, 800

Table 2. TLC analysis of aglycone and sugar components of obelmycins.

Compound	Aglycone spot	Aglycone detected	Sugar spot	Sugar detected
	Rf value[a] (color)		Rf value[b] (color[c])	
Obelmycin A	0.36 (purple)	β-Isorhodomycinone	0.12 (Sky blue)	L-Rhodosamine
Obelmycin B	0.36 (purple)	β-Isorhodomycinone	0.12 (Sky blue),	L-Rhodosamine
			0.71 (Green)	L-Rhodinose
Obelmycin C	0.36 (purple)	β-Isorhodomycinone	0.12 (Sky blue),	L-Rhodosamine
			0.56 (Grayish blue),	2-Deoxy-L-fucose
			0.71 (Green)	L-Rhodinose
Obelmycin D	0.36 (purple)	β-Isorhodomycinone	0.12 (Sky blue),	L-Rhodosamine
			0.56 (Grayish blue),	2-Deoxy-L-fucose
			0.71 (Green)	L-Rhodinose
Obelmycin E	0.45 (purple)	γ-Isorhodomycinone	0.12 (Sky blue),	L-Rhodosamine
			0.71 (Green)	L-Rhodinose
Obelmycin F	0.45 (purple)	γ-Isorhodomycinone	0.12 (Sky blue),	L-Rhodosamine
			0.56 (Grayish blue),	2-Deoxy-L-fucose
			0.71 (Green)	L-Rhodinose
Obelmycin G	0.45 (purple)	γ-Isorhodomycinone	0.12 (Sky blue)	L-Rhodosamine

[a] $CHCl_3$-MeOH (20:1).
[b] BuOH-acetic acid-H_2O (4:1:1).
[c] Visualization was carried out with *p*-anisaldehyde.

the presence of hydrogen bonded carbonyl (1600 cm^{-1}) which is characteristic of anthracyclines. The molecular formulas were determined by mass spectra.

On total acid hydrolysis obelmycins gave two kinds of purple aglycones and some sugar components. The aglycone of obelmycins A, B, C and D was found to be β-isorhodomycinone by direct comparison with an authentic sample on TLC using $CHCl_3$-MeOH (20:1) (Rf value: 0.36, Table 2) and its identity was established by mass spectrum (*m/z* 402 M^+) with a molecular formula of $C_{20}H_{18}O_9$ and ^1H and ^{13}C NMR spectra[1]. Similarly the aglycone of obelmycins E, F and G was identified as γ-isorhodomycinone by mass spectrum (*m/z* 387 $(M+H)^+$ with a molecular formula of $C_{20}H_{18}O_8$ and ^1H and ^{13}C NMR spectra[1].

The sugar components were determined by comparison with authentic samples (prepared from aclacinomycins) and the resuls are shown in Table 2. Obelmycins A and G had only RN. Three different sugars, RN, dF and R, were detected in preparations of obelmycins C, D and F; by comparison two sugars, RN and R, were found in obelmycins B and E. The stereo-configuration of the sugars were determined to be L by their optical rotations. $[\alpha]_D$ values of rhodosamine hydrochloride, 2-deoxyfucose and rhodinose were $-43.2°$ (*c* 0.1, H_2O) (literature 10: $-48.2°$, H_2O), $-56.3°$ (*c* 0.17, H_2O) (literature 11: $-61.6°$, H_2O) and $-7.1°$ (*c* 0.12, $CHCl_3$) (literature 12: $-11°$, $CHCl_3$), respectively.

On mild acid hydrolysis obelmycins B, C and D gave β-isorhodomycin II (7,10-*O*-dirhodosaminyl-β-isorhodomycinone)[5] together with obelmycin A (7-*O*-rhodosaminyl-β-isorhodomycinone) with a molecular formula of $C_{28}H_{33}O_{11}N$ (*m/z* 559 M^+), while obelmycins E and F gave G (10-*O*-rhodosaminyl-γ-isorhodomycinone) with a molecular formula of $C_{28}H_{33}O_{10}N$ (*m/z* 543 M^+).

On hydrogenolysis obelmycins B and C gave obelmycin E as a result of a reductive deglycosidation at C-7, and D gave F similarly, indicating that the sugar chains attached at C-10 were the same with respect to obelmycins B, C and E. Similarly, the same was true in the case of D and F. Obelmycin A gave an aglycone, γ-isorhodomycinone.

Table 3. ^{1}H NMR chemical shifts of obelmycins.

Proton	Obelmycin A	Obelmycin B	Obelmycin C	Obelmycin D	Obelmycin E	Obelmycin F	Obelmycin G
Aglycone moiety:							
2-H	7.29 d (10)*	7.29 d (10)*	7.31 d (10)*	7.30 d (10)*	7.26 d (10)*	7.25 d (10)*	7.29 d (10)*
3-H	7.26 d (10)*	7.27 d (10)*	7.29 d (10)*	7.28 d (10)*	7.23 d (10)*	7.23 d (10)*	7.26 d (10)*
7-Ha	—	—	—	—	2.92~3.00 m (20, 8, 2)	2.91~3.00 m (20, 8, 2)	2.93~3.02 m (20, 8, 2)
7-Hb	—	—	—	—	2.81~2.91 m (20, 9, 8)	2.81~2.91 m (20, 9, 8)	2.83~2.93 m (20, 9, 8)
7-H	5.14 br d (3)	5.14 br d (3)	5.15 br d (3)	5.14 br d (3)	—	—	—
8-Ha	2.26 d (15)	2.2	2.2	2.2	2.05	2.1	2.05~2.12 m (14, 9, 8)
8-Hb	2.12 dd (15, 4)				1.8	1.8	1.87
10-H	4.89 s	5.03 s	5.02 s	5.02 s	4.98 s	4.97 s	4.97 s
14-CH_3	1.13 t (7.5)	1.07 t (7)	1.10 t (7)	1.10 t (7)	1.09 t (7)	1.08 t (7)	1.10 t (7)
Sugar moeity attached to C-7:							
1′-H	5.50 d (3.5)	5.51 d (3.5)	5.48 d (3.5)	5.48 d (3.5)	—	—	—
4′-H	3.71 br s	3.79 br s	3.73 br s	3.72 br s*	—	—	—
5′-H	4.08 q (7.5)	4.02 q (7)	4.06 q (7)	3.99 q (7)	—	—	—
6′-CH_3	1.41 d (7.5)	1.28 d (7)	1.27 d (7)	1.27 d (7)	—	—	—
3′-$N(CH_3)_2$	2.21 s	2.18 s	2.16 s	2.15 s	—	—	—
1″-H	—	4.96	5.03	5.02	—	—	—
3″-H	—	—	3.98 br d	4.07 br	—	—	—
3″-CH_2	—	1.6~2.1	—	—	—	—	—
4″-H	—	3.48 br s	3.56 br s	3.57 br s*	—	—	—
5″-H	—	4.45 q (7)	4.53 q (7)	4.52 q (7)	—	—	—
6″-CH_3	—	1.12 d (7)	1.14 d (7)	1.14 d (7)*	—	—	—
1‴-H	—	4.83 d (3.5)	4.85 br s	4.85 br s	—	—	—
4‴-H	—	3.58 br s	3.66 br s*	3.65 br s	—	—	—
5‴-H	—	4.06 q (7)	4.06 q (7)	4.19 q (7)*	—	—	—
6‴-CH_3	—	1.16 d (7)	1.16 d (7)	1.20 d (7)*	—	—	—
Sugar moiety attached to C-10:							
1′-H	—	5.43 d (3.5)	5.44 d (3.5)	5.42 d (3.5)	5.37 d (3.5)	5.37 d (3.5)	5.37 d (3.5)
4′-H	—	3.73 br s	3.73 br s	3.67 br s*	3.75 br s	3.69 br s	3.67 br s
5′-H	—	3.88 q (7)	3.88 q (7)	3.77 q (7)	3.92 q (7)	3.90 q (7)	3.94 q (7)
6′-CH_3	—	1.24 d (7)	1.24 d (7)	1.25 d (7)	1.26 d (7)	1.27 d (7)	1.37 d (7)
3′-$N(CH_3)_2$	—	2.18 s	2.16 s	2.15 s	2.20 s	2.19 s	2.23 s
1″-H	—	4.93 br s	4.93 br s	5.02	4.94 d (3.5)	5.02 br s	—
3″-H	—	—	—	4.07 br	—	4.02 br	—
3″-CH_2	—	1.6, 2.1	1.7, 2.0	—	1.7~	—	—
4″-H	—	3.45 br s	3.45 br s	3.52 br s*	3.45 br s	3.53 br s	—
5″-H	—	4.39 q (7)	4.40 q (7)	4.48 q (7)	4.42 q (7)	4.50 q (7)	—
6″-CH_3	—	1.08 d (7)	1.07 d (7)	1.13 d (7)*	1.07 d (7)	1.14 d (7)	—
1‴-H	—	4.81 d (3.5)	4.81 d (3.5)	4.82 br s	4.81 br s	4.83 d (3.5)	—
4‴-H	—	3.58 br s	3.58 br s*	3.65 br s	3.58 br s	3.65 br s	—
5‴-H	—	4.08 q (7)	4.22 q (7)	4.21 q (7)*	4.07 q (7)	4.21 q (7)	—
6‴-CH_3	—	1.18 d (7)	1.22 d (7)	1.22 d (7)	1.16 d (7)	1.20 d (7)	—

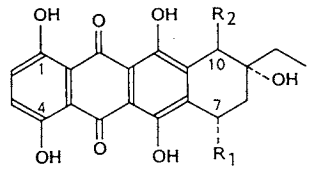
Spectra were measured in $CDCl_3$ at 400 MHz. Chemical shifts are expressed by δ (ppm) (J=Hz) from internal TMS. Similar values asterisked may be interchanged.

Table 4. ^{13}C NMR chemical shifts of obelmycins (OBM).

Carbon	OBM-A	OBM-B	OBM-C	OBM-D	OBM-E	OBM-F	OBM-G	Remarks
1	158.03*	157.82*	157.91*	157.86*	157.68	157.63	157.64	Aglycone moiety
2	129.80*	129.57*	129.58*	129.61*	129.30*	129.27*	129.29*	
3	129.70*	129.45*	129.48*	129.45*	128.99*	128.92*	128.97*	
4	158.09*	157.96*	157.99*	157.99*	157.68	157.63	157.64	
4a	112.66	112.75	112.84	112.80	112.97	112.92	112.88	
5	189.23*	189.14*	189.27*	189.24*	189.40*	189.36*	189.29*	
5a	112.26	112.11	112.19	112.18	110.31	110.93	110.92	
6	156.77	157.18	157.19	157.15	156.49	156.42	156.40	
6a	135.12	136.57	136.59	136.53	136.70	136.64	136.48	
7	70.85	70.92	70.96	70.97	21.04	21.04	21.05	
8	32.83	32.97	33.02	33.00	27.03	26.97	26.98	
9	71.91	71.73	71.78	71.77	71.91	71.85	71.81	
10	62.63	70.38	70.40	70.33	69.98	70.08	70.62	
10a	138.19	137.86	137.82	137.76	141.23	141.13	141.10	
11	156.93	157.23	157.23	157.21	157.60	157.56	157.56	
11a	111.77	111.96	112.03	112.03	110.31	110.28	110.26	
12	189.17*	189.03*	189.18*	189.11*	188.92*	188.85*	188.82*	
12a	112.62	112.75	112.84	112.80	110.97	112.92	112.88	
13	30.45	30.72	30.74	30.72	30.96	30.91	30.95	
14	6.59	6.63	6.63	6.63	6.65	6.63	6.63	
1′	101.46	102.02	101.98	101.98	—	—	—	Sugar moeity
2′	28.79	29.56	29.32	29.30	—	—	—	attached to C-7
3′	59.67	61.49	61.52*	61.40	—	—	—	
4′	66.02	73.87	74.09*	74.09*	—	—	—	
5′	67.53	68.58	68.38	68.36	—	—	—	
6′	17.00	17.85	17.80	17.80	—	—	—	
3′-N(CH_3)$_2$	42.01	43.23	43.23	43.29	—	—	—	
1″	—	98.63	99.49	99.44	—	—	—	
2″	—	24.56	34.44	34.41	—	—	—	
3″	—	24.74*	65.66	65.64*	—	—	—	
4″	—	75.36	83.74	83.71*	—	—	—	
5″	—	66.86	66.90	66.90	—	—	—	
6″	—	17.10	17.10	16.94	—	—	—	
1‴	—	99.52	100.35	100.33	—	—	—	
2‴	—	23.65	23.99	23.97	—	—	—	
3‴	—	26.02	25.55	25.54	—	—	—	
4‴	—	67.53	67.27*	67.24	—	—	—	
5‴	—	66.69	66.69	68.03*	—	—	—	
6‴	—	17.10	17.10	17.01	—	—	—	
1′	—	97.31	97.35	97.20	96.94	96.86	96.78	Sugar moiety
2′	—	29.82	29.86	29.75	29.73	29.64	29.22	attached to
3′	—	61.57	61.43*	61.40	61.58	61.46	59.77	C-10
4′	—	74.13	74.17*	74.33*	74.22	74.38	66.24	
5′	—	68.75	68.78	68.55	68.82	68.58	66.61	
6′	—	18.04	18.06	18.03	18.15	18.10	17.26	
3′-N(CH_3)$_2$	—	43.23	43.23	43.22	43.36	43.33	42.03	
1″	—	98.63	98.64	99.44	98.70	99.46	—	
2″	—	24.56	24.57	34.31	24.59	34.32	—	
3″	—	24.82*	24.76	65.60*	24.76	65.59	—	
4″	—	75.34	75.38	83.63*	75.39	83.67	—	
5″	—	66.83	66.83	66.83	66.86	66.85	—	
6″	—	17.10	17.10	16.94	17.14	17.01*	—	
1‴	—	99.46	99.46	100.28	99.51	100.28	—	
2‴	—	23.65	23.68	23.97	23.69	23.94	—	
3‴	—	26.02	26.02	25.54	26.04	25.53	—	
4‴	—	67.53	67.56*	67.24	67.59	67.25	—	
5‴	—	66.69	68.08	68.08*	66.72	68.02	—	
6‴	—	17.10	17.10	17.01	17.14	16.95*	—	

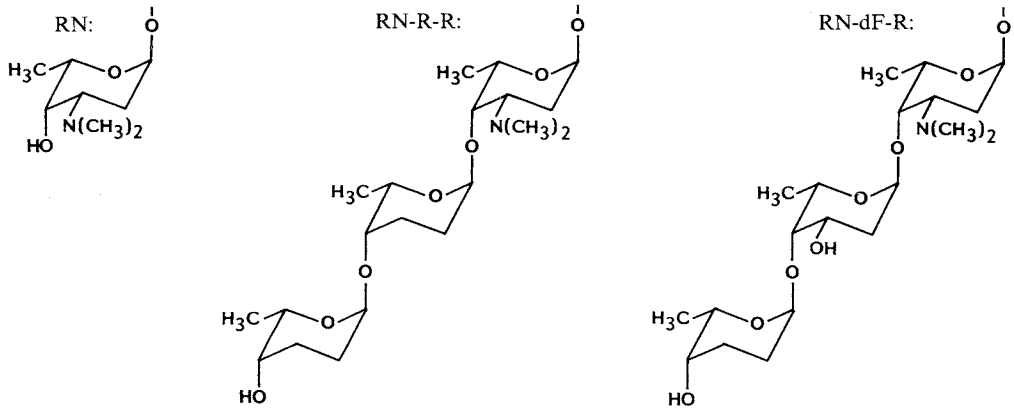
Spectra were measured in $CDCl_3$ at 100 MHz. Chemical shifts are expressed by δ (ppm) from internal TMS. Similar values asterisked may be interchanged.

Fig. 1. Structures of obelmycins.



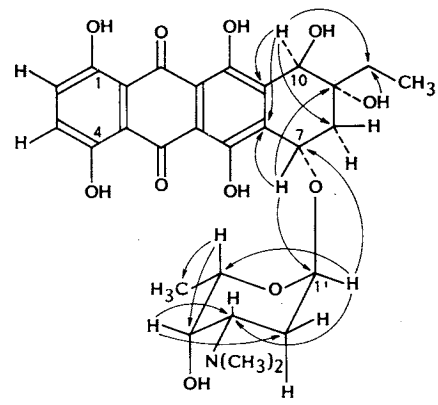
Obelmycins	R_1	R_2
A	RN	OH
B	RN-R-R	RN-R-R
C	RN-dF-R	RN-R-R
D	RN-dF-R	RN-dF-R
E	H	RN-R-R
F	H	RN-dF-R
G	H	RN

RN: L-Rhodosamine, dF: 2-deoxy-L-fucose, R: L-rhodinose.



The chemical shift assignments of ^1H and ^{13}C NMR spectra of obelmycins were carried out by means of the pulse technique, DEPT, ^1H-^1H and ^1H-^{13}C COSY, and are shown in Tables 3 and 4, respectively. The number of anomeric protons in the ^1H NMR indicates that obelmycins E and F have three and B, C and D have six molecules of hexoses, respectively. Both ^{13}C NMR chemical shifts of C-7 and C-10 of obelmycins B, C and D (about 70.9 ppm and 70.4 ppm, respectively) shifted to lower field approximately 5~9 ppm in comparison with those of β-isorhodomycinone (about 61.7 ppm and 65.4 ppm), although other chemical shift assignments between the aglycone moiety and the free aglycone were similar. These down-field shifts are attributable to the sugar linkages at C-7 and C-10 as observed in diglycosidic anthracyclines[3,4]. Similarly the chemical shift of C-7 of obelmycin A shifted to lower field

Fig. 2. HMBC experiment of obelmycin A. Arrows indicate ^1H-^{13}C long range couplings.



about 5.5 ppm due to its sugar linkage at C-7. The chemical shifts of the sugar moieties of obelmycins B, C, D, E and F were almost identical with those of A447 C[13], cosmomycins C, D, A and B[14,15], respectively. The small coupling constants ($J=3.5$ Hz) of all anomeric protons in obelmycins indicate the configurations of the glycosidic bonds are α.

From all these findings the structures of obelmycins were determined as illustrated in Fig. 1. The structure of obelmycin A was further confirmed by ^{1}H detected heteronuclear multiple-bond connectivity (HMBC) experiment and the long range couplings detected are shown in Fig. 2. The long range couplings

Table 5. Antimicrobial activity of some obelmycins and related anthracycline antibiotics.

Microorganism	MIC (μg/ml)						
	OBM-A	OBM-B	OBM-E	OBM-G	A447 C	COM-A	β-RM-I
Staphylococcus aureus FDA 209P	6.25	0.20	6.25	3.13	0.20	6.25	12.50
Bacillus subtilis ATCC 6633	3.13	0.05	6.25	3.13	0.10	6.25	3.13
B. cereus ATCC 9634	1.57	0.10	3.13	3.13	0.20	6.25	6.25
B. megaterium NRRL B-938	3.13	0.05	6.25	3.13	0.10	6.25	6.25
Micrococcus luteus ATCC 9341	3.13	0.20	6.25	3.13	0.39	12.50	3.13
M. flavus	3.13	0.10	6.25	6.25	0.10	12.50	6.25
Corynebacterium bovis 1810	1.57	0.10	6.25	3.13	0.10	6.25	3.13
Mycobacterium smegmatis ATCC 607	0.78	3.13	3.13	0.78	1.57	1.57	3.13
Pseudomonas fluorescens NIHJ B-25	1.57	0.10	6.25	6.25	0.10	6.25	3.13
Candida albicans IAM 4905	12.50	1.57	25.00	12.50	3.13	50.00	25.00

Abbreviations: OBM: Obelmycin, COM: cosmomycin, RM: rhodomycin.

Table 6. Inhibitory activity of obelmycins and related anthracyclines on the growth and nucleic acid synthesis of murine leukemic L1210 cell culture.

Compound	IC_{50} (μg/ml)			DNA/RNA
	Growth	DNA synthesis	RNA synthesis	
Obelmycin A	0.001	0.58	0.14	4.1
Obelmycin B	0.0009	0.26	0.025	10.4
Obelmycin C	0.001	0.38	0.043	8.8
Obelmycin D	0.004	1.20	0.18	6.7
Obelmycin E	0.062	1.30	0.78	1.7
Obelmycin F	0.092	1.43	0.60	2.4
Obelmycin G	0.020	1.50	1.10	1.4
β-Rhodomycin I (1-Deoxy-OBM-A)	0.01	0.21	0.06	3.5
A447 C (1-Deoxy-OBM-B)	0.003	0.44	0.041	10.7
COM C (1-Deoxy-OBM-C)	0.005	0.28	0.031	9.0
COM D (1-Deoxy-OBM-D)	0.005	0.80	0.055	14.5
COM A (1-Deoxy-OBM-E)	0.085	1.90	0.61	3.1
Iremycin (1-Deoxy-OBM-G)	0.15	0.80	0.80	1.0
Aclarubicin	0.01	0.65	0.085	7.6
Doxorubicin	0.02	1.40	0.55	2.5

In the inhibition test for nucleic acid synthesis, the drugs were exposed for 60 minutes to L1210 cell culture (8×10^5 cells/ml) with supplemented ^{14}C-labeled uridine or thymidine (0.05 μCi/ml), and the incorporation of the radioisotopes into acid insoluble material was measured. For the growth inhibition test, the drugs were exposed for 48 hours to L1210 cell culture (5×10^4 cells/ml) and the viable cells were counted by coulter counter.

IC_{50} is expressed as a drug concentration required to inhibit by a 50% control of the growth, and DNA and RNA syntheses of cultured L1210 cells.

Abbreviations: OBM: Obelmycin, COM: cosmomycin.

observed between 7-H and C-1′, and 1′-H and C-7 proved its sugar linkage at C-7.

Biological Activity

Antimicrobial activities of obelmycins are shown in Table 5. All obelmycins exhibited antimicrobial activity against all of the Gram-positive and Gram-negative bacteria tested and weak activity against *Candida albicans*. Obelmycin B was the most active among the obelmycins tested and proved to be more active than A447 C (1-deoxyobelmycin B) with the same sugar chain. Obelmycin A was also more active than β-rhodomycin I (1-deoxyobelmycin A). These results suggest that a hydroxyl group at C-1 plays an important role in the antimicrobial activity. γ-Isorhodomycinone glycosides (obelmycins E and G) were less active than β-isorhodomycinone glycosides (obelmycins A and B).

The activities of obelmycins and related compounds against cultured L1210 cells were examined and the results are shown in Table 6. Obelmycins A, B, C and D had very potent cytotoxic activity in comparison with the anthracyclines, aclarubicin and doxorubicin, which are used therapeutically. A comparison of the cytotoxicity of related compounds showed that the presence of a hydroxyl group at C-1 and sugar at C-7 is also important for the cytotoxicity exhibited against L1210 cells. As noted with aclarubicin, obelmycins A, B, C and D inhibited RNA synthesis more strongly than DNA synthesis whereas obelmycins E, F, and G without glycosidic sugar at C-7 inhibited both syntheses to a similar extent.

Antitumor effects *in vivo* of obelmycins on mice bearing leukemia L1210 were tested by daily ip administration from day 1 to 10. Obelmycins A, B and E exhibited a maximum antitumor activity of 155, 120 and 108% (T/C) at an optimum dose of 100, 3.1 and 12.5 μg/mouse/day, respectively.

Discussion

We describe here the chemical and biological properties of new anthracycline antibiotics, obelmycins, produced by a variant strain SE2-2385 of *S. violaceus* A262. Obelmycins A, B, C and D were shown to be β-isorhodomycinone glycosides with sugar chains at C-7 and C-10 while obelmycins E, F and G were γ-isorhodomycinone glycosides with a sugar chain at C-10. Ordinarily, it is difficult to separate small quantities of isorhodomycins from their counterpart compounds, β-rhodomycins, but we could readily isolate them. This was attributable to the high yields of isorhodomycins synthesized by the mutant strain SE2-2385 which was effective in oxidation at the C-1 position. By structural analysis it was found that obelmycins A, B, C, D, E, F and G were 1-hydroxy analogs of β-rhodomycin I, A447 D[13], cosmomycin C[14], cosmomycin D[14], cosmomycin A[15], cosmomycin B[15] and iremycin[16], respectively. This relationship is similar to that observed between cinerubins and aclacinomycins[17].

Biological activities of obelmycins were compared with those of some 1-deoxyobelmycins and the obelmycins were found to be more active than the 1-deoxyobelmycins. This suggests that a hydroxyl group at C-1 may play an important role in the cytotoxic activity of the compounds. Obelmycins B, C and D, diglycosidic anthracyclines, were more cytotoxic in comparison with monoglycosidic anthracyclines such as obelmycin A and betaclamycin A[18], however their antitumor activities *in vivo* were not always as striking.

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