ANTITUMOR ANTHRACYCLINE ANTIBIOTICS, ACLACINOMYCIN A AND ANALOGUES

II. STRUCTURAL DETERMINATION

TOSHIKAZU OKI, IWAO KITAMURA, YASUE MATSUZAWA, NORIO SHIBAMOTO, TATSUO OGASAWARA, AKIHIRO YOSHIMOTO and TAIJI INUI

Central Research Laboratories, Sanraku-Ocean Co. Ltd., Johnan, Fujisawa, Kanagawa, Japan

HIROSHI NAGANAWA, TOMIO TAKEUCHI and HAMAO UMEZAWA

Institute of Microbial Chemistry, Kamiosaki, Shinagawa-ku, Tokyo, Japan

(Received for publication April 23, 1979)

The structures of aclacinomycins A and B and 19 analogues were determined by a combination of chemical conversions and degradations and spectral interpretations.

In the preceding papers[1~4] we have reported *Streptomyces galilaeus* MA144-M1 to produce new antitumor anthracycline antibiotics aclacinomycins A and B, and 19 analogues. These 21 compounds can be devided into two groups by color; the first group consists of yellow-colored pigments, named aclacinomycins, having aklavinone as the aglycone and the second is red-colored group consisting of 7 components assigned to cinerubin group[5,6] having ϵ-pyrromycinone as the aglycone.

This paper describes the structural determinations of the aclacinomycins and some chemical and enzymatic conversions.

Results and Discussion

General Structural Characteristics of the 21 Compounds

The 21 compounds isolated from the cultured broth of *Streptomyces galilaeus* MA144-M1 can be classified into four groups from their color, chromatographic behavior and the results of acid hydrolysis. On silica gel TLC chromatography, the yellow components were usually accompanied by red subcomponents. The yellow compounds were numbered with the suffix 1 and the corresponding red ones with suffix 2, for example components A1 and A2. By acid hydrolysis, these components can be classified into two groups; one includes glycosidic components consisting of an aglycone and mono-, di- or trisaccharide, and the other is aglycones which do not change on hydrolysis.

Among glycosidic components, the yellow ones; A1, B1, M1, N1, G1, Y1, S1, T1, L1 and K1 have aklavinone[7,8] and the red ones: A2, B2, M2, S2 and T2 have ϵ-pyrromycinone[8,9] as an aglycone. These aglycones released by acid hydrolysis were determined by analysis of mass, ^{1}H-NMR (PMR), ^{13}C-NMR (CMR) and CD spectra, while their sugar moieties were examined for melting points, optical rotations, PMR spectra, Rf values and color development on silica gel TLC plate by direct comparison of the acid hydrolysates with authentic hexoses from cinerubin A[5], cinerubin B[6], streptolydigin[10], amicetin[11] and adriamycin[12] as shown in Table 1.

Sugar components thus determined are summarized in Table 2, that is, A1, A2 and G1: rhodosamine[13], 2-deoxyfucose[14] and cinerulose A[15]; B1 and B2: rhodosamine, 2-deoxyfucose and cinerulose B; K1: daunosamine[19], 2-deoxyfucose and cinerulose A; L1: N-monomethyldaunosamine, 2-deoxyfucose and cinerulose A; M1 and M2: rhodosamine, 2-deoxyfucose and amicetose[16]; N1: rhodosamine, 2-deoxyfucose and rhodinose[17]; S1 and S2: rhodosamine and 2-deoxyfucose; T1 and T2: rhodosamine; Y1: rhodosamine, 2-deoxyfucose and aculose[18].

Table 1. Rf values and color development of sugars.

Sugar	Rf value*	Color**
Cinerulose A	0.82	Greenish blue
Cinerulose B	0.80	Greenish blue
Aculose	0.80	Greenish blue
Amicetose	0.74	Yellowish brown
Rhodinose	0.71	Green
2-Deoxyfucose	0.56	Grayish blue
Rhodosamine	0.12	Sky-blue
N-Monomethyl daunosamine	0.23	Sky-blue
Daunosamine	0.32	Sky-blue

* Silica gel thin-layer (60 F_{254}, Merck Co.) with *n*-butanol - acetic acid - water (4: 1: 1)

** Visualization was carried out with *p*-anisaldehyde.

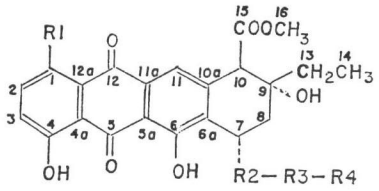
Components C1, C2, D1, D2, E1 and F1 were aglycone-type compounds. C1 and E1 are 7-deoxyaklavinone[8] and 7,7′-dideoxy-7,7′-biaklavinone, respectively, which were formed by catalytic reduction of A1, B1, M1, N1, G1, Y1, S1, T1, L1 and K1 with Pd-$BaSO_4$. Both were also found as the products of reductive glycosidic cleavage at C-7 position of A1 and aklavinone glycosides by hepatic microsomal NADPH-cytochrome P450 reductase (EC 1.6.2.4.)[20]. D1 is aklavinone which was an acid hydrolysis product of A1 and aklavinone glycosides, and F1 is bisanhydroaklavinone[8], dehydration product of aklavinone. C1 and D1 were accompanied by corresponding red compounds C2 and D2 on silica gel TLC plate, which are ζ-pyrromycinone (1-hydroxy C1)[9] and ε-pyrromycinone (1-hydroxy D1), respectively.

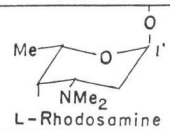
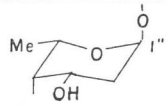
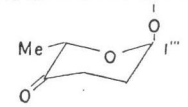
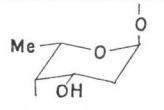
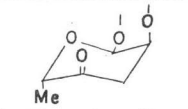
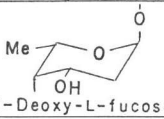
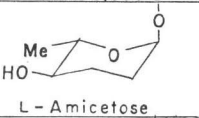
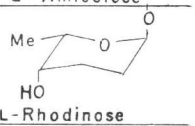
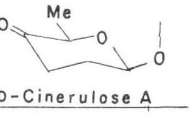
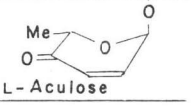
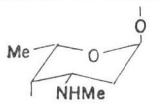
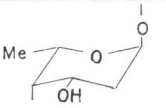
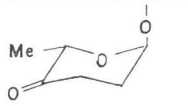
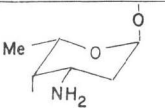
The structures of the aclacinomycins were determined as follows:

1. A1

A1 is a yellow crystalline powder having a molecular formula of $C_{42}H_{53}NO_{15}$. The ultraviolet spectrum of A1 is quite similar to that of aklavin[21] and suggests that A1 is a anthracycline antibiotic. Its visible spectrum has a maximum at 431 nm, which moves to 523 nm in base. The infrared spectrum indicates the presence of hydroxyl groups (3400~3300 cm^{-1}), a ketonic or ester carbonyl (1740 cm^{-1}), typical carbonyl hydrogen bonded to a *peri* hydroxyl group (1620 cm^{-1}), and a nonbonded carbonyl (1670 cm^{-1}) in an anthracycline. The CMR spectrum of A1 has chemical shifts at δ 192.7 and 181.3 arising from the carbonyl groups of the anthraquinone system. The down field carbonyl peak is hydrogen bonded, and the other is not. Acid hydrolysis of A1 with 0.1 N hydrochloric acid gave a yellow aglycone, and three sugar moieties. The aglycone was identified by comparison of its CD curve and UV, IR, PMR and mass spectra with those of aklavinone. The mass spectrum of the aglycone showed peaks at *m/e* 412 (M^+), 394, 376, corresponding to those of aklavinone[8]. The PMR spectrum showed a doublet of doublets at δ 5.32 (J=2, 5 Hz), assigned to the proton at C-7. By spin decoupling experiments, two doublet of doublets at δ 2.24 (J=2, 15 Hz) and δ 2.52 (J=5, 15 Hz) were assigned to C-8 H_B and C-8 H_A protons. From the PMR data, the configuration of the aglycone was different from those of aklavinones I and II, proposed by ECKARDT *et al.*[25] However, it was consistent with that of aklavinone proposed by BROCKMANN *et al.*[24] The CD curve of the aglycone supported the absolute configuration; 7S, 9R and 10R. The sugar moieties were determined to be rhodosamine, 2-deoxyfucose and cinerulose A by direct comparison with authentic samples. The CMR spectrum demonstrated that A1 consisted

Table 2. Compositions of sugars and aglycone in aclacinomycins.



Compound	R1		R2	R3	R4
	* Yellow series	Red series			
A	H	OH	 L-Rhodosamine	 2-Deoxy-L-fucose	 L-Cinerulose A
B	H	OH	〃	 2-Deoxy-L-fucose	 L-Cinerulose B
M	H	OH	〃	 2-Deoxy-L-fucose	 L-Amicetose
N	H	—	〃	〃	 L-Rhodinose
G	H	—	〃	〃	 D-Cinerulose A
Y	H	—	〃	〃	 L-Aculose
S	H	OH	〃	〃	
T	H	OH	〃		
L	H	—	 N-Monomethyl-L-daunosamine	 2-Deoxy-L-fucose	 L-Cinerulose A
K	H	—	 L-Daunosamine	〃	〃

* Yellow series: Suffix I, Aklavinone. Red series: Suffix 2, ε-Pyrromycinone.

Table 3. Physicochemical properties of methyl glycosides from aclacinomycins.

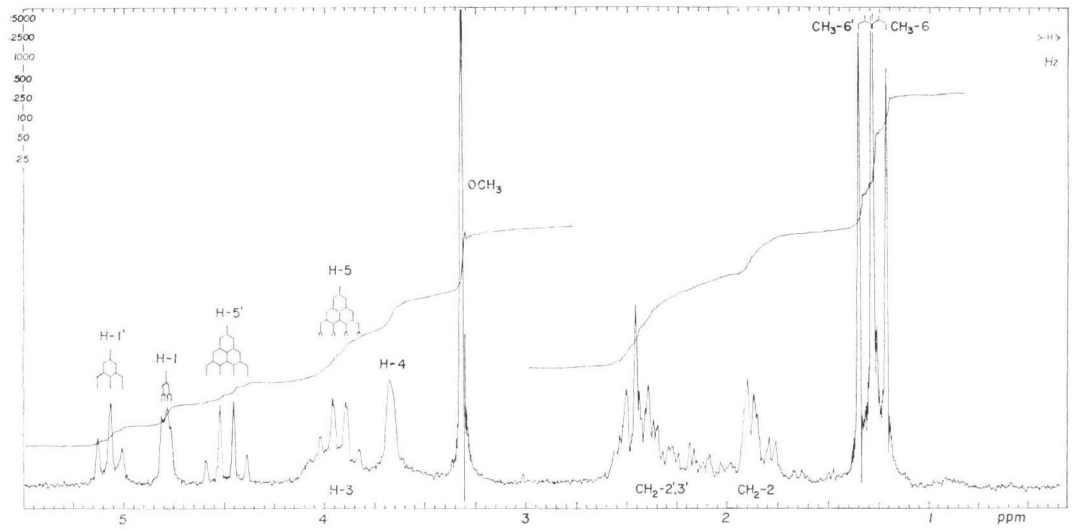
Methyl glycoside from	$[\alpha]_D$*	mp (°C)	Rf value**	Color***
A1	−300°	Oil	0.59	Grayish blue
B1	−232°	129~130	0.80	Grayish blue
M1	−161°	102~104	0.40	Greenish brown
G1	−114°	105~108	0.52	Grayish blue
Y1	−65°	109~110	0.61	Greenish blue

* in $CHCl_3$

** Silica gel thin-layer (60 F_{254}, Merck Co.) with ethyl acetate.

*** Visualization was carried out with *p*-anisaldehyde.

Fig. 1. PMR spectrum of methyl glycoside (**I**) from aclacinomycin A (100 MHz, in $CDCl_3$).



of one mole each of these sugars and aklavinone. Catalytic reduction of A1 with Pd-$BaSO_4$ caused partial cleavage of A1 into 7-deoxyaklavinone and a trisaccharide moiety, indicating that the sugar terminal attaches to C-7 position of aklavinone. On the other hand, treatment of A1 in methanolic hydrogen chloride led to the formation of a methyl glycoside (**I**) and 1-deoxypyrromycin[1] (rhodosaminyl aklavinone), to which aklavin[21,22] was recently identified. This compound **I** gave grayish blue color development by spraying with *p*-anisaldehyde on TLC plate, as shown in Table 3, and was identical with the methyl glycoside obtained from cinerubin A[5]. The IR spectrum of **I** gave distinct absorption at 1730 cm^{-1}, indicating the presence of a carbonyl group. In the PMR spectrum (Fig. 1) of **I**, the methyl groups at δ 1.23 and 1.31 were shown to be coupled with the methines at δ 3.96 and 4.49, respectively. The coupling constants were 6.5 Hz, and assigned to CH_3–CH in C-6–C-5 and C-6′–C-5′ of the two sugar moieties. According to its chemical shift and coupling with a methylene group in the area of δ 1.87, the doublet of doublets at δ 4.79 was assigned to the proton at C-1 of the 2-deoxyfucose moiety. The small coupling constants, 2 and 3 Hz, indicated that the configuration of glycoside at C-1 was α. The broad peak in the area of δ 3.82 to 4.12 suggested an axial proton at C-3. The signal at δ 3.66 showed the half height width of 4.5 Hz, and this value agreed with that of the equatorial proton

Table 4. ^{13}C-Chemical shift-assignments.

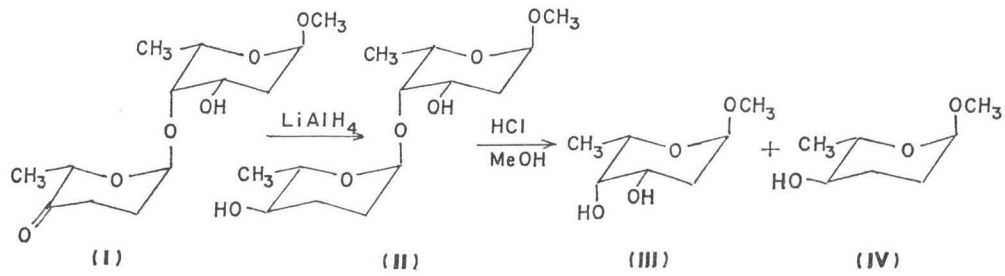
C	A1	B1	M1	N1	G1	Y1	S1	T1	L1	K1	AKN	E1*
5	192.7	192.6	193.0	192.8	193.1	192.8	192.8	193.1	193.0	192.7	192.8	194.1
12	181.3	181.1	181.6	181.3	181.6	181.3	181.3	181.5	181.6	181.2	181.3	181.6
15	171.3	171.3	171.6	171.4	171.6	171.4	171.3	171.6	171.6	171.3	171.5	172.1
4	162.6	162.5	162.9	162.6	162.9	162.6	162.6	162.9	162.9	162.6	162.8	162.9
6	162.2	162.1	162.5	162.2	162.5	162.2	162.2	162.5	162.5	162.2	161.4	161.7
10a	142.7	142.7	143.0	142.7	143.0	142.8	142.7	143.0	142.9	142.8	142.9	146.1
2	137.3	137.3	137.5	137.3	137.6	137.4	137.3	137.6	137.5	137.3	137.7	137.6
12a	133.5	133.4	133.8	133.6	133.8	133.6	133.5	133.8	133.8	133.5	133.6	134.8
6a	132.9	132.8	133.1	132.9	133.1	132.9	133.2	133.2	133.1	132.9	133.1	134.6
11a	131.5	131.3	131.8	131.5	131.7	131.5	131.4	131.6	131.6	131.4	132.7	132.3
3	124.8	124.7	125.0	124.6	125.0	124.9	124.8	125.0	125.0	124.8	125.0	124.6
11	120.9	120.9	121.2	121.0	121.2	121.0	120.9	121.2	121.1	121.0	121.5	119.9
1	120.1	120.1	120.3	120.1	120.3	120.2	120.1	120.4	120.3	120.1	120.4	119.8
4a	115.9	115.7	116.1	116.0	116.0	115.9	115.8	116.0	116.0	115.8	115.8	117.1
5a	114.7	114.6	114.9	114.8	114.9	114.7	114.6	114.9	114.9	114.7	114.7	114.7
9	71.7	71.6	71.7	71.7	71.8	71.7	71.7	71.9	71.8	71.6	71.9	73.1
7	70.6	70.7	70.8	70.6	70.8	70.7	70.7	71.3	71.1	70.9	62.5	36.2
10	57.2	57.2	57.3	57.2	57.3	57.2	57.2	57.3	57.3	57.2	56.7	59.9
16	52.5	52.5	52.5	52.5	52.5	52.5	52.5	52.6	52.6	52.4	52.6	52.0
8	33.8	33.8	33.9	33.8	33.9	33.8	33.8	33.9	33.9	33.9	34.9	35.0
13	32.2	32.2	32.2	32.2	32.3	32.2	32.2	32.2	32.2	32.2	32.5	26.7
14	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	6.7	7.4
1′	101.6	101.6	101.9	101.7	101.9	101.6	101.6	101.6	102.2	101.9		
2′	29.3	29.3	29.4	29.3	29.3	29.4	29.3	28.6	32.2	34.6		
3′	61.6	61.6	61.7	61.6	61.7	61.6	61.6	60.1	54.9	46.8		
4′	74.1	74.2	74.2	74.1	74.2	74.2	74.3	66.1	77.4	81.9		
5′	66.8	65.3	67.0	67.0	66.6	66.6	66.2	66.8	67.4	67.3		
6′	17.0	17.9	17.1	17.0	17.0	17.0	16.7	17.0	17.2	17.1		
NMe_2	43.3	43.2	43.3	43.3	43.3	43.3	43.2	43.3	33.5			
1″	100.2	99.1	99.6	100.4	99.7	99.4	99.1		100.8	100.7		
2″	34.4	27.0	34.5	34.4	34.4	34.3	33.0		34.4	34.4		
3″	65.4	67.3	65.8	65.7	65.0	65.8	65.9		65.3	65.2		
4″	83.0	68.3	83.9	83.8	83.4	83.8	71.6		72.5	82.3		
5″	68.4	66.9	68.6	68.5	68.5	68.4	68.4		68.3	68.2		
6″	17.9	16.0	17.9	17.9	17.9	17.9	17.9		17.7	17.5		
1‴	99.4	91.5	99.6	99.5	101.8	95.7			100.8	100.1		
2‴	27.7	63.0	27.3	24.0	30.0	142.3			27.7	27.7		
3‴	33.5	39.7	29.6	25.6	34.9	127.2			33.5	33.5		
4‴	210.0	208.1	71.7	67.3	207.7	195.7			210.1	209.7		
5‴	71.8	77.9	71.8	68.1	76.6	71.5			72.0	71.9		
6‴	14.8	16.2	17.9	17.0	15.6	15.2			14.8	14.8		

In ppm (δ), obtained from $CDCl_3$ solutions containing TMS as internal reference. (* in dioxane-d_8)

at C-4 in the 2-deoxyfucose moiety. The signal of the proton at C-1′ at δ 5.07 appeared as a sharp triplet. The coupling constant between the proton at C-1′ and methylene protons at C-2′ was 6 Hz. This coupling constant corresponded to those of the disaccharide from cinerubin A (a triplet of J=5 Hz). Torsion angles between the proton at C-1′ and two protons at C-2′ were calculated from the KARPLUS equation[23] to be 30° and 144°, respectively. Therefore, the torsion angles obtained above suggested that L-cinerulose A in A1 probably took a flatter conformation, due to its C-4′ carbonyl group, and this might be changeable to D-configuration. The CMR spectrum of A1 is shown in Table 4 together with the spectra of other aclacinomycin analogues. The assignments of the carbons of the aglycone were made by the comparison of the spectra with ^{13}C-labeled aklavinones which were biosynthesized from 1-^{13}C-acetate and 2-^{13}C-acetate and 1,2-^{13}C-acetate (unpublished data). The ^{13}CH coupling constants were observed by application of gated decoupling method in the CMR spectrum of A1. These $^1J[^{13}CH(1)]$ values of rhodosamine, 2-deoxyfucose and cinerulose A were 170, 168 and 166 Hz, respectively. The assignments of anomeric carbons in A1 were determined by the selective irradiations of anomeric protons. The slightly small $^1J[^{13}CH(1)]$ value of cinerulose A suggested the same conclusion as in PMR. Thus, the glycosidic linkages at C-1′ and C-1″ are α based on the coupling constants of the protons of C-1′, C-1″ and C-2′, C-2″, and the $^1J[^{13}CH(1)]$ values in A1.

Mild hydrolysis of A1 in 5% HCl gave a small amount of S1, 2-deoxyfucosyl-rhodosaminyl-aklavinone, with liberation of cinerulose A. Thus, this indicates that the distal sugar of A1 is cinerulose A. In the CMR spectrum, the signals of C-4″ were observed at δ 83.0 and 71.6 in A1 and S1, respectively. These chemical shifts indicate that cinerulose A attaches to the C-4″ of 2-deoxyfucose moiety.

Methyl glycoside **I** was reduced with lithium aluminum hydride in ethyl ether, and the product (**II**), having greenish brown color with *p*-anisaldehyde on TLC plate, was further degraded with methanolic hydrogen chloride into two sugar components. The sugar components were identified as methyl 2-deoxy-α-L-fucoside (**III**) and methyl-α-L-amicetoside (**IV**) by comparison of PMR and α_D value with their reported data[5]. Another methanolysis product 1-deoxypyrromycin (identical with T1) was further hydrolyzed to an aglycone, aklavinone, and a sugar component, rhodosamine. Rhodosamine was proved to be α-L-rhodosamine by the analysis of PMR spectrum and α_D value.



(I) (II) (III) (IV)

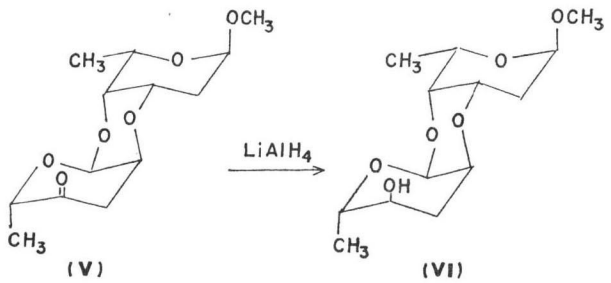
From the above-mentioned results, the total structure of A1 was determined to be L-cinerulosyl-2-deoxy-L-fucosyl-L-rhodosaminyl aklavinone, as shown in Fig. 4.

Red component A2 gave almost the same Rf value as A1 on silica gel TLC in a development system of $CHCl_3$ - MeOH (20: 1). From the spectral data of A2, it was identified as cinerubin A, L-cinerulosyl-2-deoxy-L-fucosyl-L-rhodosaminyl ϵ-pyrromycinone. In a comparative study with A1, the difference was found only in the aglycone; A2 has red 1-hydroxyaklavinone (ϵ-pyrromycinone), while A1 has

yellow aklavinone, and the sugar moiety was the same. Similarly, the components with the suffix 2 differ only in the aglycone structure from the components with suffix 1.

2. B1

By methanolysis, B1 was hydrolyzed into 1-deoxypyrromycin and a methyl glycoside (V). B1 was more resistant to the hydrolysis than A1, indicating comparatively stronger binding between rhodosamine and 2-deoxyfucose. Compound V obtained as colorless needles from cyclohexane-acetone gave two signals in the methylene region of the PMR spectrum, one of which at δ 2.59 is a doublet (J = 3 Hz) indicating the presence of only one vicinal proton. In addition, C-1′ proton splits into doublet (J = 3 Hz), while in the case of A1 this proton was a triplet (J = 6 Hz). Thus, C-2′ of V is a methine. These data demonstrated that cinerulose A is attached to 2-deoxyfucose by the same two bondings at C-1′ and C-2′ as in cinerubin B. The PMR spectrum, mp and α_D values of V obtained from B1 showed good agreement with those of the corresponding degradation product of cinerubin B[6]. Moreover, IR and PMR spectral data and mp of the crystals (VI) obtained by reducing the carbonyl group of V with lithium aluminum hydride in ethyl ether, coincided with those data of the crystalline disaccharide obtained from cinerubin B. The X-ray crystallographic study of the disaccharide moiety of cinerubin B demonstrated one glycosidic and one ethereal bonding between 2-deoxy-L-fucose and L-cinerulose B[6].



3. M1, N1, G1, Y1

The structures of M1, N1, G1 and Y1 are similar to A1 and B1 consisting of aklavinone and a trisaccharide. All of them yielded 1-deoxypyrromycin and a methyl glycoside on methanolysis.

M1: The methyl glycoside obtained from M1 was crystallized from cyclohexane-acetone mixture and gave a greenish brown spot on TLC plate by *p*-anisaldehyde, and mp, IR, PMR and α_D completely agreed with those of II from A1. According to the procedure described for A1, its structure was determined to be methyl-L-amicetosyl-2-deoxy-L-fucoside. The amicetose moiety attaches to the C-4 position of 2-deoxyfucose in α glycosidic linkage.

The presence of L-amicetose as a terminal sugar in M1 was also supported by the PMR spectrum of the acetylated product (VII) of II. The product (VII) showed two acetyl signals at δ 2.07 and 2.09 in the PMR spectrum (Fig. 2). One hydroxyl group was attributable to the C-3 of 2-deoxyfucose, because of the lower field shift of the C-3 proton (δ 5.18). The C-5′ proton at δ 4.13 showed large coupling constant of 10.0 Hz in addition to 6.5 Hz with C-6′ methyl protons, and thus the C-4′ proton is axial.

N1: Methanolysis of N1 gave 1-deoxypyrromycin, methyl-2-deoxyfucoside and another sugar product, which gave a green spot on TLC plate with *p*-anisaldehyde. This product could not be recovered because of volatility during the purification process. In mild

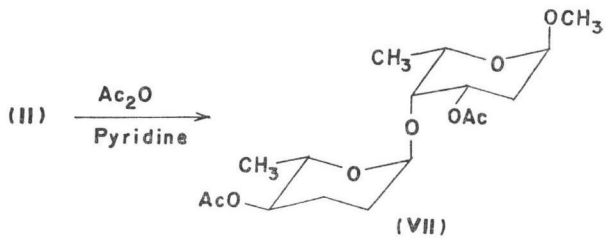
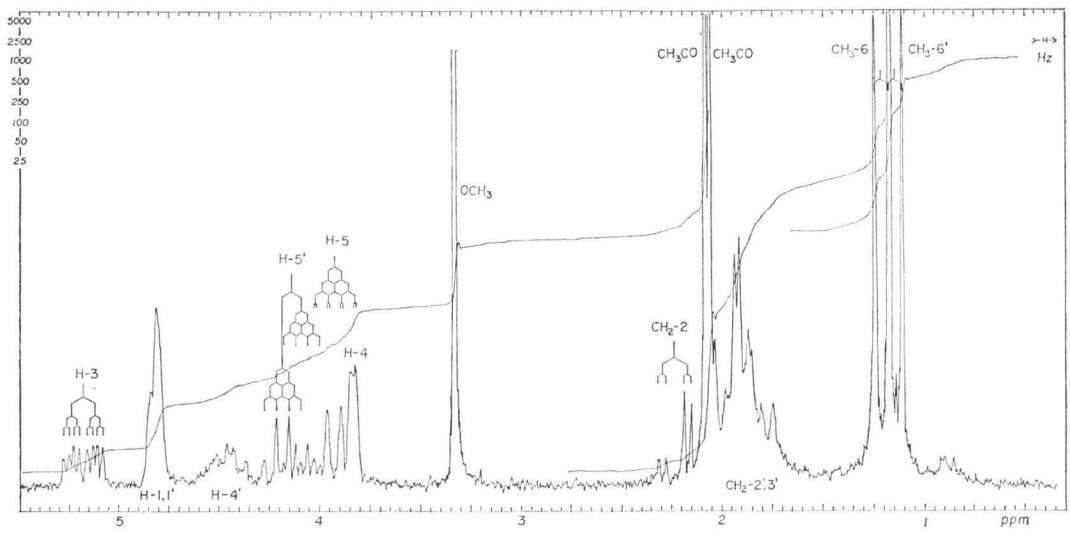


Fig. 2. PMR spectrum of methyl glycoside diacetate (**VII**) from MA144 M1 (100 MHz, in $CDCl_3$).

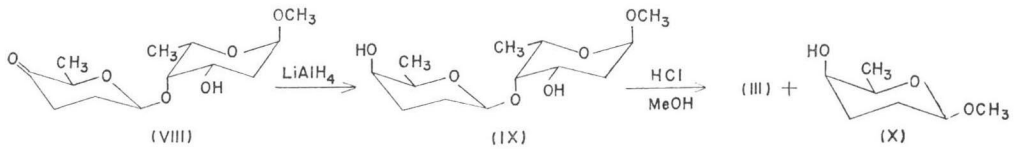


hydrolysis using 0.5% hydrochloric acid, N1 released S1 and L-rhodinose in good yield. The sugar moiety was identical with rhodinose obtained from streptolydigin[10,17] by comparison of its α_D and Rf value on TLC.

By the reduction either using rat liver homogenate, a hepatic microsomal enzyme preparation, in the presence of NADH or NADPH, or using sodium borohydride, A1 was converted to M1 or its isomer N1[20]. The C-4''' carbonyl group of L-cinerulose A was reduced to C-4'''-hydroxyl isomers; L-amicetose or L-rhodinose.

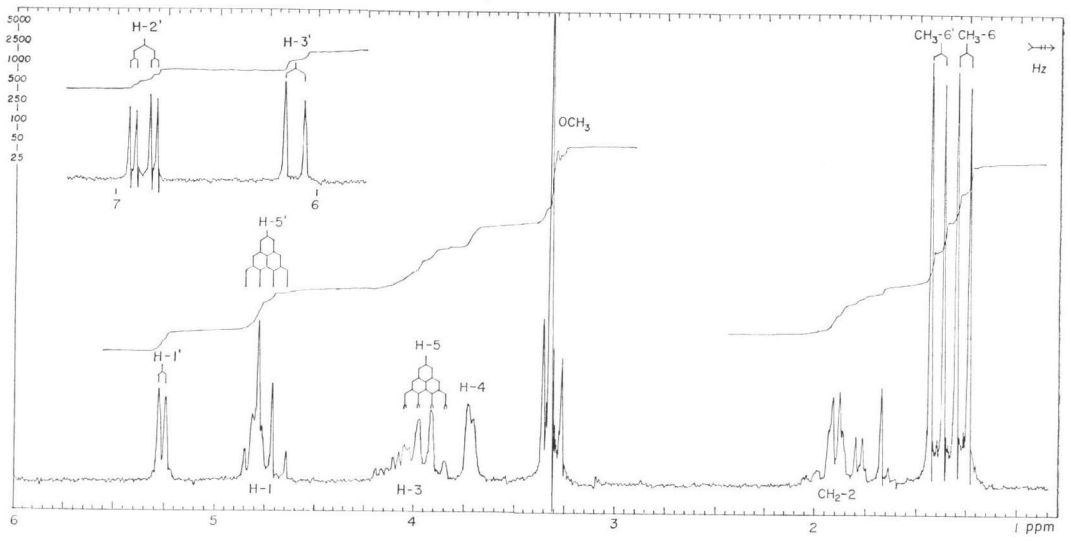
G1: The methyl glycoside (**VIII**) from G1 gave a grayish blue spot with *p*-anisaldehyde on a TLC plate. Its PMR spectrum showed a close resemblance in coupling pattern, except for the chemical shifts attributable to the terminal sugar moiety, to those of **I** from A1. Compound **VIII** has the same proton and carbon number as those of **I**. A chemical shift at δ 207.7 in the CMR of G1 together with a strong absorption at 1730 cm^{-1} in the IR spectrum indicated the presence of a carbonyl group.

The reduced methyl glycoside (**IX**) of G1 obtained by reduction with lithium aluminum hydride gave a greenish brown spot on TLC plate by *p*-anisaldehyde spraying, which showed the same Rf value as **II** of A1. Methanolysis of **IX** yielded methyl-2-deoxy-L-fucoside and a product (**X**) which has the same Rf value as methyl-L-amicetoside. Product **X** giving a yellowish brown spot on TLC with *p*-anisaldehyde and the α_D value of +128° was deduced to be the D-enantiomer of L-amicetose.

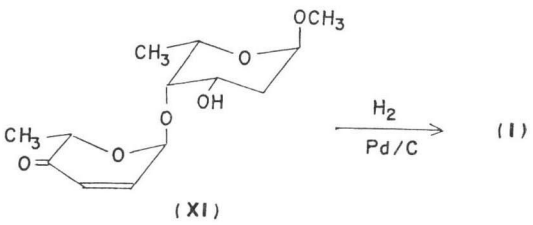


When A1 was adsorbed on silica gel in ethyl acetate and left standing for several hours at room temperature, it was converted to G1. Thus, it is supposed that the configurational change from L-cinerulose A to D-cinerulose A may occur in very mild acidic conditions through enolization of the C-4''' carbonyl group of aclacinomycin A.

Fig. 3. PMR spectrum of methyl glycoside (**XI**) from aclacinomycin Y (100 MHz, in $CDCl_3$).



Y1: The infrared absorption at 1680 cm^{-1} and UV spectrum, λ_{max}^{MeOH} nm (ε): 209 (6726), of methyl glycoside (**XI**) obtained from Y1 indicated the presence of a α, β unsaturated carbonyl group in the structure. This was also supported by the PMR (Fig. 3) and CMR spectra. By spin decoupling experiments on PMR, a doublet of doublets at δ 6.86 (J=3.5 and 10.0 Hz) and two doublets at δ 6.11 (J=10.0 Hz) and 5.26 (J=3.5 Hz) were assigned to the protons at C-2', C-3' and C-1', respectively. On the CMR spectrum, two vinyl carbons, C-2' and C-3', were observed at δ 142.1 and 127.3, respectively, and the chemical shift of the ketone at C-4' (δ 195.6) of **XI** was at higher field than that of **I** (δ 209.9).



XI was hydrogenated with a palladium catalyst, and the product reduced at the double bond to methylene gave the same Rf value on TLC, IR spectrum and α_D value as those of **I** from A1.

From the results, a sugar moiety other than 2-deoxy-L-fucose contained in **XI** was identified as 2,3,6-trideoxy-L-*hex*-2-enopyranos-4-ulose, which is attached to C-4 of 2-deoxy-L-fucose. This sugar has been reported in an early study on total synthesis of monosaccharides[26,27], but not previously found in natural products, and has been named aculose.

Aculose is unstable even on silica gel or in organic solvents. Forty percent of compound **XI** was converted to B1-methyl glycoside (**V**), after eluation from silica gel column chromatography with benzene and ethyl acetate. Also, Y1 was easily converted to B1 by stirring with silica gel in toluene.

It must be noted that Y1 was formed in equivalent amount from A1 in the enzymatic reaction using enzyme preparations from *Streptomyces* cultures[18].

4. S1

S1 gave 1-deoxypyrromycin and methyl 2-deoxy-L-fucoside by methanolysis. The presence of two sugars in S1 was also proved from the carbon number of 36 in the CMR spectrum. The difference of the chemical shifts at C-4'; δ 66.1 in 1-deoxypyrromycin and δ 74.3 in S1, indicates glycosidation

shift. It was, thus, estimated S1 has a sugar sequence of rhodosaminyl 2-deoxy-L-fucoside.

5. T1

T1 was identified as 1-deoxypyrromycin, which was recently reported by KUMAR *et al.*[22], from the comparisons of their mp, IR, PMR, α_D and Rf value on TLC. It consists of aklavinone having absolute configuration; 7S, 9R and 10R, and L-rhodosamine bonding to the C-7 with an α-glycoside linkage.

6. L1, K1

L1, which was found and isolated from the culture broth of *S. galilaeus*, was also obtained by irradiating A1 in ethyl ether under a germicidal lamp. K1 was not found in the culture broths, but was transformed photochemically from A1 through L1 in chloroform. The transformation mechanism from A1 to L1 and K1 is under investigation and will be reported elsewhere.

Methanolysis of L1 and K1 resulted in the release of the same methyl glycoside, **I** as from A1, and different glycosidic compounds than 1-deoxypyrromycin. The resulting glycosides were hydrolyzed to yield aklavinone and respective aminosugars. The aminosugar from L1 gave a larger Rf value on silica gel TLC than that of rhodosamine (*n*-butanol - acetic acid - water, 4: 1: 1) and that from K1 showed a far larger Rf value corresponding to that of daunosamine. On the PMR spectrum, the aminosugar from L1 gave a singlet of three protons (δ 2.36) attributable to one methyl group attached to the nitrogen atom. On the CMR spectrum of A1, the C-3′, bearing $-N(CH_3)_2$, gave a peak at δ 61.6 as shown in Table 4, while the C-3′ peak in L1 shifted to δ 54.9, and the peak assigned to $N-CH_3$ shifted to δ 33.5 from δ 43.3 of $-N(CH_3)_2$ in A1. Therefore, the aminosugar in L1 was demonstrated to be N-monomethyl daunosamine.

The aminosugar in K1 was demonstrated to be daunosamine from the PMR and CMR spectra together with other physicochemical properties.

7. C1, D1, F1

C1, D1 and F1 were resistant to acid hydrolysis, and formed from A1 and other aklavinone glycosides.

C1: C1 was identified by direct comparison with an aglycone produced by hydrogenolysis of A1 on palladium catalyst and by the reductive cleavage reaction of A1 with rat hepatic microsomal NADPH-cytochrome P450 reductase[20]. The infrared absorption spectrum of C1 showed strong absorption at 3550 cm^{-1} and the PMR spectrum in $CDCl_3$ indicated the presence of a methylene group at C-7 in the area of δ 2.7 to 3.1. The mass spectrum of C1 showed significant peaks at *m/e* 396 (M^+), 378, 376 and 319. These fragment ions can arise from 7-deoxyaklavinone[8].

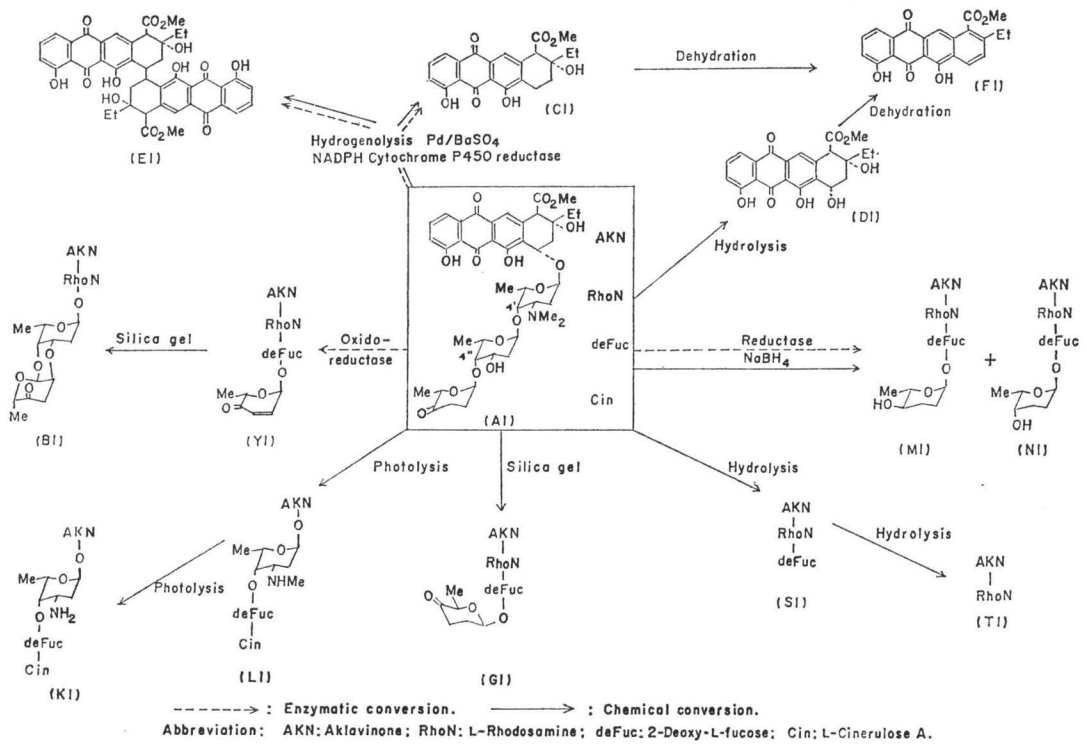
D1: D1 was identified as aklavinone by comparison with the aglycone produced by hydrolysis of aklavinone glycosides.

F1: The ultraviolet and visible light absorption spectra in methanol had λ_{max} at 453 nm with $\epsilon = 17670$. This absorption suggests the aromatization of the cyclohexene ring. The mass spectrum showed peaks at *m/e* 376 (M^+), 361, 345 and 344. From the analysis of mass and PMR spectra, F1 was identified as bisanhydroaklavinone[8].

8. E1

The presence of sugar moiety being denied by preliminary examination, E1 was an aglycone compound, and gave a smaller Rf value on TLC, than C1 (7-deoxyaklavinone), and different mp, while the highest *m/e* peak was the same as that of C1. The molecular weight was determined to be 799 by vapor pressure osmometry. Therefore, it was deduced that E1 is the dimer of 7-deoxyaklavinone.

Fig. 4. Enzymatic and chemical conversion of aclacinomycin A.



In the PMR spectrum of E1, the C-7 proton was observed at higher field (δ 4.72 in dioxane-d8) than that of aklavinone (δ 5.32 in $CDCl_3$). This result suggested the absence of oxygen at C-7. Acetylation of E1 resulted in the introduction of two acetyl groups to aromatic hydroxyl groups at C-4 and C-6, confirming the absence of hydroxyl group at C-7. Furthermore, C-7 was observed at δ 36.2 in the CMR spectrum. This chemical shift indicates no oxygen atom on the C-7 methine carbon. Therefore, E1 is the dimer of 7-deoxyaklavinone linked by C–C bonding at C-7 position.

To summarize the present study, the structural relationships among the 21 components of aclacinomycin produced by *Streptomyces galilaeus* MA144-M1 are shown in Fig. 4 with their chemical structures and chemical and enzymatic conversions.

Experimental

General:

Melting points were determined on a Kofler hot-stage microscope and are uncorrected. UV spectra were determined on a Hitachi EPS-3T and IR spectra on a Hitachi EPI-G2 spectrophotometer (KBr pellets or carbontetrachloride). NMR spectra were recorded on a Varian XL-100 spectrometer or a Hitachi R-24 spectrometer. Chemical shifts are expressed in values (ppm) with tetramethylsilane as an internal standard. Proton-noise decoupled FT-^{13}C-NMR spectra were taken at 25.2 MHz on a Varian XL-100 spectrometer using tetramethylsilane as reference. Abbreviation: s=singlet, d=doublet, dd=doublet of doublets, t=triplet, q=quartet, dq=doublet of quartets, bs=broad singlet and m=multiplet. The mass spectra were taken by a Hitachi RMU-6 mass spectrometer with direct inlet system. Molecular weights were measured by a vapor pressure osmometry (Mechrolab Osmometer 301A), corrected by calibration with dibenzoyl.

Silica gel plates F_{254} (Merck Co.) were developed with ethyl acetate and a mixture of *n*-butanol - acetic acid - water (4: 1: 1), sprayed with 5% *p*-anisaldehyde and 5% sulfuric acid in ethanol, and then heated at 90°C to examine color development of sugar. Column chromatography was carried out with silica gel (Wako), Wakogel C-200, 74~149 μ and Sephadex LH-20 (Pharmacia Fine Chemicals Co.).

MA144 A1, A2, B1, B2, C1, C2, D1, D2, E1, F1, G1, K1, L1, M1, M2, N1, S1, S2, T1, T2 and Y1:

These compounds were prepared in pure form from a culture broth of *Streptomyces galilaeus* as reported previously[4].

Total hydrolysis of A1 and other glycosidic compounds:

A solution A1 (100 mg) in 10 ml of 0.1 N hydrochloric acid was heated on a water bath at 85°C for 30 minutes. A yellow precipitate (30 mg) was obtained, and recrystallized from benzene to yield aklavinone (20 mg) as orange-yellow needles, mp. 167~170°C (lit.[7] 170°C), λ_{max}^{MeOH} nm (ϵ) 229 (40129), 259 (24720), 290 (9476), 431 (11866), MS: *m/e* 412 (M^+), 394, 376, ν_{max}^{KBr} cm^{-1}: 1730, 1620, PMR ($CDCl_3$) δ in ppm: 1.11 (3H, t, J=7, H-14), 1.61 (2H, q, J=7, H-13), 2.24 (1H, dd, J=2,15, H_B-8), 2.52 (1H, dd, J=5,15, H_A-8), 3.7 (3H, s, OMe), 4.08 (1H, s, H-10), 5.32 (1H, dd, J=2,5, H-7), 7.22 (1H, dd, J=3,7, H-3), 7.54 (1H, s, H-11), 7.6 (1H, t, J=5,7, H-2), 7.68 (1H, dd, J=3,5, H-1). The HCl solution was neutralized by addition of silver carbonate and extracted with chloroform. In the aqueous layer three sugars corresponding to rhodosamine, 2-deoxyfucose and cinerulose A were detected at Rf 0.12, 0.56 and 0.82, respectively, on TLC.

Other glycosidic compounds were also hydrolyzed and analyzed by the same method as in A1. Rf values of sugar residues are shown in Table 1.

Partial methanolysis of A1:

To a solution of A1 (1.0 g) in dry acetone (100 ml) were added dry methanol (10 ml) and 0.1 N hydrochloric acid in absolute methanol (20 ml). The mixture was allowed to stand at room temperature for 45 minutes, and neutralized by addition of silver carbonate and filtered, and then the filtrate was evaporated. The residue was extracted several times with a total of 100 ml of ether. The ether insoluble material was dissolved in 0.1 M acetate buffer (pH 3.0) with added EDTA solution to remove metals, and extracted with chloroform at pH 7.0. The extract was washed with water, dried over Na_2SO_4 and concentrated to a yellowish brown residues. The pigments were chromatographed on silica gel (10 g) using chloroform - methanol (10: 1), and the eluate was concentrated and precipitated with *n*-hexane to give 75 mg of 1-deoxypyrromycin, mp. 121~125°C, solidified at 154~173°C and remelted at 230~235°C, ν_{max}^{KBr} cm^{-1}: 1735, 1620, 1290, 1010, and NMR spectra were identical with those of authentic sample.

The residues obtained by the evaporation of the ether extract were chromatographed on silica gel (65 g) using benzene - ethyl acetate (6: 3.5). The eluate was concentrated to give 156 mg of a colorless oil (**I**), $[\alpha]_D^{23}$ −300° (*c* 0.5, $CHCl_3$) (lit.[5] $[\alpha]_D$ −270° (*c* 0.41, $CHCl_3$)), $\nu_{max}^{CCl_4}$ cm^{-1}: 3450, 1730, 1120, 1100, 1040. PMR ($CDCl_3$) δ in ppm: 1.23 (3H, d, J=6.5, H-6), 1.31 (3H, d, J=6.5, H-6′), 1.87 (2H, m, H-2), 2.31 (2H, m, H-2′), 2.48 (2H, m, H-3′), 3.33 (3H, s, OMe), 3.66 (1H, bs, H-4), 3.96 (1H, m, H-3), 3.96 (1H, dq, J=1, 6.5, H-5), 4.49 (1H, q, J=6.5, H-5′), 4.79 (1H, dd, J=3,2, H-1), 5.07 (1H, t, J=6, H-1′).

Reduction of methyl glycoside (**I**):

A solution of **I** (156 mg) in dry ether (30 ml) was dropped into 59 mg of lithium aluminum hydride in dry ether (30 ml) and stirred at room temperature for 15 minutes. The excess of reagent was decomposed by stirring with 50 ml of wet ether and was filtered.

The combined ether layer was evaporated and the residue (135 mg) was purified by preparative silica gel TLC (ethyl acetate) and on Sephadex LH-20 column with methanol to yield a colorless powder (83 mg). Recrystallization from acetone and cyclohexane gave 69 mg of colorless platelets (**II**): mp. 103~104°C, $[\alpha]_D^{24}$ −176° (*c* 0.5, $CHCl_3$), ν_{max}^{KBr} cm^{-1}: 3460, 3420, 3400, 1040, 1000. PMR ($CDCl_3$) δ in ppm: 1.22 (3H, d, J=6.5, H-6′), 1.28 (3H, d, J=6.5, H-6), 1.87 (6H, m, H-2,2′ and 3′), 3.32 (3H, s, OMe), 3.55 (1H, bs, H-4), 3.7~4.05 (3H, m, H-5,5′ and 3), 4.28 (1H, m, H-4′), 4.77 (2H, bs, H-1 and 1′).

Hydrolysis of compound II:

Compound II (60 mg) in dry methanol (3 ml) and 4 N hydrochloric acid in absolute methanol (0.3 ml) was treated at 80°C for 10 minutes, neutralized with silver carbonate and filtered. The filtrate was evaporated to yield a colorless mixture (42 mg), which was separated by preparative TLC with cyclohexane - acetone (3: 1) into methyl-α-L-amicetoside (2.2 mg) and methyl-2-deoxy-α,β-L-fucoside (17.9 mg).

Methyl-α-L-amicetoside (IV): Colorless liquid, $[\alpha]_D^{24}$ −113° (*c* 0.13, $CHCl_3$) (lit.[5] $[\alpha]_D$ −129° (*c* 0.44, $CHCl_3$)), $\nu_{max}^{CCl_4}$ cm^{-1}: 3400, 1130, 1055, PMR ($CDCl_3$) δ in ppm: 1.27 (3H, d, J=6, H-6), 1.6~2.0 (4H, m, H-2 and 3), 3.2~3.4 (1H, m, H-4), 3.35 (3H, s, OMe), 3.58 (1H, dq, J=6, 10, H-5), 4.63 (1H, bs, H-1).

The mixture of two anomeric methyl 2-deoxy-L-fucosides from the above preparative thin-layer was further purified by preparative TLC with ethyl acetate to obtain 3.1 mg of the α anomer: $[\alpha]_D^{24}$ −85° (*c* 0.1, $CHCl_3$) (lit.[5] $[\alpha]_D$ −119° (*c* 0.68, $CHCl_3$)), $\nu_{max}^{CCl_4}$ cm^{-1}: 3400, 1040, PMR ($CDCl_3$) δ in ppm:1.27 (3H, d, J=6.5, H-6), 1.84 (2H, dd, J=2.5, 8, H-2), 3.32 (3H, s, OMe), 3.4~4.0 (3H, m, H-3, 4 and 5), 4.77 (1H, t, J=2.5, H-1).

Hydrogenolysis of A1:

A solution of A1 (136 mg) in methanol (30 ml) was hydrogenated over 5% $Pd/BaSO_4$ (308 mg) at room temperature and atmospheric pressure for 2 hours. The reaction mixture was filtered and evaporated to 2~3 ml. The residue was separated into aglycones and sugar moiety on a Sephadex LH-20 column with methanol. The faster eluate, containing C1 and E1, was evaporated and purified by preparative TLC with chloroform - methanol (50: 1).

C1; Recrystallized from benzene to yield 12 mg, mp. 229~230°C (lit.[28] mp. 224~225°C), ν_{max}^{KBr} cm^{-1}: 3550, 1730, 1620, PMR ($CDCl_3$) δ in ppm: 1.05 (3H, t, J=7.0, H-14), 1.5 (2H, q, J=7.0, H-13), 2.1~2.5 (2H, m, H-8), 2.7~3.1 (2H, m, H-7), 3.71 (3H, s, OMe), 3.92 (1H, s, H-10), 7.3 (1H, dd, J=2, 7, H-3), 7.66 (1H, t, J=7, H-2), 7.66 (1H, s, H-11), 7.82 (1H, dd, J=2, 7, H-1), 12.05 and 12.45 (OH, exchange with D_2O), MS: *m/e* 396 (M^+), 376, M.W. 396 (by vapor pressure osmometry).

E1; Recrystallized from chloroform to yield 32 mg, mp. 266~272°C, ν_{max}^{KBr} cm^{-1}: 3550, 1740, 1615, PMR (dioxane-d_8) δ in ppm: 0.8 (3H, t, J=7, H-14), 1.30 (2H, q, J=7, H-13), 1.5~2.0 (2H, m, H-8), 3.78 (3H, s, OMe), 4.05 (1H, s, H-10), 4.72 (1H, bs, H-7), 7.25 (1H, dd, J=2, 7, H-3), 7.38 (1H, s, H-11), 7.64 (1H, t, J=7, H-2), 7.76 (1H, dd, J=2, 7, H-1), 11.88 and 12.88 (OH, exchange with D_2O), MS: *m/e* 396, M.W. 799 (by vapor pressure osmometry), (Found: C, 66.71; H, 5.05; O, 28.27; Calc. for $C_{44}H_{40}O_{14}$: C, 66.66; H, 5.09; O, 28.25%; M.W. 792.8).

The later eluates, containing sugar moiety, were decolorized with active charcoal, and evaporated to yield colorless oil (4 mg), $\nu_{max}^{CCl_4}$ cm^{-1}: 3450, 1730, 1100, 1040, 1020. Acid hydrolysate of this compound revealed three sugar spots, corresponding to rhodosamine, 2-deoxyfucose and cinerulose A; Rf 0.12, 0.56 and 0.82, respectively, on silica gel thin-layer.

Reductive cleavage of A1 by rat microsomal NADPH-cytochrome P450 reductase (EC 1.6.2.4.):

To a solution of A1 (400 mg) in 5 mM acetic acid (10 ml) were added 500 ml of 0.1 M Tris-HCl, pH 8.0, 10 mg of purified enzyme from the microsomal fraction of rat liver and 80 mg of NADPH. The reaction was carried out at 37°C for 2 hours under nitrogen. After adding a chloroform - methanol mixture (1: 1) 500 ml, the chloroform layer was evaporated to dryness and purified by preparative TLC with chloroform - methanol (50: 1). The product was separated into two bands, the faster was identified with C1 (17 mg) and the slower was E1 (38 mg).

Partial methanolysis of B1 and formation of methyl glycoside (V):

A solution of B1 (1.0 g) in dry acetone (100 ml) with 4 ml of 2 N methanolic hydrogen chloride was kept at room temperature for 30 minutes. After completing the reaction, the solution was neutralized with silver carbonate, filtered and evaporated to dryness. After extracting the residue several times with ether, the remaining insoluble material was washed vigorously with EDTA solution in the acetate buffer, and extracted with chloroform at pH 7.0. After concentration of the extract the residue was chromatographed on silica gel using chloroform - methanol (10: 1). The eluate was concentrated and precipitated with *n*-hexane to give 90 mg of 1-deoxypyrromycin.

The ether extract was evaporated and chromatographed on silica gel (65 g) using benzene - ethyl acetate (2 : 1). The eluate was concentrated to give colorless needles, which was recrystallized from cyclohexane and acetone, yielding methyl glycoside (**V**) (139 mg), mp. 129~130°C, $[\alpha]_D^{22}$ −232° (*c* 0.5, $CHCl_3$) (lit.[6] mp. 129~130°C, $[\alpha]_D$ −196° (*c* 0.46, $CHCl_3$)), ν_{max}^{KBr} cm^{-1}: 1730, PMR ($CDCl_3$) δ in ppm: 1.29 (3H, d, J=6.5, H-6′), 1.40 (3H, d, J=6.5, H-6), 1.75 (1H, m, J=1, 5.5, 13, H_A-2), 2.38 (1H, dd, J=3.5, 13, H_B-2), 2.59 (2H, d, J=3, H-3′), 3.35 (3H, s, OMe), 3.92 (1H, dq, J=1.5, 6.5, H-5), 3.98 (1H, bs, H-4), 4.1~4.3 (1H, m, H-3), 4.37 (1H, q, J=3, H-2′), 4.75 (1H, q, J=6.5, H-5′), 4.9 (1H, dd, J=1, 4, H-1), 5.2 (1H, d, J=3, H-1′).

Reduction of methyl glycoside (**V**):

Compound **V** (210 mg) was dissolved in 30 ml of dry ether, was dropped into 60 mg of lithium aluminum hydride in dry ether (45 ml), and was stirred at room temperature for 15 minutes. The excess of reagent was decomposed with wet ether and the solution was filtered. The insoluble material was extracted with ether and ethyl acetate. The extracts were evaporated to give a residue (134 mg), which was purified by preparative silica gel TLC using ethyl acetate and on Sephadex LH-20 column with methanol to yield colorless needles (81 mg). Recrystallization from cyclohexane and acetone gave 63 mg of colorless needles (**VI**): mp. 133~136°C (lit.[6] mp. 133~135°C), $[\alpha]_D^{24}$ −67° (*c* 0.5, $CHCl_3$), ν_{max}^{KBr} cm^{-1}: 3500.

Partial methanolysis of M1:

To a solution of M1 (256 mg) in dry acetone (100 ml) was added 0.1 N methanolic hydrogen chloride (20 ml). The mixture was allowed to stand at 10°C for 35 minutes, and then neutralized with silver carbonate, filtered and evaporated. The residue was extracted several times with ether. The ether-insoluble material consisted mainly of 1-deoxypyrromycin. The ether extract was evaporated and the residue was chromatographed on silica gel (15 g) using benzene - ethyl acetate (3: 2). The eluate was concentrated to give a colorless powder, followed by recrystallization from cyclohexane and acetone to give 6.1 mg of colorless platelets (**II**): mp. 102.5~104°C, $[\alpha]_D^{23}$ −161° (*c* 1.0, $CHCl_3$), ν_{max}^{KBr} cm^{-1}: 3460, 3420, 1040, 1000.

Acetylation of methyl glycoside (**II**):

A solution of **II** (18.4 mg) in pyridine (0.5 ml) and acetic anhydride (0.4 ml) were allowed to stand at room temperature for 24 hours. The excess acetic anhydride was decomposed by pouring into cold sodium bicarbonate solution. The extract with chloroform was washed with water, dried over Na_2SO_4, concentrated to dryness and purified by preparative silica gel TLC with chloroform, followed by Sephadex LH-20 column chromatography with methanol. The eluate was concentrated to yield a colorless oil (**VII**), PMR ($CDCl_3$) δ in ppm: 1.14 (3H, d, J=6.5, H-6′), 1.21 (3H, d, J=6.5, H-6), 1.7~2.1 (4H, m, H-2′ and 3′), 2.07 (3H, s, OAc), 2.09 (3H, s, OAc), 2.18 (2H, dd, J=3.5, 12.5, H-2), 3.33 (3H, s, OMe), 3.83 (1H, bs, H-4), 3.93 (1H, dq, J=1, 6.5, H-5), 4.13 (1H, dq, J=6.5, 10.0, H-5′), 4.3~4.6 (1H, m, H-4′), 4.81 (1H, bs, H-1′), 4.83 (1H, bs, H-1), 5.18 (1H, 2×dd, J=3, 5, 12.5, H-3).

Partial hydrolysis of N1:

Purified N1 (1.0 g) in 0.5% hydrochloric acid (500 ml) was allowed to stand at room temperature for 1 hour. After neutralization with silver carbonate, the solution was filtered and extracted with chloroform. The extract was concentrated and precipitated with *n*-hexane, to yield 370 mg of S1, mp. 145~146°C, ν_{max}^{KBr} cm^{-1}: 1730, 1620, 1290, 1010.

The neutralized aqueous layer was concentrated and purified by preparative silica gel TLC with chloroform - methanol (3: 7) as a developing solvent and gel filtration on Sephadex LH-20 with methanol to yield L-rhodinose as a colorless oil (12 mg), $[\alpha]_D^{24}$ −10° (*c* 0.5, $CHCl_3$) (lit.[17] $[\alpha]_D^{20}$ −11°).

Chemical conversion of A1 to M1 and N1:

A solution of A1 (504 mg) in ethyl acetate (20 ml) was vigorously shaken with sodium borohydride (51 mg) in 20 ml of water at room temperature for 30 minutes. The solvent layer was washed with EDTA solution and water, and then evaporated to dryness under reduced pressure. The residue was purified by preparative silica gel TLC with chloroform - methanol (10: 1). The product was separated into two bands; the faster was identified with M1 and the slower was N1. Each eluate was

concentrated and precipitated with *n*-hexane to yield yellow powder; M1 (177 mg), mp. 149~150°C, ν_{max}^{KBr} cm^{-1}: 1740, 1680, 1620, 1010; N1 (22 mg), mp. 146~147°C, ν_{max}^{KBr} cm^{-1}: 1735, 1675, 1620, 1000.

Partial methanolysis of G1:

To a solution of G1 (950 mg) in dry acetone (100 ml) were added dry methanol (10 ml) and 0.1 N methanolic hydrogen chloride (20 ml). The mixture was allowed to stand at room temperature for 50 minutes with stirring, and then neutralized by addition of silver carbonate, filtered, and evaporated. The residue was extracted several times with ether. The ether-insoluble material consisted mainly of 1-deoxypyrromycin, while the ether extract was evaporated and chromatographed on silica gel (65 g) using benzene - ethyl acetate (6: 3.5). The eluate was concentrated to give a colorless powder which was recrystallized from benzene, yielding methyl glycoside (**VIII**) (55 mg) as colorless platelets: mp. 105~108°C, $[\alpha]_D^{24}$ −114° (*c* 0.5, $CHCl_3$), ν_{max}^{KBr} cm^{-1}: 3450, 1730, 1040, PMR ($CDCl_3$) δ in ppm: 1.26 (3H, d, J=6.5, H-6), 1.37 (3H, d, J=6.5, H-6′), 1.87 (2H, dd, J=2, 6.5, H-2), 2.34 (2H, m, H-2′), 2.55 (2H, m, H-3′), 3.33 (3H, s, OMe), 3.67 (1H, bs, J=1.5, H-4), 3.92 (1H, q, J=6.5, H-5), 3.92 (1H, m, H-3), 4.11 (1H, q, J=6.5, H-5′), 4.77 (1H, dd, J=2, 3, H-1), 4.90 (1H, dd, J=4.5, 6, H-1′).

Reduction of methyl glycoside (**VIII**):

Compound **VIII** (77 mg) was dissolved in 15 ml of dry ether, dropped into 30 mg of lithium aluminum hydride in dry ether (15 ml) and the mixture was stirred at room temperature for 15 minutes. The excess of reagent was decomposed with wet ether and the solution was filtered. The insoluble material was washed with ether and ethyl acetate. The combined extracts were evaporated and the residue (60 mg) was purified by preparative silica gel TLC with ethyl acetate and by Sephadex LH-20 column chromatography with methanol to yield 19.5 mg of colorless oil (**IX**), $[\alpha]_D^{24}$ −122° (*c* 0.5, $CHCl_3$), $\nu_{max}^{CCl_4}$ cm^{-1}: 3400, 1040, PMR ($CDCl_3$) (60 MHz) δ in ppm: 1.23 (3H, d, J=6.5), 1.31 (3H, d, J=6.0), 1.5~2.4 (6H, m, H-2, 2′ and 3′), 3.3 (3H, s, OMe), 3.4~4.1 (4H, m), 4.32 (1H, bs), 4.47 (1H, bs), 4.77 (1H, t, J=2).

Hydrolysis of the reduced methyl glycoside (**IX**)

A solution of **IX** (19 mg) in dry methanol (1 ml) with 0.1 ml of 4 N methanolic hydrogen chloride was kept at room temperature for 15 minutes and neutralized with silver carbonate. The filtrate was evaporated to yield a colorless mixture (15 mg), which was separated by preparative silica gel TLC with cyclohexane - acetone (3: 1) into methyl-α-D-amicetoside (**X**): colorless liquid, $[\alpha]_D^{24}$ +128° (*c* 0.1, $CHCl_3$) (lit.[5] Methyl-L-amicetoside: $[\alpha]_D$ −129° (*c* 0.44, $CHCl_3$)), $\nu_{max}^{CCl_4}$ cm^{-1}: 3400, 1130, 1055.

A mixture of two anomeric methyl glycosides from the above preparative thin-layer was further purified by preparative TLC with ethyl acetate to obtain 2.0 mg of methyl-2-deoxy-α-L-fucoside (**III**): $[\alpha]_D^{24}$ −72° (*c* 0.1, $CHCl_3$), $\nu_{max}^{CCl_4}$ cm^{-1}: 3400, 1040.

Partial methanolysis of Y1:

To a solution of Y1 (850 mg) in dry acetone (100 ml) were added dry methanol (10 ml) and 0.1 N methanolic hydrogen chloride (15 ml). The mixture was allowed to stand at room temperature for 45 minutes with stirring, and then neutralized with silver carbonate, filtered and evaporated. The residue was extracted several times with ether. The insoluble material consisted mainly of 1-deoxypyrromycin, and the ether extract was evaporated and chromatographed on silica gel (60 g) column using benzene - ethyl acetate (2: 1). The first fraction contained 66 mg of methyl glycoside (**V**); mp 128~130°C, $[\alpha]_D^{24}$ −230° (*c* 0.5, $CHCl_3$), ν_{max}^{KBr} cm^{-1}: 1730, and successive eluates containing methyl glycoside (**XI**) (42 mg) were collected. Recrystallization from benzene to give colorless needles, mp. 109~110°C, $[\alpha]_D^{22}$ −65° (*c* 1.0, $CHCl_3$), λ_{max}^{MeOH} nm (ε): 209 (6726), ν_{max}^{KBr} cm^{-1}: 1680, PMR ($CDCl_3$) δ in ppm: 1.26 (3H, d, J=6.8, H-6), 1.4 (3H, d, J=6.8, H-6′), 1.8~2.1 (2H, m, H-2), 3.32 (3H, s, OMe), 3.74 (1H, bs, H-4), 3.94 (1H, dq, J=6.8, 1, H-5), 4.0 (1H, m, H-3), 4.73 (1H, q, J=6.8, H-5′), 4.8 (1H, bs, H-1), 5.26 (1H, d, J=3.5, H-1′), 6.11 (1H, d, J=10.0, H-3′), 6.86 (1H, dd, J=3.5, 10.0, H-2′), (Found: C, 57.77; H, 7.31, Calc. for $C_{13}H_{20}O_6$: C, 57.34; H, 7.40; O, 35.26%).

Reduction of Y1-methyl glycoside (**XI**):

Compound **XI** (31 mg) obtained above was dissolved in 5 ml of methanol added to 20 mg of 5% palladium on charcoal in methanol (5 ml), and hydrogenated at room temperature under atmospheric

pressure. After 1.5 hours, the catalyst was removed by filtration and the filtrate was evaporated to dryness, followed by Sephadex LH-20 column chromatography using methanol. The eluate was concentrated to yield a colorless oil (**I**) (8 mg), $[\alpha]_D^{24}$ −297° (*c* 0.5, $CHCl_3$), $\nu_{max}^{CCl_4}$ cm^{-1}: 3450, 1730, 1120, 1100, 1040.

Partial methanolysis of S1:

To a solution of S1 (50 mg) in dry acetone (10 ml) were added dry methanol (0.5 ml) and 0.1 N methanolic hydrogen chloride (1 ml). The mixture was allowed to stand at room temperature for 30 minutes, and then neutralized with silver carbonate, filtered and evaporated. The residue was extracted several times with ether. The insoluble material consisted mainly of 1-deoxypyrromycin, and the ether extract was evaporated and purified by preparative silica gel TLC with ethyl acetate and on Sephadex LH-20 column with methanol to yield methyl-2-deoxy-L-fucoside (**III**) (2.3 mg) of colorless oil, $[\alpha]_D^{24}$ −108° (*c* 0.1, $CHCl_3$), $\nu_{max}^{CCl_4}$ cm^{-1}: 3400, 1040.

Hydrolysis of T1:

A solution of T1 (2.0 g) in 200 ml of 0.1 N hydrochloric acid was treated at 95°C for 1 hour. The yellow precipitate was filtered, chromatographed on silica gel (100 g) with chloroform - methanol (100: 1) and recrystallized from benzene to yield aklavinone (520 mg).

The filtrate from the above hydrolysate was treated with Amberlite IRA 400 (OH-form) resin to remove all the chloride ions and concentrated under reduced pressure. The residue was chromatographed on cellulose powder (100 g) using *n*-butanol saturated with water as a developing solvent. The eluate was evaporated under reduced pressure to yield an oily residue which was recrystallized from methanol to yield α,β-anomeric rhodosamine as colorless crystals (37 mg), mp. 138.5~139.5°C, $[\alpha]_D^{20}$ −46.7° (*c* 0.1, water) (lit.[13] L-rhodosamine hydrochloride, mp. 152~153°C, $[\alpha]_D^{20}$ −48.2° (water)), ν_{max}^{KBr} cm^{-1}: 3400, 2980, 2930, 2700, 1100, 1080, 1040, 980.

Partial hydrolysis of L1:

Concentrated hydrochloric acid (0.12 ml) was added to a solution of L1 (600 mg) in dry acetone (60 ml) and the mixture was stirred at room temperature for 40 minutes. The yellow precipitate was filtered, washed with dry acetone and recrystallized from methanol and acetone. N-Monomethyl daunosaminyl aklavinone hydrochloride (181 mg) was obtained as yellow needles, mp. 165~167°C, remelted at 217°C after solidified, ν_{max}^{KBr} cm^{-1}: 1735, 1620, λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 259 (425), 290 (177), 430 (217), PMR (CD_3OD) δ in ppm: 1.11 (3H, t, J=6.5, H-14), 1.33 (3H, d, J=6.5, H-6′), 1.63 (2H, q, J=6.5, H-13), 2.0~2.25 (2H, m, H-2′), 2.4 (2H, m, H-8), 2.67 (3H, s, N-Me), 3.74 (3H, s, OMe), 3.84 (1H, bs, H-4′), 4.08 (1H, s, H-10), 4.27 (1H, dq, J=1.5, 6.5, H-5′), 5.11 (1H, dd, J=2, 5, H-7), 5.5 (1H, dd, J=2, 3.5, H-1′), 7.17 (1H, dd, H-3), 7.43 (1H, s, H-11), 7.55 (1H, t, H-2), 7.6 (1H, dd, H-1), (Found: C, 58.19; H, 5.66; N, 2.27; O, 28.04; Cl, 6.05, Calc. for $C_{29}H_{33}NO_{10}\cdot HCl$: C, 58.83; H, 5.79; N, 2.37; O, 27.02; Cl, 5.99%).

Partial methanolysis of L1:

A solution of L1 (213 mg) in dry acetone (45 ml) and 0.1 N methanolic hydrogen chloride (9 ml) was allowed to stand at room temperature for 40 minutes, and then the mixture was neutralized, filtered and evaporated. The residue was extracted several times with ether, and the ether extract was evaporated and chromatographed on silica gel (10 g) using benzene - ethyl acetate (6: 3.5). The eluate was concentrated to yield methyl glycoside (**I**) (25 mg) as colorless oil, Rf 0.59 (on silica gel with ethyl acetate), $[\alpha]_D^{24}$ −290° (*c* 0.5, $CHCl_3$).

The ether-insoluble material was dissolved in 0.1 M acetate buffer (pH 3.0), EDTA solution was added to remove metals, and the solution was extracted with chloroform at pH 7.0. The extract was washed with water, dried over Na_2SO_4, concentrated to a yellowish brown residue, and purified by preparative silica gel TLC with chloroform - methanol (5: 1) and Sephadex LH-20 chromatograph with methanol. The eluate was concentrated and precipitated with chloroform and *n*-hexane to give N-methyl daunosaminyl aklavinone, 32 mg of yellowish brown powder, mp. 111~116°C, remelted at 217°C after solidified, ν_{max}^{KBr} cm^{-1}: 1735, 1620.

Photochemical transformation of A1 to L1:

A solution of A1 (5 g) in 250 ml of ethyl ether was exposed overnight to a germicidal lamp (National Germicidal Lamp, 15 W; distance: 30 cm) at room temperature, concentrated to dryness under reduced pressure, and purified by preparative silica gel TLC with chloroform - methanol (10: 1). The eluate was concentrated and precipitated with *n*-hexane to yield yellowish brown powder of L1 (178 mg), mp. 134~136°C, $[\alpha]_D^{24}$ −3.7° (*c* 1.0, $CHCl_3$), λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 230 (480), 259 (296), 290 (118), 433 (148), ν_{max}^{KBr} cm^{-1}: 1735, 1680, 1625, 1295, (Found: C, 61.83; H, 6.33; N, 1.74; O, 30.15, Calc. for $C_{41}H_{51}NO_{15}$: C, 61.72; H, 6.44; N, 1.76; O, 30.08%).

Photochemical transformation of A1 to K1:

A solution of A1 (8.0 g) in chloroform (400 ml) was allowed to stand at room temperature under UV lamp (as in the above section). After 2 days of irradiation, 0.1 M acetate buffer (pH 3.0) was added to the solution and shaken. The aqueous layer was adjusted to pH 7.0 and extracted with chloroform. The extract was evaporated and purified by preparative silica gel TLC with chloroform - methanol (10: 1). The eluate was concentrated and precipitated with *n*-hexane to yield a yellowish brown powder of K1 (440 mg), mp. 150~151°C, $[\alpha]_D^{23}$ −13° (*c* 0.5, $CHCl_3$), λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 229.5 (517), 258.5 (313), 290 (129), 434 (153), ν_{max}^{KBr} cm^{-1}: 1730, 1670, 1620, 1290, (Found: C, 61.53; H, 6.24; N, 1.70, Calc. for $C_{40}H_{49}NO_{15}$: C, 61.29; H, 6.30; N, 1.79; O, 30.62; M.W. 783.8).

L1 (1.0 g) in chloroform (100 ml) was irradiated under the same conditions as in A1, and 85 mg of K1 were obtained.

Partial hydrolysis of K1:

To a solution of K1 (550 mg) in acetone (55 ml) was added conc. hydrochloric acid (0.1 ml), and stirred at room temperature for 40 minutes. The yellow precipitate was filtered, washed with dry acetone and recrystallized from acetone and methanol. Daunosaminyl aklavinone hydrochloride (280 mg) was obtained as yellow needles, mp. 191~192°C, $[\alpha]_D^{24}$ +177° (*c* 0.5, MeOH), ν_{max}^{KBr} cm^{-1}: 3400, 1730, 1620, 1280, 1010, λ_{max}^{MeOH} nm (ε): 229.5 (38032), 259 (22831), 290 (8843), 432 (11560), PMR ($CDCl_3 + CD_3OD$) δ in ppm: 1.11 (3H, t, J=7.0, H-14), 1.33 (3H, d, J=6.5, H-6′), 1.55 (2H, q, J=7.0, H-13), 2.0 (2H, m, H-2′), 2.5 (2H, m, H-8), 3.4~3.65 (1H, m, H-3′), 3.74 (3H, s, OMe), 4.09 (1H, s, H-10), 4.24 (1H, q, J=6.5, H-5′), 5.15 (1H, dd, J=2, 5, H-7), 5.50 (1H, bs, H-1′), 7.24 (1H, dd, H-3), 7.55 (1H, s, H-11), 7.66 (1H, t, H-2), 7.69 (1H, dd, H-1), (Found: C, 57.50; H, 5.72; N, 2.17; O, 28.36; Cl, 6.05, Calc. for $C_{28}H_{31}NO_{10}\cdot HCl$: C, 58.18; H, 5.58; N, 2.42; O, 27.68; Cl, 6.13%).

Partial methanolysis of K1:

A solution of K1 (100 mg) in dry acetone (10 ml) and 0.1 N methanolic hydrogen chloride (2 ml) was held at room temperature for 45 minutes, neutralized by addition of silver carbonate and filtered, and then the filtrate was evaporated. The residue was extracted several times with ether. The ether extract was concentrated and purified by preparative TLC with ethyl acetate, following by Sephadex LH-20 column chromatography with methanol. The eluate was evaporated to give 17 mg of a colorless oil (I), Rf 0.59 (on silica gel with ethyl acetate), $[\alpha]_D^{24}$ −295° (*c* 0.5, $CHCl_3$).

The ether-insoluble material was dissolved in 0.1 M acetate buffer (pH 3.0) with added EDTA solution to remove metals, and extracted with chloroform at pH 7.0. The extract was washed with water, dried and concentrated to a yellowish brown residue, which was purified by preparative TLC with chloroform - methanol (5: 1). The eluate was concentrated and precipitated with *n*-hexane to yield 43 mg of daunosaminyl aklavinone, mp. 124~129°C, remelted at 230°C after solidified, ν_{max}^{KBr} cm^{-1}: 1735, 1620.

Acetylation of E1:

E1 (35 mg) was kept in a solution containing 3.5 ml of acetic anhydride and 7 ml of pyridine overnight at room temperature. The solution was poured onto a mixture of ice and 10% hydrochloric acid, and extracted with chloroform. The extract was washed with water, dried over Na_2SO_4 and evaporated to dryness under reduced pressure. Recrystallization from methanol gave pale yellow platelets (25 mg), mp. 263~267°C, ν_{max}^{KBr} cm^{-1}: 3530, 3450, 1785, 1775, 1735, 1680, PMR (dioxane-d_8) δ in ppm: 0.8 (3H, t, J=7.0, H-14), 1.27 (2H, q, J=7.0, H-13), 1.5~2.0 (2H, m, H-8), 2.35 (3H, s, OAc),

2.49 (3H, s, OAc), 3.79 (3H, s, OMe), 4.12 (1H, s, H-10), 4.0~4.3 (1H, m, H-7), 7.32 (1H, dd, J=2, 8, H-3), 7.71 (1H, t, J=8, H-2), 7.8 (1H, s, H-11), 8.12 (1H, dd, J=2, 8, H-1).

Acetylation of C1:

A solution of C1 (50 mg) in pyridine (10 ml) and acetic anhydride (5 ml) was allowed to stand overnight at room temperature and the reaction mixture was poured onto ice water and extracted with chloroform. The extract was washed with 10% hydrochloric acid and water, dried, and concentrated *in vacuo* to dryness. Recrystallization from benzene gave pale yellow needles (32 mg), mp. 186~190°C, ν_{max}^{KBr} cm^{-1}: 3450, 1780, 1740, 1680, PMR (dioxane-d_8) δ in ppm: 1.02 (3H, t, J=7, H-14), 1.55 (2H, q, J=7, H-13), 1.89 (2H, m, H-8), 2.38 (3H, s, OAc), 2.41 (3H, s, OAc), 2.79 (2H, m, H-7), 3.64 (3H, s, OMe), 3.97 (1H, s, H-10), 7.35 (1H, dd, J=2, 8, H-3), 7.7 (1H, t, J=7.5, 8, H-2), 7.92 (1H, s, H-11), 8.11 (1H, dd, J=2, 7.5, H-1).

Acknowledgements

This study was supported, in part, by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture of Japan, and by Contract (NO1-CM-57009) from the Division of Cancer Treatment, National Cancer Institute, U.S.A.

References

1) Oki, T.; Y. Matsuzawa, A. Yoshimoto, K. Numata, I. Kitamura, S. Hori, A. Takamatsu, H. Umezawa, M. Ishizuka, H. Naganawa, H. Suda, M. Hamada & T. Takeuchi: New antitumor antibiotics, aclacinomycins A and B. J. Antibiotics 28: 830~834, 1975
2) Oki, T.; N. Shibamoto, Y. Matsuzawa, T. Ogasawara, A. Yoshimoto, I. Kitamura, T. Inui, H. Naganawa, T. Takeuchi & H. Umezawa: Production of nineteen anthracyclic compounds by *Streptomyces galilaeus* MA144-M1. J. Antibiotics 30: 683~687, 1977
3) Oki, T.: New anthracycline antibiotics. Jap. J. Antibiotics 30 (Suppl.): S70~S84, 1977
4) Oki, T.; I. Kitamura, A. Yoshimoto, Y. Matsuzawa, N. Shibamoto, T. Ogasawara, T. Inui, A. Takamatsu, T. Takeuchi, T. Masuda, M. Hamada, H. Suda, M. Ishizuka, T. Sawa & H. Umezawa: Antitumor anthracycline antibiotics, aclacinomycin A and analogues. I. Taxonomy, production, isolation and physicochemical properties. J. Antibiotics 32: 791~800, 1979
5) Keller-Schierlein, W. & W. Richle: Metabolic products of microorganisms. LXXXVI. Structure of cinerubin A. Antimicr. Agents & Chemoth. -1970: 68~70, 1971
6) Richle, W.; E. K. Winkler, D. M. Hawley, M. Dobler & W. Keller-Schierlein: Stoffwechselprodukte von Mikroorganismen. 104. Die Struktur des Cinerubins B. Helv. Chim. Acta 55: 467~480, 1972
7) Gordon, J. J.; L. M. Jackman, W. D. Ollis & I. O. Sutherland: Aklavinone. Tetrahedron Lett. 1960-8: 28~34, 1960
8) Brockmann, Jr., H.; H. Budzikiewicz, C. Djerassi, H. Brockmann & J. Niemeyer: Das massenspektroskopische Fragmentierungsverhalten der Anthracyclinone. Chem. Ber. 98: 1260~1269, 1965
9) Brockmann, H. & W. Lenk: Über Actinomycetenfarbstoffe. VI. Pyrromycinone. Chem. Ber. 92: 1880~1903, 1959
10) Rinehart, Jr., K. L.; J. R. Beck, D. B. Borders & D. Krauss: Streptolydigin. III. Chromophore and structure. J. Amer. Chem. Soc. 85: 4038~4039, 1963
11) Stevens, C. L.; K. Nagarajan & T. H. Haskell: The structure of amicetin. J. Org. Chem. 27: 2991~3005, 1962
12) Arcamone, F.; C. Franceschi, S. Penco & A. Selva: Adriamycin (14-hydroxydaunomycin), a novel antitumor antibiotic. Tetrahedron Lett. 1969-13: 1007~1010, 1969
13) Brockmann, H.; E. Spohler & T. Waehnelt: Rhodosamin, Isolierung, Konstitution und Konfiguration. Chem. Ber. 96: 2925~2936, 1963
14) Iselin, B. & T. Reichstein: 2-Deoxy-1-fucose. Helv. Chim. Acta 27: 1200~1203, 1944
15) Keller-Schierlein, W. & W. Richle: Neuartige Zucker aus Anthracyclin-Antibiotika. Chimia 24: 35~36, 1970
16) Stevens, C. L.; P. Blumbergs & D. L. Wood: Stereochemical identification and synthesis of amicetose and the stereochemical identification of rhodinose and the sugar from streptolydigin. J. Am. Chem. Soc. 86: 3592~3594, 1964
17) Brockmann, H. & T. Waehnelt: Rhodinose, eine Tridesoxyhexose. Naturwissenschaften 50: 43, 1963
18) Yoshimoto, A.; T. Ogasawara, I. Kitamura, T. Oki, T. Inui, T. Takeuchi & H. Umezawa: Enzymatic

conversion of aclacinomycin A to Y by a specific oxidoreductase in *Streptomyces*. J. Antibiotics 32: 472～481, 1979

19) ARCAMONE, F.; G. GASSINELLI, P. OREZZI, G. FRANCESCHI & R. MONDELLI: Daunomycin. II. The structure and stereochemistry of daunosamine. J. Am. Chem. Soc. 86: 5335～5336, 1964
20) OKI, T.; T. KOMIYAMA, H. TONE, T. INUI, T. TAKEUCHI & H. UMEZAWA: Reductive cleavage of anthracycline glycosides by microsomal NADPH cytochrome C reductase. J. Antibiotics 30: 613～615, 1977
21) STRELITZ, F.; H. FLON, U. WEISS & I. N. ASHESOV: Aklavin, an antibiotic substance with antiphage activity. J. Bact. 72: 90～94, 1956
22) KUMAR, V.; W. A. REMERS & R. GRULICH: The structure of aklavin. J. Antibiotics 30: 881～882, 1977
23) KARPLUS, M.: Vicinal proton coupling in nuclear magnetic resonance. J. Am. Chem. Soc. 85: 2870～2871, 1963
24) BROCKMANN, H.; H. BROCKMANN, Jr. & J. NIEMEYER: Die absolute Konfiguration der Anthracyclinone. Tetrahedron Lett. 1968-45: 4719～4724, 1968
25) ECKARDT, K.; D. TRESSELT & J. TAX: Antibiotika aus Actinomyceten. Isolierung und Konstitution von Aklavinone-I und Aklavinone-II, zwei neuen Anthracyclinonen aus Fermentationsansätzen von *Streptomyces galilaeus*. Tetrahedron 30: 3787～3791, 1974
26) ACHMATOWICZ, Jr., O.; P. BUKOWSKI, B. SZECHNER, Z. ZWIERZXCHOWSKA & A. ZAMOJSKI: Synthesis of methyl 2,3-dideoxy-DL-*alk*-2-enopyranosides from fran compounds. A general approach to the total synthesis of monosaccharides. Tetrahedron 27: 1973～1996, 1971
27) PAULSEN, H. & W. KOEBERNICK: Synthese von verzweigten Zuckern durch 1,4-Addition an Pyranosid-Enone. Carbohydr. Res. 56: 53～66, 1977
28) ECKARDT, K.: Rote Antibiotika aus Actinomyceten. Zur Identifizierung einer Reihe von Anthracyclinonpigmenten aus *Streptomyces galilaeus* Stamm JA 3034 (Galirubine und Galirubinone). Chem. Ber. 100: 2561～2568, 1967