THE ABSOLUTE STRUCTURES OF RUBEOMYCINS A AND A_1 (CARMINOMYCINS II AND III) AND RUBEOMYCINS B AND B_1 (4-HYDROXYBAUMYCINOLS A_1 AND A_2)

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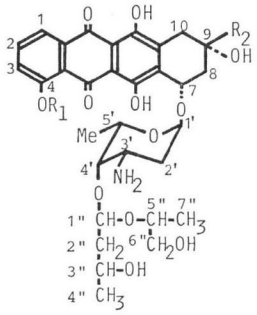
The absolute configurations of rubeomycins A and A_1 (corresponding to carminomycins II and III) and rubeomycins B and B_1 (corresponding to 4-hydroxybaumycinols A_1 and A_2), except at the C-1″ position, were determined by comparison of the optical rotations and other spectral data of rubeomycin derivatives with those of daunomycin and L-(+)-lactic acid.

We have previously reported the isolation, characterization and biological activities of rubeomycins A, A_1, B and B_1, and on the basis of their ^{13}C NMR and mass spectral data, the planar structures shown in Fig. 1 were assigned[1]. Rubeomycins were new anthracycline glycoside antibiotics with potent antitumor activity against P388 leukemia in mice, which were isolated from the fermented mycelial cake of a new actinomycete isolate, designated as *Actinomadura roseoviolacea* var. *biwakoensis* nov. var.

The related anthracycline antibiotics, baumycins A_1 and A_2[2~3], carminomycins II and III[4] (corresponding to rubeomycins A and A_1) and 4-hydroxybaumycinols A_1 and A_2[5] (corresponding to rubeomycins B and B_1) were reported at almost the same time as the rubeomycins. For these antibiotics, the each absolute configuration of the anthracyclinone and amino sugar moieties was confirmed or inferred from that of daunomycin[6~11], however the absolute structure of the acetal moiety was not established until recently.

In 1982, the absolute structures of the acetal moiety in carminomycins II and III were determined on the basis of the optical and spectral properties of the derived dinitrobenzoates from the degraded carminomycins, *i.e.*, carminomycin II; 3″(*S*), 5″(*R*): carminomycin III; 3″(*S*), 5″(*S*)[12]. These results suggested that carminomycin II corresponded to rubeomycin A and carminomycin III corresponded to rubeomycin A_1 respectively, by the comparison of the absolute structures of the acetal moiety with those for rubeomycins A and A_1, which were described in detail later.

Fig. 1. Planar structures of rubeomycins and related anthracyclines.



Rubeomycins A and A_1 (carminomycins II and III)
R_1=H, R_2=$COCH_3$
Rubeomycins B and B_1 (4-hydroxybaumycinols A_1 and A_2)
R_1=H, R_2=$CH(OH)CH_3$
Baumycins A_1 and A_2
R_1=CH_3, R_2=$COCH_3$

(Each pair of compounds are epimers with the respect to the carbon atom at the 1″, 3″ or 5″ position.)

It is important to determine the absolute structure of the acetal moiety in both the study of the biosynthesis and of the correlation of structure with antitumor activity.

In this paper, we wish to report the chemical degradation of rubeomycins in order to confirm the proposed planar structures, and to report determination of the absolute configuration of rubeomycins, except at the C-1″ position, by conversion to the derivative of daunomycin or those of L-(+)-lactic acid, whose absolute structures are already established.

Degradation of Rubeomycins A and A_1

Acid hydrolysis of rubeomycin A with 0.3 N sulfuric acid at 85°C for 30 minutes gave the aglycone as a precipitate (**1a**), which was identified as carminomycinone by comparison of their mass spectra[18], and the amino sugar (**2**) from the aqueous layer, was identified as daunosamine by the mass spectrum of the trimethylsilyl derivative. Mild acid hydrolysis of rubeomycin A with 0.3 N sulfuric acid at 25°C for 1.5 hours gave carminomycin I (**3a**), whose ^{13}C NMR spectrum agreed with the reported one[18].

Hydrogenolysis of rubeomycin A with palladium charcoal/barium sulfate afforded 7-deoxycarminomycinone (**4a**) and the sugar moiety. The latter product was treated with 0.3 N sulfuric acid to give daunosamine, and acetylated with acetic anhydride in pyridine to give the peracetyl sugar moiety, whose

Scheme 1. Degradations of rubeomycins.

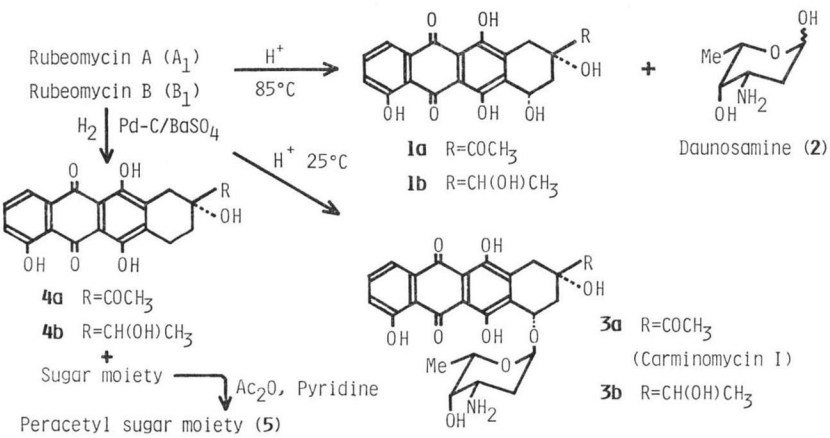
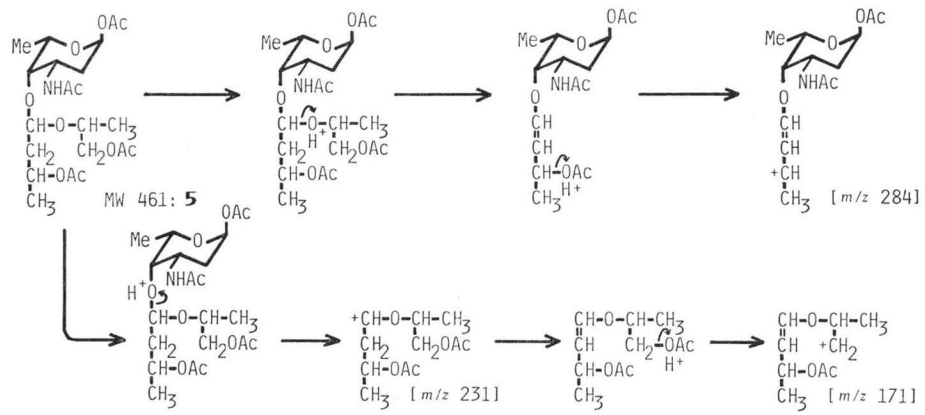


Fig. 2. Assigments of the mass fragments for peracetyl sugar moiety (**5**) by CI(NH_3)-MS: [ ]=found fragments.



structure was inferred as **5** from the mass spectral fragmentation data, see Fig. 2. These experimental results confirmed the proposed planar structure.

Degradation of rubeomycin A_1 by the same methods gave the same products as rubeomycin A in each case, therefore rubeomycin A_1 was the epimer of rubeomycin A at the sugar moiety (Scheme 1).

Degradation of Rubeomycins B and B_1

Rubeomycins B and B_1 were degraded respectively by the procedures described above. Acid hydrolysis of rubeomycin B gave 13-dihydrocarminomycinone (**1b**) and daunosamine (**2**), and its milder acid hydrolysis gave 13-dihydrocarminomycin I (**3b**). Hydrogenolysis of rubeomycin B afforded 7-deoxy-13-dihydrocarminomycinone (**4b**) and the sugar moiety. The latter product was identical with the sugar moiety of rubeomycin A. Accordingly these experimental results suggested that rubeomycin B was 13-dihydrorubeomycin A.

The degradation of rubeomycin B_1 by the same methods gave the same products as rubeomycin B in each case, then rubeomycin B_1 was the epimer of rubeomycin B at the sugar moiety (Scheme 1).

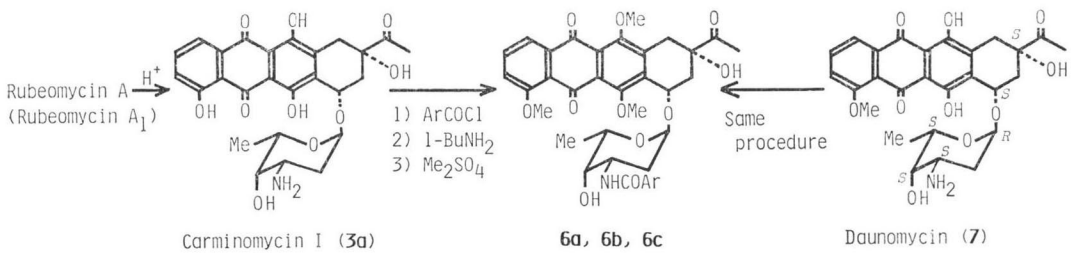
Absolute Configuration of Rubeomycins A and A_1

In order to determine the absolute configuration, rubeomycin A with nine asymmetric carbons, was divided into two parts, carminomycin I and the acetal moiety. Then each moiety was converted to the derivative of daunomycin or those of L-(+)-lactic acid (*S*-lactic acid), whose absolute structures are known[7,8].

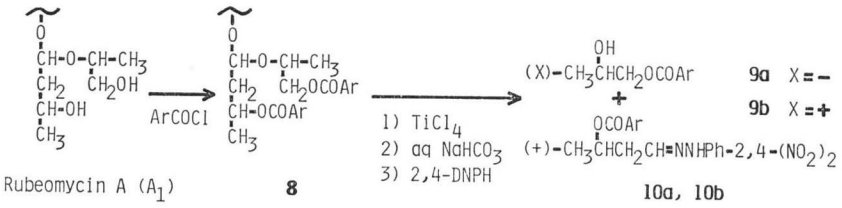
The carminomycin I (**3a**) derived from rubeomycin A with mild acid hydrolysis was treated with *p*-methoxybenzoyl chloride as a suspension of chloroform and saturated sodium bicarbonate at 0°C for 15 minutes, followed by quenching of excess *p*-methoxybenzoyl chloride with *n*-butylamine to give carminomycin *N*-*p*-methoxybenzoate, which was methylated with dimethyl sulfate and potassium carbonate in acetone under reflux for 2.5 hours to afford 4,6,11-*O*-trimethylcarminomycin *N*-*p*-methoxybenzoate (**6a**: $[\alpha]_D^{25}$ +45.3°, *c* 0.14, $CHCl_3$) in 81% overall yield from **3a**. **6a** was identified by direct comparison with the authentic sample (**6c**: $[\alpha]_D^{25}$ +45.7°, *c* 0.12, $CHCl_3$), which was synthesized from daunomycin (**7**) by the same procedures (Scheme 2). Accordingly, it was confirmed that the absolute configuration of the carminomycin I moiety in rubeomycin A was the same as that of daunomycin (**7**).

Rubeomycin A was treated with *p*-methoxybenzoyl chloride in pyridine and chloroform at 50°C for 48 hours to give hexakis(*p*-methoxybenzoyl)rubeomycin A (**8**) in 65% yield. Hydrolysis of **8** with mineral acid in aqueous THF even at the lowest temperature that the reaction proceeded, gave the corresponding alcohol, and crotonaldehyde instead of 3-*p*-methoxybenzoyloxybutanal. However, cleavage of the acetal group in **8** was accomplished by exposure to titanium tetrachloride[14] in methylene chloride

Scheme 2. Conversion of rubeomycin A (A_1) to 6,11-*O*-dimethyldaunomycin *N*-*p*-methoxybenzoate; Determination of the absolute configuration in carminomycin I moiety: (Ar=Ph-OMe-*p*).



Scheme 3. Cleavage of the acetal moiety in rubeomycin A (A_1) by titanium tetrachloride; Determination of the absolute configuration at C-3″ and C-5″ position: (Ar=Ph-OMe-*p*).

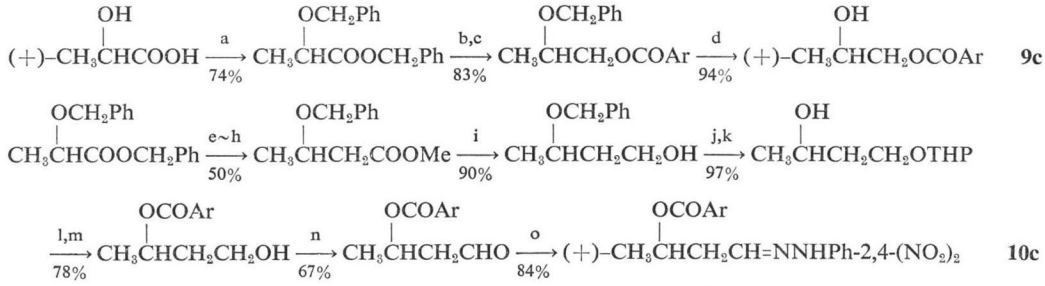


at −40°C for 45 minutes, followed by hydrolysis with saturated sodium bicarbonate at 0°C to afford the corresponding alcohol and aldehyde, and then, as the latter was unstable, the crude mixture was immediately treated with 2,4-dinitrophenylhydrazine to give 1-*p*-methoxybenzoyloxy-2-propanol (**9a**: $[\alpha]_D^{25}$ −18.1°, *c* 0.73, $CHCl_3$) in 87% yield and 2,4-dinitrophenylhydrazone of 3-*p*-methoxybenzoyloxybutanal (**10a**: $[\alpha]_D^{25}$ +93.9°, *c* 0.60, $CHCl_3$) in 84% yield (Scheme 3).

The ^1H NMR and IR spectra of **9a** and **10a** were consistent with those of the authentic samples (**9c** and **10c**) respectively, which were synthesized from L-(+)-lactic acid as shown in Scheme 4. As far as the optical rotations are concerned, **9a** was levo-rotatory, whereas the authentic sample (**9c**: $[\alpha]_D^{25}$ +11.1°, *c* 1.17, $CHCl_3$) was dextro-rotatory, and **10a** was dextro-rotatory, the same as the authentic sample (**10c**: $[\alpha]_D^{25}$ +53.8°, *c* 0.90, $CHCl_3$). Accordingly, it was established that the absolute configuration at the C-3″ was *S* and that at the C-5″ was *R* in rubeomycin A.

The absolute configuration of rubeomycin A_1 was also determined by the same procedures. The 4,6,11-*O*-trimethylcarminomycin *N*-*p*-methoxybenzoate (**6b**: $[\alpha]_D^{25}$ +44.9°, *c* 0.12, $CHCl_3$) derived from rubeomycin A_1 was identified with **6c** and these optical rotations were the same. The 1-*p*-methoxybenzoyloxy-2-propanol (**9b**: $[\alpha]_D^{25}$ +15.7°, *c* 0.14, $CHCl_3$) and the 2,4-dinitrophenylhydrazone of 3-*p*-methoxybenzoyloxybutanal (**10b**: $[\alpha]_D^{25}$ +93.9°, *c* 0.19, $CHCl_3$) derived from rubeomycin A_1 were compared with the authentic samples (**9c** and **10c**) respectively, and both the optical rotations were dextro-rotatory. Accordingly, the absolute configuration of the carminomycin I moiety in rubeomycin A_1

Scheme 4. Syntheses of authentic samples (**9c** and **10c**) from L-(+)-lactic acid: (Ar=Ph-OMe-*p*).



a) Benzyl bromide, NaH, hexamethylphosphoric triamide, THF, reflux 2 hours; b) $LiAlH_4$, ether, 0°C 1.5 hours; c) ArCOCl, pyridine, chloroform, 0°C 1.5 hours; d) hydrogen, 5%-Pd/C, ether, room temperature 15 hours; e) aq KOH, MeOH, room temperature 2.5 hours; f) thionyl chloride, benzene, room temperature 18 hours; g) diazomethane, ether, 0°C 15 minutes, then room temperature 2 hours; h) silver oxide, MeOH, reflux 45 minutes; i) $LiAlH_4$, ether, 0°C 1 hour; j) Dihydropyrane, *p*-toluenesulfonic acid, methylene chloride, room temperature 1 hour; k) hydrogen; 5%-Pd/C, ether, room temperature 3 hours; l) ArCOCl, pyridine, chloroform, 30°C 3 hours; m) aq HCl, THF, room temperature 2 hours; n) chromium trioxide, pyridine, methylne chloride, room temperature 15 minutes; o) 2,4-Dinitrophenylhydrozine, sulfuric acid, EtOH, water, room temperature 15 minutes.

Table 1. The optical rotations and configuration of the derivatives from rubeomycins A and A_1, daunomycin and L-(+)-lactic acid.

	Derivatives	Optical rotations	Configuration
6a	(from rubeomycin A)	+45.3°	7(*S*), 9(*S*), 1′(*R*) 3′(*S*), 4′(*S*), 5′(*S*)
6b	(from rubeomycin A_1)	+44.9°	
6c	(from daunomycin)	+45.7°	
9a	(from rubeomycin A)	−18.1°	5″ (*R*)
9b	(from rubeomycin A_1)	+15.7°	5″ (*S*)
9c	(from lactic acid)	+11.1°	*S*
10a	(from rubeomycin A)	+93.9°	3″ (*S*)
10b	(from rubeomycin A_1)	+93.9°	3″ (*S*)
10c	(from lactic acid)	+53.8°	*S*

was also the same as that of daunomycin (**7**), and both the absolute configuration at the C-3″ and C-5″ were *S*.

Table 1 showed the optical rotations and configuration of these derivatives derived from rubeomycins A and A_1, daunomycin and L-(+)-lactic acid.

Absolute Configuration of Rubeomycins B and B_1

The absolute configuration at the C-13 position of rubeomycin B was determined by the NOE measurement between the C-14 methyl group and the C-8 or C-10 methylene group, after the conformation of the hydroxyethyl group was fixed.

Rubeomycin B was transformed to the 9,13-*O*-isopropylidene derivatives (**13** and **14**) shown in Scheme 5 by the following procedures: Rubeomycin B was treated with *p*-methoxybenzoyl chloride in a suspension of chloroform and saturated sodium bicarbonate at 0°C for 15 minutes, followed by quenching of excess *p*-methoxybenzoyl chloride with *n*-butylamine to give rubeomycin B *N*-*p*-methoxybenzoate, which was mildly hydrolyzed with 0.3 N hydrochloric acid in THF at 25°C for 3 hours to afford 13-dihydrocarminomycin *N*-*p*-methoxybenzoate. Treatment of this diol with 2,2-dimethoxypropane and

Scheme 5. Conversion of rubeomycin B for NOE measurements; Determination of the absolute configuration at C-13 position: (Ar=Ph-OMe-*p*).

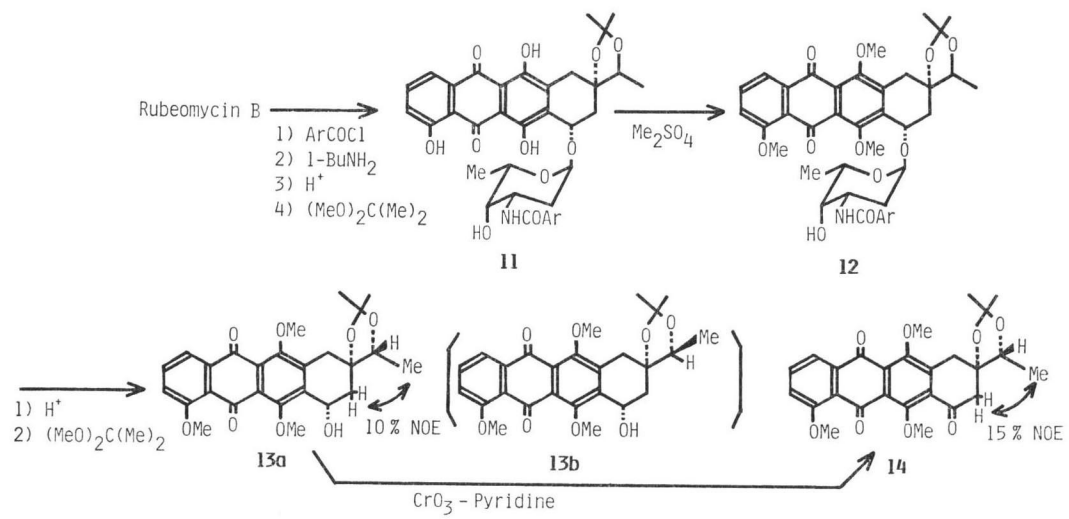
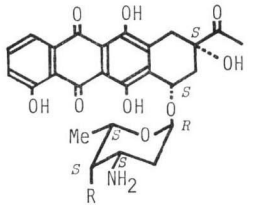
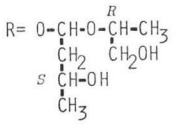


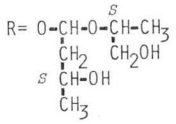
Fig. 3. Absolute structures of rubeomycins.

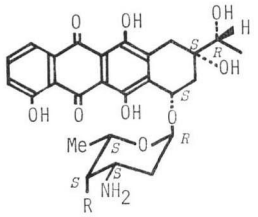


Rubeomycin A

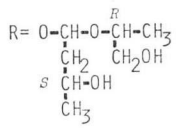


Rubeomycin A_1

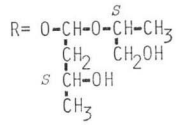




Rubeomycin B



Rubeomycin B_1



pyridinium *p*-toluenesulfonate in chloroform at 0°C for 20 minutes gave 13-dihydro-9,13-*O*-isopropylidenecarminomycin *N*-*p*-methoxybenzoate (**11**) in 86% overall yield from rubeomycin B. **11** was methylated with dimethyl sulfate and potassium carbonate in acetone under reflux for 2 hours to give 13-dihydro-9,13-*O*-isopropylidene-4,6,11-*O*-trimethylcarminomycin *N*-*p*-methoxybenzoate (**12**) in 68% yield. **12** was hydrolyzed with 3.0 N hydrochloric acid in THF at 50°C for 1.5 hours to afford 13-dihydro-4,6,11-*O*-trimethylcarminomycinone, followed by treatment with 2,2-dimethoxypropane and *p*-toluenesulfonic acid in chloroform at 0°C for 10 minutes to give 13-dihydro-9,13-*O*-isopropylidene-4,6,11-*O*-trimethylcarminomycinone (**13**) in 91% overall yield from **12**.

In the NOE measurement of **13**, 10% NOE was observed between the C-14 methyl group and the C-8 methylene group, but no NOE was observed between the C-14 methyl group and C-10 methylene group. Accordingly, it was elucidated that the structure of **13** was not **13b** but **13a**, then the absolute configuration at the C-13 position of rubeomycin B was *R*.

To confirm the absolute configuration, **13** was transformed to 7-dehydro-13-dihydro-9,13-*O*-isopropylidene-4,6,11-*O*-trimethylcarminomycinone (**14**) in 81% yield by the oxidation with chromium trioxide-pyridine complex in methylene chloride.

In the NOE measurement of **14**, 15% NOE was observed between the C-14 methyl group and the C-8 methylene group, but no NOE was also observed between the C-14 methyl group and the C-10 methylene group.

In conclusion, the absolute configuration of rubeomycins were determined respectively as shown in Fig. 3, *i.e.*, rubeomycin A; 7(*S*), 9(*S*), 1′(*R*), 3′(*S*), 4′(*S*), 5′(*S*), 3″(*S*), 5″(*R*): rubeomycin A_1; 7(*S*), 9(*S*), 1′(*R*), 3′(*S*), 4′(*S*), 5′(*S*), 3″(*S*), 5″(*S*): rubeomycin B; 7(*S*), 9(*S*), 13(*R*), 1′(*R*), 3′(*S*), 4′(*S*), 5′(*S*), 3″(*S*), 5″(*R*): rubeomycin B_1; 7(*S*), 9(*S*), 13(*R*), 1′(*R*), 3′(*S*), 4′(*S*), 5′(*S*), 3″(*S*), 5″(*S*). The elucidation of the absolute configuration at C-1″ position is the subject of further studies.

Experimental

Melting points were determined with a Yanagimoto micro melting point apparatus, type MP-S3, and were not corrected. IR absorption spectra were measured with a Jasco A-102 spectrometer, and the position of the absorption bands are reported in wavenumber (cm^{-1}). NMR spectra were recorded on

a Jeol JNM-PMX-60 spectrometer and a Jeol JNM-FX-60 spectrometer. Chemical shifts are given in δ values with tetramethylsilane as an internal standard. Abbreviation: s singlet, d doublet, t triplet, q quartet and m multiplet. Mass spectra were obtained with a Shimadzu GCMS-6020 spectrometer and a Shimadzu GCMS-LKB-9000 spectrometer. Optical rotations were determined with a Jasco DIP-4 automatic polarimeter.

Preparative TLC was conducted on 20×20 cm glass plates coated in this laboratory with a 0.6~0.7 mm thickness of Silica Gel GF_{254} (E. Merck Co.). Column chromatography was carried with Silica Gel 60 (E. Merck Co.) and Sephadex LH-20 (Pharmacia Fine Chemical Co.).

Acid Hydrolysis of Rubeomycin A

Rubeomycin A (10 mg, 0.015 mmol) was dissolved in 0.3 N sulfuric acid (1.0 ml), and the mixture was stirred at 85°C for 30 minutes. After stirring, the mixture was extracted with chloroform. The extract was concentrated to give carminomycinone (**1a**, 2 mg, 0.005 mmol) as red needle crystals in 33% yield.

The acidic aqueous layer was neutralized with saturated barium hydroxide, and the neutral solution was concentrated, after centrifuging, to give crude daunosamine (**2**, 2 mg, 0.008 mmol) in 53% yield.

A solution of hexamethyldisilazane and trimethylchlorosilane in anhydrous pyridine (TMS-HT, Tokyo Kasei Co., Ltd.) (0.5 ml) was added to crude daunosamine, and the mixture was stored for 10 minutes. This solution was then submitted to GC mass spectral analysis.

Carminomycinone (**1a**): mp 206~210°C; MS *m/z* 384 (M^+), 366, 348, 323; MW 384.

Trimethylsilyl derivative of carminomycinone: MS *m/z* 729 (M^+-15), 714 (M^+-30), 701 (M^+-43), 611 ($M^+-43-90$); MW 744.

Trimethylsilyl derivative of daunosamine: MS *m/z* 363 (M^+), 348 (M^+-15); MW 363.

Mild Acid Hydrolysis of Rubeomycin A

Rubeomycin A (90 mg, 0.137 mmol) was dissolved in 0.3 N sulfuric acid (2.7 ml), and the mixture was stirred at 25°C for 1.5 hours. After stirring, the mixture was neutralized with dil sodium hydroxide, then extracted with chloroform. The extract was washed with saturated brine, dried and concentrated to afford crystals, which were purified by recrystallization from chloroform - ether to give carminomycin I (**3a**, 62 mg, 0.121 mmol) in 88% yield: mp 202.5~204.0°C; ^{13}C NMR ($CDCl_3$-AcOH) δ 16.6, 24.4, 29.6, 33.5, 35.3, 47.4, 67.1, 67.4, 69.6, 76.5, 100.1, 110.5, 111.3, 116.6, 119.6, 124.8, 133.4, 134.0, 136.9, 137.0, 156.6, 156.7, 162.7, 186.1, 190.5, 211.5; $[\alpha]_D^{25}$ +180° (*c* 0.048, $CHCl_3$).

Hydrogenolysis of Rubeomycin A

5% Palladium charcoal/ barium sulfate (300 mg) was added to a solution of rubeomycin A (100 mg, 0.152 mmol) in methanol (30 ml), and the mixture was stirred under hydrogen at room temperature for 30 minutes. After stirring, the mixture was filtered through Celite, and the filtrate was concentrated to afford crude products, which were purified by column chromatography (Sephadex LH-20, eluted with methanol) to give 7-deoxycarminomycinone (**4a**, 43 mg, 0.117 mmol) in 77% yield and the sugar moiety (30 mg, 0.102 mmol) in 67% yield.

TMS-HT (0.5 ml) was added to the former product (**4a**, *ca* 2 mg), and the mixture was stored for 10 minutes. This solution was then submitted to GC mass spectral analysis.

Pyridine (0.5 ml) and acetic anhydride (0.5 ml) were added to the latter product (20 mg, 0.068 mmol), and the mixture was stirred at room temperature for 20 hours. After stirring, the mixture was poured into saturated sodium bicarbonate, and then extracted with chloroform. The extract was washed with saturated brine, dried and concentrated to give the crude peracetyl sugar moiety (**5**, *ca* 35 mg), which was then submitted to GC mass spectral analysis (Fig. 2) without a further purification.

0.3 N Sulfuric acid (1.0 ml) was added to the latter product (*ca* 2 mg, 0.007 mmol), and the mixture was stirred at 25°C for 1.5 hours. After stirring, the mixture was neutralized with saturated barium hydroxide, and the neutral solution was concentrated, after centrifuging, to give crude daunosamine (**2**, *ca* 1 mg), which was identified by direct comparison with the authentic sample on TLC (BuOH - AcOH - H_2O, 4: 1: 1).

Trimethylsilyl derivative of 7-deoxycarminomycinone: MS *m/z* 641 (M^+-15); MW 656.

Acid Hydrolysis of Rubeomycin B

Rubeomycin B (10 mg, 0.015 mmol) was hydrolyzed by the procedure described above to give 13-dihydrocarminomycinone (**1b**, 2 mg, 0.005 mmol) in 33% yield, and crude daunosamine (**2**, 2 mg, 0.008 mmol) in 53% yield.

13-Dihydrocarminomycinone (**1b**): mp 198~203°C; MS *m/z* 386 (M^+), 368, 350, 323; MW 386.

Trimethylsilyl derivative of 13-dihydrocarminomycinone: MS *m/z* 803 (M^+-15), 788 (M^+-30), 701 (M^+-117), 611 ($M^+-117-90$); MW 818.

Trimethylsilyl derivative of daunosamine: MS *m/z* 363 (M^+), 348 (M^+-15); MW 363.

Mild Acid Hydrolysis of Rubeomycin B

Rubeomycin B (30 mg, 0.045 mmol) was mildly hydrolyzed by the procedure described above to give 13-dihydrocarminomycin I (**3b**, 19 mg, 0.037 mmol) in 82% yield: mp 220~223°C; ^{13}C NMR ($CDCl_3$ - AcOH) δ 16.3, 16.7, 28.6, 32.8, 34.6, 47.9, 66.6, 67.2, 70.0, 72.2, 73.5, 99.5, 110.4, 111.3, 116.1, 119.6, 124.7, 133.5, 134.8, 136.9, 138.2, 156.8, 157.2, 162.7, 186.1, 190.6.

Hydrogenolysis of Rubeomycin B

Rubeomycin B (50 mg, 0.076 mmol) was hydrogenated by the procedure described above to give 7-deoxy-13-dihydrocarminomycinone (**4b**, 20 mg, 0.054 mmol) in 71% yield, and the sugar moiety (15 mg, 0.051 mmol) in 67% yield. The latter product was identified with the authentic sample obtained from rubeomycin A by direct comparison of their TLC (BuOH - AcOH - H_2O, 4: 1: 1).

Trimethylsilyl derivative of 7-deoxy-13-dihydrocarminomycinone: MS *m/z* 715 (M^+-15); MW 730.

4,6,11-*O*-Trimethylcarminomycin *N*-*p*-Methoxybenzoate (**6a**) from **3a**

A suspension of the carminomycin I (**3a**, 14 mg, 0.027 mmol) derived from rubeomycin A in chloroform (2.7 ml) and saturated sodium bicarbonate (2.7 ml) was reacted with *p*-methoxybenzoyl chloride (93 mg, 0.545 mmol) at 0°C. After addition, the mixture was stirred at 0°C for 10 minutes, and at 25°C for 10 minutes, then *n*-butylamine (0.14 ml) was added, and the resulting mixture was stirred at 0°C for 10 minutes, and at 25°C for 10 minutes. After stirring, the mixture was acidified with dil hydrochloric acid (pH 3~4) and extracted with chloroform. The extract was washed with water and saturated brine, dried and concentrated to afford crude product, which was purified by preparative TLC ($CHCl_3$ - MeOH, 10: 1) to give carminomycin *N*-*p*-methoxybenzoate (17 mg, 0.026 mmol) in 96% yield: mp 185~189°C; ^1H NMR ($CDCl_3$) δ 1.30 (3H, d, J=7 Hz), 2.43 (3H, s), 3.77 (3H, s), 12.23 (1H, s), 13.47 (1H, s), 13.90 (1H, s).

Potassium carbonate (77 mg, 0.558 mmol) and dimethyl sulfate (53 mg, 0.421 mmol) were added to a solution of the above carminomycin *N*-*p*-methoxybenzoate (9 mg, 0.014 mmol) in acetone (2.8 ml), and the mixture was refluxed for 2.5 hours. After refluxing, the mixture was slightly acidified with dil hydrochloric acid at 0°C, and saturated sodium bicarbonate was added to it, then the mixture was stirred at room temperature for 30 minutes. After stirring, the mixture was extracted with chloroform, and the extract was washed with saturated brine, dried and concentrated to afford crude product, which was purified by preparative TLC ($CHCl_3$ - EtOEt - MeOH, 10: 10: 1) to give 4,6,11-*O*-trimethylcarminomycin *N*-*p*-methoxybenzoate (**6a**, 8 mg, 0.012 mmol) in 84% yield: mp 141~144°C; IR ($CHCl_3$) 3520, 3450, 3000, 2940, 1710, 1670, 1610, 1590, 1495, 1460, 1330, 1320, 1270, 1255, 1188, 1110, 1085, 1030, 1005, 980, 945, 840 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.33 (3H, d, J=7 Hz), 2.40 (3H, s), 3.77 (3H, s), 3.90 (3H, s), 3.93 (3H, s), 3.97 (3H, s), 5.28 (1H, m), 5.38 (1H, m), 6.70 (2H, d, J=9 Hz), 7.57 (2H, d, J=9 Hz); ^{13}C NMR ($CDCl_3$) δ 117.3 (C-1), 136.9 (C-2), 119.0 (C-3), 158.8 (C-4), 121.2 (C-4a), 182.8 (C-5), 126.5 (C-5a), 154.8 (C-6), 134.1 (C-6a), 70.3 (C-7), 35.8 (C-8), 77.8 (C-9, in CD_3COCD_3), 33.9 (C-10), 134.1 (C-10a), 154.4 (C-11), 123.6 (C-11a), 182.8 (C-12), 136.5 (C-12a), 211.8 (C-13), 24.5 (C-14), 99.9 (C-1′), 30.5 (C-2′), 46.0 (C-3′), 67.3 (C-4′), 69.7 (C-5′), 16.8 (C-6′), 56.6 (4-OMe), 63.2 (6-OMe), 61.8 (11-OMe), 55.3, 113.8, 126.7, 128.7, 162.3, 166.2 (*p*-methoxybenzoyl group); $[\alpha]_D^{25}$ +45.3° (*c* 0.14, $CHCl_3$).

6,11-*O*-Dimethyldaunomycin *N*-*p*-Methoxybenzoate (**6c**) from **7**

Daunomycin (**7**, 23 mg, 0.044 mmol) was treated with *p*-methoxybenzoyl chloride by the procedure described above to give daunomycin *N*-*p*-methoxybenzoate (27 mg, 0.041 mmol) in 94% yield: mp 200.5~202.0°C; ^1H NMR ($CDCl_3$) δ 1.32 (3H, d, J=7 Hz), 2.42 (3H, s), 3.80 (3H, s), 4.00 (3H, s), 13.47

(1H, s), 14.17 (1H, s).

The above daunomycin *N*-*p*-methoxybenzoate (11 mg, 0.017 mmol) was methylated with dimethyl sulfate by the procedure described above to give 6,11-*O*-dimethyldaunomycin *N*-*p*-methoxybenzoate (**6c**, 8 mg, 0.012 mmol) in 71% yield: mp 141~144°C; IR ($CHCl_3$), ^1H NMR ($CDCl_3$), ^{13}C NMR ($CDCl_3$) (perfectly consistent with those of **6a** respectively); $[\alpha]_D^{25}$ +45.7° (*c* 0.12, $CHCl_3$).

Hexakis(*p*-methoxybenzoyl)rubeomycin A (**8**) from Rubeomycin A

p-Methoxybenzoyl chloride (518 mg, 3.04 mmol) was added to a solution of rubeomycin A (40 mg, 0.061 mmol) in chloroform (1.2 ml) and pyridine (1.2 ml), and the mixture was stirred at 50°C for 48 hours. After stirring, *n*-butylamine was added to it, and the resulting mixture was neutralized with dil hydrochloric acid and extracted with chloroform. The extract was washed with water and saturated brine, dried and concentrated to afford crude product, which was purified by preparative TLC ($CHCl_3$ - EtOEt - MeOH, 40: 30: 1) to give hexakis(*p*-methoxybenzoyl)rubeomycin A (**8**, 58 mg, 0.040 mmol) in 65% yield: ^{13}C NMR ($CDCl_3$) δ 0.90~1.36 (3H×3, m), 2.17 (3H, s), 3.77 (3H, s), 3.80 (3H×4, s), 3.90 (3H, s), 6.50~7.20 (12H, m), 7.50~8.30 (15H, m).

Cleavage of the Acetal Group in **8** with Titanium Tetrachloride

Titanium tetrachloride (73 mg, 0.384 mmol) was added to a well stirred solution of hexakis(*p*-methoxybenzoyl)rubeomycin A (**8**, 56 mg, 0.038 mmol) in anhydrous methylene chloride (3.8 ml) at −40°C. The suspension was stirred at −40°C for 45 minutes, then poured into ice cold-aqueous sodium bicarbonate with vigorous stirring and extracted with methylene chloride. The extract was washed with saturated brine, dried and concentrated to afford crude products, which were dissolved in ethanol (1.0 ml) and chloroform (1.0 ml), and 0.1 N 2,4-dinitrophenylhydrazine in ethanol-water-sulfuric acid (3.8 ml) was added to the solution. After addition, the mixture was stirred at room temperature for 15 minutes, then extracted with methylene chloride. The extract was washed with saturated brine, dried and concentrated to afford crude products, which were purified by preparative TLC ($CHCl_3$ - EtOEt - C_6H_{14}, 1: 1: 1) to give 1-*p*-methoxybenzoyloxy-2-propanol (**9a**, 7 mg, 0.033 mmol) in 87% yield, and 2,4-dinitrophenylhydrazone of 3-*p*-methoxybenzoyloxybutanal (**10a**, 13 mg, 0.032 mmol) in 84% yield.

1-*p*-Methoxybenzoyloxy-2-propanol (**9a**): mp 58.0~58.5°C; IR ($CHCl_3$) 3620, 3480, 2980, 1710, 1610, 1515, 1280, 1260, 1175, 1105, 1035, 850 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.27 (3H, d, J=7 Hz), 2.30 (1H, s), 3.85 (3H, s), 4.00~4.40 (3H, m), 6.88 (2H, d, J=9 Hz), 7.95 (2H, d, J=9 Hz); $[\alpha]_D^{25}$ −18.1° (*c* 0.73, $CHCl_3$).

2,4-Dinitrophenylhydrazone of 3-*p*-methoxybenzoyloxybutanal (**10a**): mp 160.0~161.5°C; IR ($CHCl_3$) 3330, 3050, 1710, 1620, 1610, 1600, 1515, 1340, 1315, 1280, 1260, 1170, 1140, 1105, 1035, 925, 850, 835 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.47 (3H, d, J=7 Hz), 2.80 (2H, t, J=7 Hz), 3.83 (3H, s), 5.45 (1H, m), 6.83 (2H, d, J=9 Hz), 7.53 (1H, t, J=7 Hz), 7.77 (1H, d, J=9 Hz), 7.92 (2H, d, J=9 Hz), 8.17 (1H, d, d, J=9 Hz, 3 Hz), 8.98 (1H, *d*, J=3 Hz); $[\alpha]_D^{25}$ +93.9° (*c* 0.60, $CHCl_3$).

Synthesis of *S*-1-*p*-Methoxybenzoyloxy-2-propanol (**9c**) from *S*-Lactic Acid

Benzyl *S*-2-Benzyloxypropionate from *S*-Lactic Acid: A solution of *S*-lactic acid (2.99 g, 33.2 mmol) in anhydrous THF (5 ml) was added to a suspension of sodium hydride (1.6 g, 66.7 mmol) in anhydrous THF (12 ml) and hexamethylphosphoric triamide (17 ml) at room temperature over 10 minutes. After the mixture was refluxed for 15 minutes with stirring, benzyl bromide (14.2 g, 83.0 mmol) was added over 10 minutes. After addition, the mixture was refluxed for a further 2 hours, then cooled with an ice-water bath and neutralized with dil hydrochloric acid. The neutral mixture was extracted with chloroform, then the extract was washed with saturated brine, dried and concentrated to afford crude product, which was purified by column chromatography (silica gel, eluted with ether - hexane) to give benzyl *S*-2-benzyloxypropionate (6.64 g, 24.6 mmol) in 74% yield: IR ($CHCl_3$) 3050, 1740, 1500, 1450, 1370, 1270, 1190, 1140, 1060, 1020 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.43 (3H, d, J=7 Hz), 4.08 (1H, q, J=7 Hz), 4.38 (1H, d, J=12 Hz), 4.70 (1H, d, J=12 Hz), 5.18 (2H, s), 7.30 (5H, s), 7.33 (5H, s); $[\alpha]_D^{25}$ −40.7° (*c* 1.78, $CHCl_3$).

S-2-Benzyloxy-1-*p*-methoxybenzoyloxypropane from Benzyl *S*-2-Benzyloxypropionate: A solution of benzyl *S*-2-benzyloxypropionate (1.00 g, 3.70 mmol) in anhydrous ether (4.5 ml) was added to a suspension of lithium aluminum hydride (211 mg, 5.55 mmol) in anhydrous ether (14 ml) at 0°C over

10 minutes. After addition, the mixture was stirred for 1.5 hours and then excess lithium aluminum hydride was decomposed with wet ether. The resulting mixture was dried over anhydrous sodium sulfate, and filtered through Celite. The filtrate was concentrated to give crude *S*-2-benzyloxypropanol, which was dissolved in chloroform (8.0 ml) and pyridine (2.0 ml), then *p*-methoxybenzoyl chloride (820 mg, 4.81 mmol) was added to the solution. After addition, the mixture was stirred for 1.5 hours, then poured into saturated sodium bicarbonate. The resulting mixture was extracted with chloroform, and the extract was washed with saturated brine, dried and concentrated to afford crude product, which was purified by column chromatography (silica gel, eluted with ether - hexane) to give *S*-2-benzyloxy-1-*p*-methoxybenzoyloxypropane (925 mg, 3.08 mmol) in 83% overall yield: IR ($CHCl_3$) 3000, 1705, 1605, 1510, 1450, 1375, 1310, 1270, 1160, 1100, 1025, 840 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.30 (3H, d, J=7 Hz), 3.83 (3H, s), 3.90 (1H, m), 4.30 (2H, d, J=6 Hz), 4.62 (2H, s), 6.78 (2H, d, J=9 Hz), 7.27 (5H, s), 7.95 (2H, d, J=9 Hz).

S-1-*p*-Methoxybenzoyloxy-2-propanol from *S*-2-Benzyloxy-1-*p*-methoxybenzoyloxypropane: 5% Palladium charcoal (40 mg) was added to a solution of *S*-2-benzyloxy-1-*p*-methoxybenzoyloxypropane (395 mg, 1.32 mmol) in ether (13 ml), and the mixture was stirred under hydrogen at room temperature for 15 hours. After stirring, the mixture was filtered through Celite, and the filtrate was concentrated to afford crude product, which was purified by preparative TLC ($CHCl_3$ - EtOEt, 1: 1) to give *S*-1-*p*-methoxybenzoyloxy-2-propanol (**9c**, 261 mg, 1.24 mmol) in 94% yield: mp 56.0~57.0°C; IR ($CHCl_3$) 3620, 3480, 2980, 1710, 1610, 1515, 1280, 1260, 1175, 1105, 1035, 850 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.27 (3H, d, J=7 Hz), 2.30 (1H, s), 3.85 (3H, s), 4.00~4.40 (3H, m), 6.88 (2H, d, J=9 Hz), 7.95 (2H, d, J=9 Hz); $[\alpha]_D^{25}$ +11.1° (c 1.17, $CHCl_3$).

Synthesis of 2,4-Dinitrophenylhydrazone of *S*-3-*p*-Methoxybenzoyloxybutanal (**10c**) from *S*-Lactic Acid

Methyl *S*-3-Benzyloxybutyrate from Benzyl *S*-2-Benzyloxypropionate: 2.0 N Potassium hydroxide (30 ml) was added to a solution of benzyl *S*-2-benzyloxypropionate (3.24 g, 12 ml) in methanol (60 ml), and the mixture was stirred at room temperature for 2.5 hours. After stirring, the methanol was evaporated under reduced pressure, and water was added to the mixture. The resulting mixture was washed with chloroform, then acidified with dil hydrochloric acid and extracted with chloroform. The extract was washed with saturated brine, dried and concentrated to give crude *S*-2-benzyloxypropionic acid (2.2 g), which was dissolved in benzene (12 ml), then thionyl chloride (3.6 g, 30 mmol) was added to the solution at 0°C. The mixture was stirred at room temperature for 18 hours, and then concentrated to give crude *S*-2-benzyloxypropionyl chloride (*ca* 3 g), which was dissolved in ether (10 ml), and a solution of diazomethane (*ca* 1.5 g, prepared from *N*-nitrosomethylurea and potassium hydroxide) in ether (50 ml) at 0°C was added, then the mixture was stirred at 0°C for 15 minutes and at room temperature for 2 hours. After stirring, anhydrous methanol (55 ml), and a suspension of freshly prepared silver (I) oxide (530 mg, 2.28 mmol) in anhydrous methanol (10 ml) was added to the solution over 30 minutes. After addition, the mixture was stirred under reflux for 45 minutes, then filtered through Celite. The filtrate was concentrated to afford crude product, which was purified by column chromatography (silica gel, eluted with ether - hexane) to give methyl *S*-3-benzyloxybutyrate (1.25 g, 6.01 mmol) in 50% overall yield from benzyl *S*-2-benzyloxypropionate: ^1H NMR ($CDCl_3$) δ 1.27 (3H, d, J=7 Hz), 2.35 (1H, d, d, J=7 Hz, 15 Hz), 2.68 (1H, d, d, J=7 Hz, 15 Hz), 3.67 (3H, s), 4.00 (1H, m), 4.50 (2H, s), 7.23 (5H, s).

S-3-Benzyloxybutanol from Methyl *S*-3-Benzyloxybutyrate: A solution of methyl *S*-3-benzyloxybutyrate (1.19 g, 5.72 mmol) in anhydrous ether (5 ml) was added to a suspension of lithium aluminum hydride (326 mg, 8.58 mmol) in anhydrous ether (15 ml) at 0°C over 5 minutes. After addition, the mixture was stirred for 1 hour, then excess lithium aluminum hydride was decomposed with wet ether. Anhydrous sodium sulfate was added to the resulting mixture and the dried suspension was filtered through Celite. The filtrate was concentrated to afford crude product, which was purified by column chromatography (silica gel, eluted with ether - hexane) to give *S*-3-benzyloxybutanol (930 mg, 5.17 mmol) in 90% yield: IR ($CHCl_3$) 3480, 2950, 1445, 1375, 1340, 1140, 1110, 1075, 1020, 960 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.23 (3H, d, J=6 Hz), 1.75 (2H, d, t, J=6 Hz, 6 Hz), 2.48 (1H, t, J=5 Hz), 3.73 (3H, m), 4.43 (1H, d, J=11 Hz), 4.70 (1H, d, J=11 Hz), 7.28 (5H, s); $[\alpha]_D^{25}$ +40.2° (c 1.90, $CHCl_3$).

1-Tetrahydropyranyloxy-*S*-3-butanol from *S*-3-Benzyloxybutanol: Dihydropyrane (240 mg, 2.86

mmol) and *p*-toluenesulfonic acid (2.5 mg) were added to a solution of *S*-3-benzyloxybutanol (255 mg, 1.42 mmol) in methylene chloride (7.0 ml) at 0°C, then stirred for 10 minutes and at room temperature for a further 1 hour. After stirring, the mixture was poured into saturated sodium bicarbonate, then extracted with chloroform. The extract was washed with saturated brine, dried and concentrated to give crude *S*-3-benzyloxy-1-tetrahydropyranyloxybutane, which was dissolved in ether (7.0 ml), then 5% palladium charcoal (50 mg) was added to the solution. The mixture was stirred under hydrogen at room temperature for 3 hours, and then was filtered through Celite. The filtrate was concentrated to afford crude product, which was purified by column chromatography (silica gel, eluted with ether - hexane) to give 1-tetrahydropyranyloxy-*S*-3-butanol (240 mg, 1.38 mmol) in 97% overall yield: ^{1}H NMR ($CDCl_3$) δ 1.20 (3H, d, J=6 Hz), 1.40~1.87 (8H, m), 2.97 (1H, m), 3.33~5.17 (5H, m), 4.58 (1H, s).

S-3-*p*-Methoxybenzoyloxybutanol from 1-Tetrahydropyranyloxy-*S*-3-butanol: *p*-Methoxybenzoyl chloride (282 mg, 1.65 mmol) was added to a solution of 1-tetrahydropyranyloxy-*S*-3-butanol (240 mg, 1.38 mmol) in chloroform (7.0 ml) and pyridine (1.4 ml) at 0°C. After addition, the mixture was stirred at 30°C for 3 hours, then poured into saturated sodium bicarbonate. The resulting mixture was extracted with chloroform, and the extract was washed with saturated brine, dried and concentrated to give crude *S*-3-*p*-methoxybenzoyloxy-1-tetrahydropyranyloxybutane, which was dissolved in THF (16 ml), then 3.0 N hydrochloric acid (8 ml) was added to the solution. After addition, the mixture was stirred at room temperature for 2 hours, then poured into saturated sodium bicarbonate. The resulting mixture was extracted with chloroform, and the extract was washed with saturated brine, dried and concentrated to afford crude product, which was purified by column chromatography (silica gel, eluted with ether-hexane) to give *S*-3-*p*-methoxybenzoyloxybutanol (240 mg, 1.07 mmol) in 78% overall yield: ^{1}H NMR ($CDCl_3$) δ 1.37 (3H, d, J=7 Hz), 1.87 (2H, d, t, J=6 Hz, 6 Hz), 2.70 (1H, s), 3.65 (2H, t, J=6 Hz), 3.82 (3H, s), 5.30 (1H, m), 6.87 (2H, d, J=9 Hz), 7.92 (2H, d, J=9 Hz).

S-3-*p*-Methoxybenzoyloxybutanal from *S*-3-*p*-Methoxybenzoyloxybutanol: Chromium trioxide (386 mg, 3.86 mmol) was added to a solution of anhydrous pyridine (0.62 ml) in anhydrous methylene chloride (10 ml) at 0°C, and the mixture was stirred at room temperature for 15 minutes. At the end of this period, a solution of *S*-3-*p*-methoxybenzoyloxybutanol (144 mg, 0.643 mmol) in anhydrous methylene chloride (3.0 ml) was added in one portion. After stirring for an additional 1 hour at room temperature, the solution was decanted from the residue, and was washed with ether and chloroform. The combined organic solution was filtered through Celite and silica gel, and the filtrate was concentrated to give crude product, which was purified by preparative TLC (EtOEt - C_6H_{14}, 2: 1) to give *S*-3-*p*-methoxybenzoyloxybutanal (95 mg, 0.428 mmol) in 67% yield: ^{1}H NMR ($CDCl_3$) δ 1.43 (3H, d, J=7 Hz), 2.73 (2H, m), 3.80 (3H, s), 5.52 (1H, m), 6.82 (2H, d, J=9 Hz), 7.85 (2H, d, J=9 Hz), 9.70 (1H, t, J=2 Hz).

2,4-Dinitrophenylhydrazone of *S*-3-*p*-Methoxybenzoyloxybutanal: 0.1 N 2,4-Dinitrophenylhydrazine in ethanol - water - sulfuric acid (2.0 ml) was added to a solution of *S*-3-*p*-methoxybenzoyloxybutanal (24 mg, 0.108 mmol) in ethanol (1.0 ml), and the mixture was stirred at room temperature for 15 minutes. After stirring, the mixture was stored at 0°C, and the resulting crystals were collected to give 2,4-dinitrophenylhydrazone of *S*-3-*p*-methoxybenzoyloxybutanal (**10c**, 36 mg, 0.090 mmol) in 84% yield: mp 155.5~157.0°C; IR ($CHCl_3$) 3330, 3050, 1710, 1620, 1610, 1600, 1515, 1340, 1315, 1280, 1260, 1170, 1140, 1105, 1035, 925, 850, 835 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.47 (3H, d, J=7 Hz), 2.80 (2H, t, J=7 Hz), 3.83 (3H, s), 5.45 (1H, m), 6.83 (2H, d, J=9 Hz), 7.53 (1H, t, J=7 Hz), 7.77 (1H, d, J=9 Hz), 7.92 (2H, d, J=9 Hz), 8.17 (1H, d, d, J=9 Hz, 3 Hz), 8.98 (1H, d, J=3 Hz); $[\alpha]_D^{25}$ +53.8° (*c* 0.90, $CHCl_3$)

13-Dihydro-9,13-*O*-isopropylidenecarminomycin *N*-*p*-Methoxybenzoate (**11**) from Rubeomycin B

p-Methoxybenzoyl chloride (283 mg, 1.66 mmol) was added to a suspension of rubeomycin B (110 mg, 0.166 mmol) in chloroform (16 ml) and saturated sodium bicarbonate (16 ml) at 0°C. After addition, the mixture was stirred at 0°C for 15 minutes, then *n*-butylamine (0.8 ml) was added, and the resulting mixture was stirred at 0°C for 5 minutes, and at 25°C for 5 minutes. After stirring, the mixture was acidified with dil hydrochloric acid (pH 3~4) and extracted with chloroform. The extract was washed with water and saturated brine, dried and concentrated to give crude rubeomycin B *N*-*p*-methoxybenzoate, which was dissolved in THF (10 ml) and 0.3 N hydrochloric acid (5 ml) and the resulting mixture was stirred at 25°C for 3 hours. After stirring, the mixture was neutralized with dil sodium

hydroxide and acidified with dil hydrochloric acid (pH 3~4), then extracted with chloroform. The extract was washed with water and saturated brine, dried and concentrated to afford crude product, which was dissolved in a little chloroform, and the solution was poured into ether - hexane (1: 1) with stirring. The resultant precipitate was collected to give 13-dihydrocarminomycin *N*-*p*-methoxybenzoate (134 mg), which was dissolved in chloroform (1.7 ml) and 2,2-dimethoxypropane (173 mg, 1.66 mmol) and pyridinium *p*-toluenesulfonate (*ca* 1 mg) was added to the solution. After addition, the mixture was stirred at 20°C for 1 hour, then poured into saturated sodium bicarbonate and neutralized with dil hydrochloric acid. The neutral mixture was extracted with chloroform, and the extract was washed with saturated brine, dried and concentrated to afford crude product, which was purified by preparative TLC ($CHCl_3$ - MeOH, 10: 1) to give 13-dihydro-9,13-*O*-isopropylidenecarminomycin *N*-*p*-methoxybenzoate (**11**, 98 mg, 0.142 mmol) in 86% overall yield from rubeomycin B: ^{1}H NMR ($CDCl_3$) δ 1.20 (3H, d, J=7 Hz), 1.27 (3H, d, J=7 Hz), 1.42 (3H, s), 1.49 (3H, s), 3.80 (3H, s), 12.31 (1H, s), 13.03 (1H, s), 13.67 (1H, s).

13-Dihydro-9,13-*O*-isopropylidene-4,6,11-*O*-trimethylcarminomycin *N*-*p*-Methoxybenzoate (**12**) from **11**

Potassium carbonate (723 mg, 5.24 mmol) and dimethyl sulfate (495 mg, 3.93 mmol) were added to a solution of 13-dihydro-9,13-*O*-isopropylidenecarminomycin *N*-*p*-methoxybenzoate (**11**, 90 mg, 0.131 mmol) in acetone (6.5 ml), and the mixture was refluxed for 2 hours. After refluxing, the mixture was slightly acidified with dil hydrochloric acid at 0°C, and saturated sodium bicarbonate was added, then the mixture was stirred at room temperature for 30 minutes. After stirring, the mixture was extracted with chloroform, and the extract was washed with saturated brine, dried and concentrated to afford crude product, which was purified by preparative TLC ($CHCl_3$ - EtOEt - MeOH, 10: 10: 1) to give 13-dihydro-9,13-*O*-isopropylidene-4,6,11-*O*-trimethylcarminomycin *N*-*p*-methoxybenzoate (**12**, 65 mg, 0.089 mmol) in 68% yield: ^{1}H NMR ($CDCl_3$) δ 1.03 (3H, d, J=7 Hz), 1.27 (3H, d, J=7 Hz), 1.40 (3H, s), 1.50 (3H, s), 3.77 (3H, s), 3.93 (3H×3, s).

13-Dihydro-9,13-*O*-isopropylidene-4,6,11-*O*-trimethylcarminomycinone (**13**) from **12**

13-Dihydro-9,13-*O*-isopropylidene-4,6,11-*O*-trimethylcarminomycin *N*-*p*-methoxybenzoate (**12**, 41 mg, 0.056 mmol) was dissolved in THF (3.8 ml) and 3.0 N hydrochloric acid (1.9 ml), and the solution was stirred at 45°C for 1.5 hours. After stirring, the solution was neutralized with saturated sodium bicarbonate, then extracted with chloroform. The extract was washed with saturated brine, dried and concentrated to afford crude product, which was purified by preparative TLC ($CHCl_3$ - MeOH, 10: 1) to give 13-dihydro-4,6,11-*O*-trimethylcarminomycinone (22 mg, 0.051 mmol) in 92% yield: mp 183.0~184.5°C; ^{1}H NMR ($CDCl_3$) δ 1.30 (3H, d, J=7 Hz), 1.75 (1H, d, d, J=14 Hz, 5 Hz), 2.50 (1H, d, d, J=14 Hz, 4 Hz), 2.58 (1H, d, J=18 Hz), 3.25 (1H, d, J=18 Hz), 3.92 (3H, s), 4.02 (3H, s), 4.08 (3H, s), 5.33 (1H, m), 7.17~7.83 (3H, m).

2,2-Dimethoxypropane (63 mg, 0.606 mmol) and *p*-toluenesulfonic acid (*ca* 0.5 mg) were added to a solution of the above 13-dihydro-4,6,11-*O*-trimethylcarminomycinone (13 mg, 0.030 mmol) in chloroform (1.5 ml). After addition the mixture was stirred at 0°C for 10 minutes, then poured into saturated sodium bicarbonate and extracted with chloroform. The extract was washed with saturated brine, dried and concentrated to afford crude product, which was purified by preparative TLC ($CHCl_3$ - EtOEt, 1: 1) to give 13-dihydro-9,13-*O*-isopropylidene-4,6,11-*O*-trimethylcarminomycinone (**13**, 14 mg, 0.030 mmol) in 99% yield: ^{1}H NMR ($CDCl_3$) δ 1.35 (3H, d, J=7 Hz), 1.42 (3H×2, s), 1.73 (1H, m), 1.77~2.17 (2H, m), 2.48 (1H, d, J=18 Hz), 3.23 (1H, d, J=18 Hz), 3.88 (3H, s), 3.97 (3H, s), 4.07 (3H, s), 5.20 (1H, m), 7.20 (1H, d, d, J=8 Hz, 3 Hz), 7.57 (1H, t, J=8 Hz), 7.73 (1H, d, d, J=8 Hz, 3 Hz).

7-Dehydro-13-dihydro-9,13-*O*-isopropylidene-4,6,11-*O*-trimethylcarminomycinone (**14**) from **13**

Chromium trioxide (13 mg, 0.13 mmol) was added to a solution of anhydrous pyridine (*ca* 0.02 ml) in anhydrous methylene chloride (0.5 ml) at 0°C, and the mixture was stirred at room temperature for 15 minutes. At the end of this period, a solution of 13-dihydro-9,13-*O*-isopropylidene-4,6,11-*O*-trimethylcarminomycinone (**13**, 10 mg, 0.021 mmol) in anhydrous methylene chloride (0.5 ml) was added in one portion. After stirring for an additional 1 hour at room temperature, the solution was decanted from the residue, and was washed with ether and chloroform. The combined organic solution was filtered

through Celite and silica gel, and the filtrate was concentrated to give crude product, which was purified by preparative TLC ($CHCl_3$ - EtOEt, 1: 1) to give 7-dehydro-13-dihydro-9,13-*O*-isopropylidene-4,6,11-*O*-trimethylcarminomycinone (**14**, 8 mg, 0.017 mmol) in 81% yield: ^1H NMR ($CDCl_3$) δ 1.32 (3H, d, J=7 Hz), 1.37 (3H×2, s), 2.78 (2H, s), 2.80 (1H, d, J=18 Hz), 3.43 (1H, d, J=18 Hz), 3.95 (3H, s), 4.00 (3H, s), 4.08 (3H, s), 7.20 (1H, d, d, J=8 Hz, 3 Hz), 7.57 (1H, t, J=8 Hz), 7.73 (1H, d, d, J=8 Hz, 3 Hz).

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