NEW ANTHRACYCLINE METABOLITES FROM MUTANT STRAINS OF *STREPTOMYCES GALILAEUS* MA144-M1

II. STRUCTURE OF 2-HYDROXYAKLAVINONE AND NEW AKLAVINONE GLYCOSIDES

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Four blocked mutants of aclacinomycin-producing *Streptomyces galilaeus* MA144-M1 produced new anthracyclinones; 2-hydroxyaklavinone, its non-esterified analog and 2-hydroxy-7-deoxyaklavinone, several new anthracyclines; 2-deoxyfucosyl-2-deoxyfucosyl-rhodosaminyl-aklavinone (MA144 U1), 2-deoxyfucosyl-rhodosaminylaklavinone (MA144 U2) and five aklavinone glycosides devoid of amino sugar, designated as MA144 U5, U6, U7, U8 and U9.

In the study of the strain improvement on the production of aclacinomycins[1], we have obtained several mutant strains producing new anthracycline analogs, together with variants being capable of highly accumulating aclacinomycin A. The isolation and characterization of these mutants were described in the preceding paper[2]. The examination and evaluation of biological efficacy of new analogs are important for development of new drugs with improved antitumor activity as well as for study on the biosynthetic pathway of aclacinomycin A.

This paper deals with the structural elucidation of new anthracycline analogs which were produced by four mutant strains of *Streptomyces galilaeus* MA144-M1.

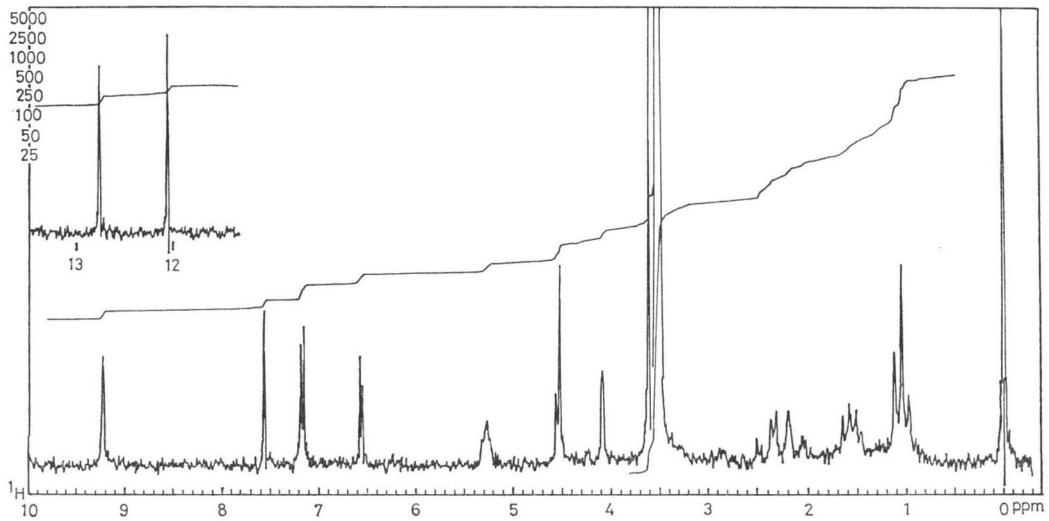
Results and Discussion

Novel Anthracyclinones 58C, 58D, and 58G

The mutant ANR-58 produced eight pigmental components and five of them could not be isolated due to the instability to autooxidation during purification process. 58C, D and G are non-glycosidic compounds and are isolated as brownish yellow crystals. 58D and C melted at 183°C and 245~247°C, and their visible spectra showed maxima at 440 and 438 nm, respectively. Their IR spectra resembled to those of aklavinone[3] and 7-deoxyaklavinone[4], respectively, in the presence of ester, hydrogen bonded and non-bonded carbonyls. Molecular formulas of 58D and C were $C_{22}H_{20}O_9$ and $C_{22}H_{20}O_8$ by elemental analysis and mass spectra (M^+ m/z 428 and 412), suggesting that 58D and C possess an additional oxygen atom in comparison with aklavinone and 7-deoxyaklavinone.

The PMR spectrum of 58D (Fig. 1) was superimposable on that of aklavinone in the high and middle fields. In the low field, it indicated the presence of one isolated hydroxyl (δ 9.22) in addition to two hydrogen bonded-phenolic hydroxyls (δ 12.05 and 12.76) and of three olefinic protons (δ 7.56,

Fig. 1. PMR spectrum of 2-hydroxyaklavinone (58D) in dioxane-d_8 (100 MHz).



7.12 and 6.56) missing one from aklavinone. The singlet at δ 7.56 was assigned to C-11 proton. The doublet peaks at δ 7.12 and 6.56 with small coupling constant were considered to be *meta* aromatic protons at C-1 and C-3. Therefore, the isolated peak at δ 9.22 is suggested to be the phenolic hydroxyl at C-2. The configuration of 58D was deduced as 7*S*, 9*R*, 10*R* from the results of spin decoupling experiments at C-7 and C-8 positions, according to the same manner as employed for the configurational determination of aklavinone. Thus, 58D was identified as 2-hydroxyaklavinone.

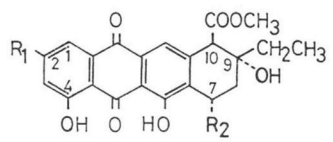
On the other hand, the PMR spectrum of 58C showed the presence of a methylene at C-7 in the vicinity of δ 2.7~3.1 instead of a methine at δ 5.28 in the spectrum of 58D. Therefore 58C was shown to be 2-hydroxy-7-deoxyaklavinone.

58G was an acidic aglycone with the molecular formula $C_{21}H_{14}O_8$ and melted at 154°C. In the IR spectrum, the ester carbonyl was missed and an additional chelated carbonyl at 1600 cm^{-1} was observed in comparison with those of 58D and C. Moreover, the UV absorption peak at 205 nm showed the presence of the α,β-unsaturated carbonyl. In the PMR spectrum, the protons at C-7 and C-8 were missed and the signal of ethyl methylene was shifted to downfield (δ 2.74). The signal of C-10 proton was also shifted to downfield (δ 5.89) and coupled with an olefinic proton at δ 6.70 in *meta* positions (J=2.0 Hz). These data allow to place the double bond between C-8 and C-9 and an α,β-unsaturated carbonyl at C-7. It is supported that 58G was esterified at C-10 with methyl iodide after protective acetylation of aromatic hydroxyls by the appearance of IR absorption peak at 1720 cm^{-1} and specific signal for the methoxyl (δ 3.88) in the PMR spectrum. From these analytical data, the structure of 58G was proposed as shown in Fig. 2.

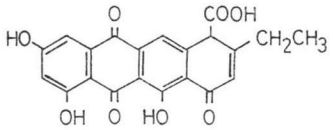
MA144 U1 and U2

Mutant 7U-491 produced two glycosidic compounds, designated as MA144 U1 and U2, which were yellow solids having elemental formulas $C_{42}H_{55}NO_{16}$ and $C_{36}H_{45}NO_{13}$, respectively. Acid hydrolysis of U1 and U2 with 0.1 N hydrochloric acid as described in the previous paper[5] gave aklavinone as aglycone and rhodosamine and 2-deoxyfucose as sugar residues. According to the same method as described before[5], mild alcoholysis gave aklavin (rhodosaminyl-aklavinone) and methyl-2-deoxyfucoside from both compounds, indicating that the structures of MA144 U1 and U2 are aklavin linked

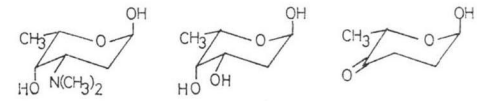
Fig. 2. Structure of metabolites obtained from mutant strains of *Streptomyces galilaeus* MA144-M1.

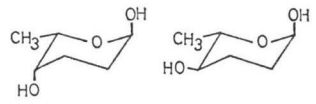


Compounds		R_1	R_2
Aglycone 58	C	OH	H
	D	OH	OH
Glycoside U	1	H	RN-dF-dF
	2	H	RN-dF
	5	H	dF-dF-C
	6	H	dF-R-R
	7	H	dF-dF-A
	8	H	dF-R
	9	H	dF-dF



Aglycone 58 G





with one or two molecules of 2-deoxyfucose. The PMR spectrum of U2 exhibited two anomeric protons at δ 5.0 and 5.52 with 6.0 and 5.5 Hz of half height width, indicating that the protons at C-1′ and C-1″ are equatorial. Thus, the structure of U2 was proposed as 2-deoxyfucosyl-rhodosaminylaklavinone. This compound is, therefore, identical to MA144 S1[5].

The PMR spectrum of U1 indicated the presence of an additional anomeric proton (α-anomer) as well as other signals attributable to the third sugar in the molecule. The CMR spectrum of U1 indicated the existence of the third sugar linked through the C-4″ carbon of 2-deoxyfucosyl residue, because of the glycosidation shift for the C-4″ carbon. These analytical data proved the structure of U1 as shown in Fig. 2.

MA144 U5, U6, U7, U8 and U9

This group of products were yellow aminosugar-lacking aklavinone glycosides. MA144 U5, U6, U7 and U8 were produced by the mutant 9U-653, and MA144 U9 was one of the major products of mutant 7N-1881 which accumulated MA144 U1 and U2. Their sugar compositions are summarized in Fig 2; that is, U5: 2-deoxyfucose and cinerulose A, U6 and U8: 2-deoxyfucose and rhodinose, U7: 2-deoxyfucose and amicetose, U9: 2-deoxyfucose. PMR spectra of U5, U6 and U7 exhibited three anomeric protons with small coupling constants, indicating the trisaccharide with α-configuration of glycosidic linkage, and U8 and U9 showed two anomeric protons with small coupling constants. Moreover, CMR spectra also revealed that U5, U6 and U7 have trisaccharide moiety, and U8 and U9 have disaccharide moiety.

Determination of sugar sequences was achieved by means of alcoholysis or mild hydrolysis[5] as follows:

U5: This was composed of aklavinone, 2-deoxyfucose and cinerulose A as mentioned above, but it was shown to have trisaccharide moiety from the PMR and CMR spectra. Mild methanolysis of U5 gave aklavinone and the methyl glycoside of cinerulosyl-2-deoxyfucoside which was identical to the authentic sample obtained from aclacinomycin A (aklavinone-rhodosamine-2-deoxyfucose-cinerulose A) in comparison with the PMR spectrum and Rf values on TLC (S5). From the results,

U5 was identified to be cinerulosyl-2-deoxyfucosyl-2-deoxyfucosylaklavinone[5].

U7: This was derived chemically from U5 by the hydration of carbonyl group at C-4 position of cinerulose A with $NaBH_4$. The treatment of U7 with methanolic hydrogen chloride gave aklavinone and the methylglycoside which was shown to be methyl amicetosyl-2-deoxyfucoside by comparison of the PMR and TLC with those obtained from an authentic sample of MA144 M1 (aklavinone-rhodosamine-2-deoxyfucose-amicetose). The mild hydrolysis of U7 released amicetose to produce MA144 U9 as described below, indicating that the terminal sugar of U7 is amicetose. Therefore, U7 was shown to be amicetosyl-2-deoxyfucosyl-2-deoxyfucosylaklavinone.

U6 and U8: Mild hydrolysis with 0.5% HCl at 20°C converted U6 to U8 by releasing rhodinose within a few minutes. The same treatment of U8 released rhodinose within 10 minutes and an additional 2-deoxyfucose within 60 minutes, successively. These results suggest that U6 and U8 are rhodinosyl-rhodinosyl-2-deoxyfucosylaklavinone and rhodinosyl-2-deoxyfucosylaklavinone, respectively.

U9: Acid hydrolysis of U9 gave only 2-deoxyfucose as the sugar moiety. However, the PMR and CMR spectra indicated the existence of disaccharide moiety. Thus, U9 is identical with 2-deoxyfucosyl-2-deoxyfucosylaklavinone.

Experimental

Thin-layer Chromatography

TLC was carried out on a silica gel $60F_{254}$ plate (E. Merck & Co.) using the solvent systems of chloroform - methanol (20: 1; S1 and/or 10: 1; S2, v/v), chloroform - methanol - acetic acid (100: 15: 0.5; S3, v/v/v), chloroform - methanol - ammonia water (100: 15: 0.2; S4, v/v/v) and ethyl acetate (S5). Detection of sugars was done by spraying TLC plate with the mixture of 5% *p*-anisaldehyde and 5% sulfuric acid in ethanol and heating at 90°C for 10 minutes.

Microorganisms and Cultivation

Mutant strains obtained from *S. galilaeus* MA144-M1 used in this experiment are strains ANR-58, 7U-491, 9U-653 and 7N-1881, and maintained on YS agar slant (0.3% yeast extract, 1.0% soluble starch, 1.5% agar, pH 7.2). Isolation procedure as well as the composition of fermentation medium and cultural conditions are described in the preceding paper[2]. For isolation of products, 10 liters of the cultured broth were prepared and the products were extracted from mycelia with about 3 liters of acetone. The re-extraction with about 1.5 liters of chloroform following the evaporation of the acetone layer yielded yellow oily or solid pigments.

Isolation and Purification of Products

58C, D and G: A crude material from the cultured broth of ANR-58 was dissolved in chloroform - methanol mixture (1: 2, v/v) and chromatographed on a Sephadex LH-20 column (ϕ4.2×33 cm) with the same solvent system. The faster eluted pigments were further purified by PLC (preparative layer chromatography) with S1 to yield 58C and D. The slower eluate containing 58G was evaporated and recrystallized from chloroform - methanol mixture.

58C: Recrystallized from acetone to yield 40 mg, m.p. 245~247°C, ν_{max}^{KBr} cm^{-1}: 1720, 1670, 1625, 1260. $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$): 226 (776), 274 (551), 438 (295). PMR ($CDCl_3$ - CD_3OD, 1: 1) δ in ppm: 1.09 (3H, t, J=7.0, CH_3-14), 1.64 (2H, q, J=7.0, CH_2-13), 1.8~3.1 (4H, m, CH_2-7, 8), 3.74 (3H, s, OCH_3), 3.95 (1H, s, H-10), 6.58 (1H, d, J=2.1, H-3), 7.18 (1H, d, J=2.1, H-1), 7.57 (1H, s, H-11). MS: m/z 412(M^+) (Found: C 64.01; H 4.91; O 31.05; Calcd. for $C_{22}H_{20}O_8$: C 64.08; H 4.89; O 31.03).

58D: Recrystallized from acetone to yield 174 mg, m.p. 183°C, ν_{max}^{KBr} cm^{-1}: 1730, 1680, 1625, 1255, $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$): 225 (755), 290 (450), 440 (281), PMR (dioxane-d_8) δ in ppm: 1.04 (3H, t, J=7.0, CH_3-14), 1.54 (2H, q, J=7.0, CH_2-13), 2.1 (1H, dd, J=2, 15, H_B-8), 2.42 (1H, dd, J=4.5, 15, H_A-8), 3.62 (3H, s, OCH_3), 4.10 (1H, s, H-10), 4.52 (1H, s, OH-9 exchanged with D_2O), 4.54 (1H, d, OH-7

exchanged with D_2O), 5.28 (1H, bs, H-7), 6.56 (1H, d, J=2.1, H-3), 7.12 (1H, d, J=2.1, H-1), 7.56 (1H, s, H-11), 9.22 (1H, s, OH-2, exchanged with D_2O), 12.05 and 12.76 (2H, s×2, OH-4 and 6, exchanged with D_2O), MS: m/z 428 (Found: C 61.65; H 4.68; O 33.72; Calcd. for $C_{22}H_{20}O_9$: C 61.68; H 4.71; O 33.61).

58G: Yield: 464 mg, m.p. 154°C, ν_{max}^{KBr} cm^{-1}: 1670, 1620, 1600, 1280, $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$): 205 (770), 222 (799), 290 (740), 442 (330), PMR (pyridine-d_5) δ in ppm: 1.22 (3H, t, J=7.0, CH_3-14), 2.74 (2H, q, J=7.0, CH_2-13), 5.89 (1H, d, J=2.0, H-10), 6.70 (1H, d, J=2.0, H-8), 6.91 (1H, d, J=2.1, H-3), 7.63 (1H, d, J=2.1, H-1), 7.82 (1H, s, H-11), MS: m/z 394 (Found: C 64.33; H 3.55; O 31.97; Calcd. for $C_{21}H_{14}O_8$; C 63.96; H 3.58; O 32.46).

U1 and U2: An oily material obtained from the cultured broth of strain 7U-491 was dissolved in chloroform and precipitated with n-hexane. The precipitate (530 mg) was chromatographed on a silica gel column (Wakogel C-200, ϕ 2×20 cm) using toluene - methanol (20: 1~10: 1) as the eluting solvent. U2 was eluted faster than U1. The fractions containing U1 or U2 were separately washed with 10^{-2} M EDTA solution, dried, evaporated to small volume and precipitated with n-hexane.

U1: Yield: 84 mg, m.p. 152~155°C, ν_{max}^{KBr} cm^{-1}: 1730, 1670, 1615, 1285, 1000, $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$): 431 (152), PMR ($CDCl_3$) δ in ppm: 1.08 (3H, t, J=7.5, CH_3-14), 1.12~1.3 (3H×3, d×3, CH_3-6′, 6″, 6‴), 1.5~2.6 (10H, m, CH_2-8, 13, 2′, 2″, 2‴), 2.18 (6H, s, N-$(CH_3)_2$), 3.70 (3H, s, OCH_3), 3.55 (1H, bs, H-4′), 4.10 (1H, s, H-10), 3.96~4.28 (m, H-5″, 5‴), 4.55 (1H, s, OH-9), 4.4~4.6 (1H, q, J=7.0, H-5′), 4.94 and 5.00 (2H, overlapped broad singlets, H-1′, 1‴), 5.25 (1H, bs, H-7), 5.50 (1H, bs, H-1′), 7.26 (1H, dd, J=1.5, 8.0, H-3), 7.64 (1H, s, H-11), 7.64 (1H, t, J=8.0, H-2), 7.79 (1H, dd, J=1.5, 8.0, H-1).

U2: Yield: 22 mg, m.p. 145~147°C, ν_{max}^{KBr} cm^{-1}: 1730, 1670, 1615, 1290, 1005, $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1cm}^{1\%}$): 431 (176), PMR ($CDCl_3$) δ in ppm: 1.09 (3H, t, J=7.5, CH_3-14), 1.20 (3H, d, J=7.0, CH_3-6′), 1.24 (3H, d, J=7.0, CH_3-6″), 1.4~2.5 (8H, m, CH_2-8, 13, 2′, 2″), 2.18 (6H, s, N$(CH_3)_2$), 3.69 (3H, s, OCH_3), 3.63 (1H, m, H-3′), 3.78 (1H, bs, H-4′), 4.10 (1H, s, H-10), 4.0 (1H, q, J=7.0, H-5″), 4.51 (1H, q, J=7.0, H-5′), 4.55 (1H, s, OH-9), 5.0 (1H, bs, H-1″), 5.25 (1H, bs, H-7), 5.50 (1H, bs, H-1′), 7.27 (1H, dd, H-3), 7.65 (1H, s, H-11), 7.65 (1H, t, H-2), 7.80 (1H, dd, H-1).

U5, U6, U7 and U8: A solid material obtained from the cultured broth of 9U-653 was chromatographed on a silica gel column (Wakogel C-200, ϕ 4×30 cm) using toluene - methanol (100: 2, v/v) as the eluting solvent. The mixture was separated into five fractions. The former two fractions contained bisanhydroaklavinone, 7-deoxyaklavinone and aklavinone. The third fraction was evaporated to dryness, and the residue was crystallized from ethyl acetate to yield 520 mg of U5 as yellow crystals. The 4th fraction was also crystallized from ethyl acetate to yield 137 mg of U6. The 5th fraction was further separated to U7 (Rf 0.63) and U8 (Rf 0.50) by PLC with solvent system S2. The precipitation with n-hexane gave 24 mg of U7 and 90 mg of U8.

U5: m.p. 155°C, ν_{max}^{KBr} cm^{-1}: 1730, 1675, 1620, 1290, 1010, λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 431 (141), PMR ($CDCl_3$) δ in ppm: 1.08 (3H, t, CH_3-14), 1.24, 1.28 and 1.32 (3H×3, d×3, CH_3-6′, 6″, 6‴), 1.5~2.5 (12H, m, CH_2-8, 13, 2′, 2″, 2‴, 3‴), 3.69 (3H, s, OCH_3), 3.59 (1H, s, H-4′), 3.75 (1H, s, H-4″), 4.10 (1H, s, H-10), 4.27 (1H, s, OH-9), 4.0~4.2 (2H, m, H-5″, 5‴), 4.48 (1H, q, J=7.0, H-5′), 4.96 (1H, bs, H-1″), 5.08 (1H, t, J=6, H-1‴), 5.22 (1H, bs, H-7), 5.48 (1H, bs, H-1′), 7.27 (1H, dd, H-3), 7.65 (1H, s, H-11), 7.56 (1H, t, H-2), 7.80 (1H, dd, H-1), 11.96 and 12.66 (1H×2, s×2, phenolic-OH).

U6: m.p. 150°C, ν_{max}^{KBr} cm^{-1}: 1735, 1675, 1620, 1295, 1005, λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 432 (151), PMR ($CDCl_3$) δ in ppm: 1.08 (3H, t, CH_3-14), 1.16~1.25 (3H×3, d×3, CH_3-6′, 6″, 6‴), 1.4~2.5 (14H, m, CH_2-8, 13, 2′, 2″, 3″, 2‴, 3‴), 3.50 (1H, bs, H-4′), 3.59 (1H, bs, H-4″), 3.69 (3H, s, OCH_3), 4.10 (1H, s, H-10), 3.85~4.3 (3H, m, H-5′, 5″, 5‴), 4.59 (1H, s, OH-9), 4.85 (1H, bs, H-1″), 4.95 (1H, bs, H-1‴), 5.29 (1H, bs, H-7), 5.42 (1H, bs, H-1′), 7.28 (1H, dd, H-3), 7.66 (1H, s, H-11), 7.66 (1H, t, H-2), 7.80 (1H, dd, H-1).

U7: m.p. 144~145°C, ν_{max}^{KBr} cm^{-1}: 1735, 1670, 1620, 1290, 1010, λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 431 (136), PMR ($CDCl_3$) δ in ppm: 1.08 (3H, t, J=7, CH_3-14), 1.18~1.33 (3H×3, d×3, CH_3-6′, 6″, 6‴), 1.4~2.5 (12H, m, CH_2-8, 13, 2′, 2″, 2‴, 3‴), 3.56, 3.62 (1H×2, bs×2, H-4′, 4″), 3.69 (3H, s, OCH_3), 3.7~3.9 (1H, m, H-5‴), 4.10 (1H, s, H-10), 4.0~4.3 (2H, m, H-5′, 5″), 4.28 (1H, s, OH-9), 4.78 (1H,

bs, H-1‴), 4.95 (1H, bs, H-1″), 5.23 (1H, bs, H-7), 5.47 (1H, bs, H-1′), 7.28 (1H, dd, H-3), 7.66 (1H, s, H-11), 7.66 (1H, t, H-2), 7.80 (1H, dd, H-1).

U8: m.p. 142°C, ν_{max}^{KBr} cm^{-1}: 1735, 1675, 1620, 1295, 1000, λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 432 (184), PMR ($CDCl_3$) δ in ppm: 1.07 (3H, t, J=7.0, CH_3-14), 1.22 (6H, d, J=6.5, CH_3-6′, 6″), 1.3~2.6 (10H, m, CH_2-8, 13, 2′, 2″, 2‴), 3.50 (1H, bs, H-4′), 3.68 (3H, s, OCH_3), 4.09 (1H, s, H-10), 3.8~4.2 (2H, m, H-5′, 5″), 4.56 (1H, s, OH-9), 4.92 (1H, bs, H-1″), 5.28 (1H, bs, H-7), 5.40 (1H, bs, H-1′), 7.24 (1H, dd, H-3), 7.63 (1H, s, H-11), 7.63 (1H, t, H-2), 7.77 (1H, dd, H-1), 11.94 and 12.61 (1H×2, s×2, phenolic OH).

U9: A oily material obtained from the cultured broth of 7N-1881 was dissolved in methanol and chromatographed on a Sephadex LH-20 column (ϕ 5.0×40 cm) using methanol as elution solvent. The early eluates were further purified by PLC with solvent S2 to separate four components, MA144 U1, U2, U9 and aclacinomycin A with Rf values of 0.14, 0.17, 0.41 and 0.70, respectively. Their yields were 240 mg of U1, 160 mg of U2, 250 mg of U9, and 38 mg of aclacinomycin A.

U9: m.p. 153~154°C, ν_{max}^{KBr} cm^{-1}: 1730, 1670, 1620, 1290, 1005, λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 432 (174), PMR ($CDCl_3$) δ in ppm: 1.06 (3H, t, J=7.0, CH_3-14), 1.28 (6H, d, J=6.5, CH_3-6′, 6″), 1.4~2.5 (8H, m, CH_2-8, 13, 2′, 2″), 3.55 (1H, bs, H-4′), 3.67 (3H, s, OCH_3), 4.10 (1H, s, H-10), 4.0~4.35 (2H, m, H-5′, 5″), 4.93 (1H, bs, H-1″), 5.25 (1H, bs, H-7), 5.46 (1H, bs, H-1′), 7.28 (1H, dd, H-3), 7.66 (1H, s, H-11), 7.66 (1H, t, H-2), 7.80 (1H, dd, H-1), 12.06 and 12.76 (1H×2, s×2, phenolic OH).

Acetylation of 58G

A solution of 58G (112 mg) in pyridine (10 ml) and acetic anhydride (5 ml) was stirred overnight at room temperature. The reaction mixture was poured onto ice water and a pale yellow precipitate (137 mg) was obtained. Purification by PLC (solvent S2) and recrystallization from the chloroform and *n*-hexane mixture gave pale yellow crystals (60 mg), m.p. 178~182°C, MS: m/z 520 (M^+), PMR (pyridine-d_5) δ in ppm: 1.18 (3H, t, J=7.5, CH_3-14), 2.73 (2H, q, J=7.5, CH_2-13), 2.28, 2.42 and 2.48 (3H×3, s×3, OAc×3), 5.90 (1H, d, J=2, H-10), 6.58 (1H, d, J=2, H-8), 7.54 (1H, d, J=2.5, H-3), 8.12 (1H, d, J=2.5, H-1), 8.16 (1H, s, H-11).

Methylation of 2,4,6-Triacetyl 58G

To a solution of 2,4,6-triacetyl 58G (60 mg) in chloroform (10 ml) and absolute methanol (2 ml) was added silver oxide (40 mg) and methyl iodide (0.1 ml). The mixture was stirred overnight at room temperature, and precipitates were filtered off. The filtrate was concentrated *in vacuo* and purified by PLC (solvent S1). The recrystallization from acetone gave a pale yellow crystal (15 mg), m.p. 225°C, ν_{max}^{KBr} cm^{-1}: 1775, 1720, 1675, 1645, 1595, 1570, 1180, PMR ($CDCl_3$) δ in ppm: 1.30 (3H, t, J=7.5, CH_3-14), 2.76 (2H, q, J=7.5, CH_2-13), 2.55 and 2.62 (3H×3, s×3, OAc×3), 3.87 (3H, s, OCH_3), 5.58 (1H, d, J=2.0, H-10), 6.07 (1H, d, J=2.0, H-8), 7.25 (1H, overlapped $CDCl_3$, H-3), 7.97 (1H, d, J=2.5, H-1), 8.16 (1H, s, H-11).

References

1) Oki, T.; I. Kitamura, A. Yoshimoto, Y. Matsuzawa, N. Shibamoto, T. Ogasawara, T. Inui, A. Takamatsu, T. Takeuchi, T. Masuda, 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
2) Yoshimoto, A.; Y. Matsuzawa, T. Oki, T. Takeuchi & H. Umezawa: New anthracycline metabolites from mutant strains of *Streptomyces galilaeus* MA144-M1. I. Isolation and characterization of various blocked mutants. J. Antibiotics 34: 951~958, 1981
3) Brockmann, H.; H. Brockmann, Jr. & J. Niemeyer: Die absolute Konfiguration der Anthracyclinon. Tetrahed. Lett. 1968: 4719~4724, 1968
4) Brockmann, Jr., H.: Dass massenspektroskopische Fragmentierungsverhalten der Anthracyclinon. Chem. Ber. 98: 1260~1269, 1965
5) Oki, T.; I. Kitamura, Y. Matsuzawa, N. Shibamoto, T. Ogasawara, A. Yoshimoto, T. Inui, H. Naganawa, T. Takeuchi & H. Umezawa: Antitumor anthracycline antibiotics, aclacinomycin A and analogues. II. Structural determination. J. Antibiotics 32: 801~819, 1979