NEW ANTHRACYCLINES, FEUDOMYCINS, PRODUCED BY THE MUTANT FROM *STREPTOMYCES COERULEORUBIDUS* ME130-A4

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Various blocked mutants were isolated from *Streptomyces coeruleorubidus* ME130-A4 by NTG and UV treatments. Among them, mutant strain 4N-140 produced new anthracycline feudomycins A and B having new aglycones in which the side chain at C-9 position of daunomycinone was ethyl and acetonyl, respectively. New aglycones feudomycinones C and D having methyl at C-9 and additional hydroxyl group at C-10 of daunomycinone were also isolated from this strain.

The isolation of high-yielding variants and the blocked mutants from *Streptomyces galilaeus* MA144-M1 capable of accumulating new anthracycline metabolites and of glycosidating various anthracyclinones without productivity of pigments was useful for study on the biosynthesis of anthracycline antibiotics and for preparing new products[1].

Mutation of *S. coeruleorubidus* ME130-A4, which produces daunomycin-related baumycins, gave unique variants and blocked mutants which varied in the production of new anthracycline compounds, and mutant strain 1U-222 biotransformed ε-pyrromycinone to 1-hydroxydaunomycin-related antibiotics as previously described[2].

In this paper we describe the isolation of various mutants from *S. coeruleorubidus* ME130-A4 and the structural elucidation of anthracycline glycosides with new aglycones, feudomycins.

Results

Isolation of Variants

The original strain ME130-A4 of *S. coeruleorubidus* produced baumycins A1, A2, B1 and B2 (4′-substituted daunomycin), 7-deoxy-13-dihydrodaunomycinone and ε-rhodomycinone. Daunomycin, dihydrodaunomycin, *N*-acetyl and *N*-formyldaunomycins, daunomycinone and 13-dihydrodaunomycinone were also accumulated at low levels under the usual fermentation condition.

Mutation of the original strain with NTG or UV induced various variants capable of producing new anthracycline metabolites including non-glycosidic aglycones and pigment-negative mutants. The isolated variants are listed in Table 1, and were characterized according to their metabolic products as follows:

Anthracycline glycoside-producing variants: Strain 1N-364 was the variant in which major metabolic product was not baumycins but dihydrodaunomycin, while strain 2N-266 accumulated preferentially daunomycin. Strain 4N-140 induced from strain 1N-364 by the successive mutation with NTG

Table 1. Variants and blocked mutants isolated from *Streptomyces coeruleorubidus* ME130-A4.

Group*	Mutant	Mutation		Major metabolites
		Original strain	Mutagen	
I	ME130-A4	—	—	Baumycins A & B
	1N-364	ME130-A4	NTG	Dihydrodaunomycin
	2N-266	1N-364	〃	Daunomycin
	2N-218	〃	〃	Feudomycins A & B and dihydrodaunomycin
	4N-140	2N-218	〃	Feudomycins A & B
II	1U-85	ME130-A4	UV	ε-Rhodomycinone (0.82)
	1U-215	〃	〃	Yellow aglycone (0.82)
	1U-223	〃	〃	Four violet aglycones (0.79, 0.69, 0.46, 0.19)
	1N-362	〃	NTG	Reddish violet aglycone (0.17)
	1N-367	〃	〃	Pink aglycone (0.30)
	1N-447	〃	〃	Five yellow aglycones (0.86, 0.80, 0.21, 0.15, 0.04)
	1N-467	〃	〃	Two pink aglycones (0.78, 0.2); Two violet aglycones (0.74, 0.23); Two gray aglycones (0.26, 0.03)
III	1U-222	ME130-A4	UV	**Non-productive, but bioconvertable
	1N-372	〃	NTG	〃 〃
	1N-415	〃	〃	Non-productive and unbioconvertable

Values in parenthesis show Rf values on TLC using $CHCl_3$ - MeOH (10 : 1).

* Group I: Anthracycline glycoside-producing strains.
Group II: Aglycone-producing strains.
Group III: Anthracyclinone (pigment)-non-producing strains.

** Conversion of exogenous aklavinone to daunomycinone glycosides.

produced new anthracycline feudomycins and related aglycones as described below.

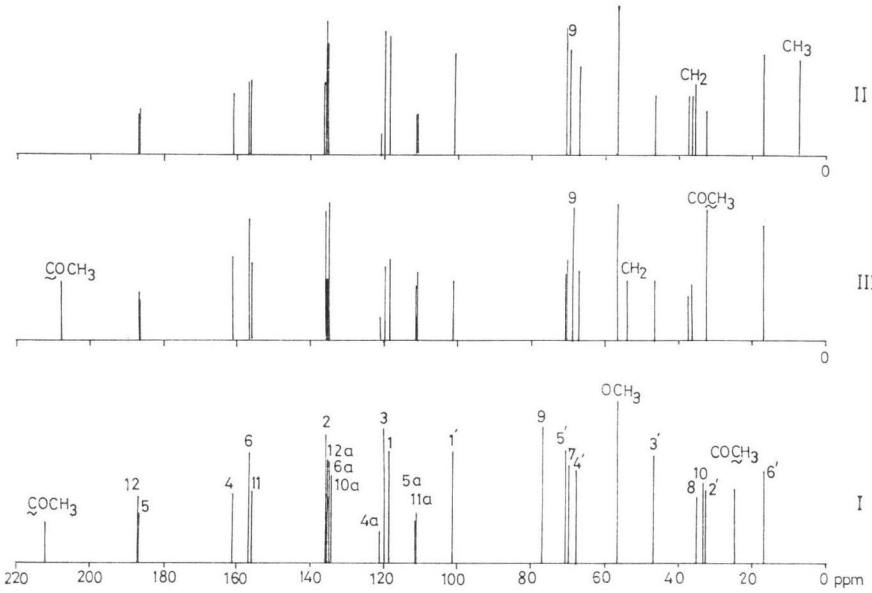
Aglycone-producing variants: In the course of mutation, the blocked mutants capable of producing only ε-rhodomycinone occurred at about 1% frequency, while daunomycinone- or dihydrodaunomycinone-accumulating mutants were not induced. Six blocked mutants produced violet, gray, pink and yellow unknown aglycone pigments.

Anthracycline-negative mutants: Strain 1U-222 and 1N-415 were selected by a loss of acetone-extractable red pigment from mycelia, and these strains did not produced any anthracycline pigment in the culture broth. Strain 1U-222 possessed the ability to produce the parent glycosides when aklavinone or ε-rhodomycinone was exogenously added to the culture[3], whereas strain 1N-415 lacked both activities of glycosidation and pigment-formation.

Feudomycins A and B Produced by a Mutant Strain 4N-140

Feudomycins A (**II**) and B (**III**) were red crystalline powders having molecular formulae $C_{27}H_{31}NO_9$ and $C_{28}H_{31}NO_{10}$, respectively. UV/Visible spectra of **II** and **III** were quite similar to that of daunomycin (**I**). The IR spectra of **II** and **III** were also very similar to that of **I**, excepting that the acetyl carbonyl peak (1700 cm^{-1}) observed in **I** and **III** was missing in **II**. Their CMR spectra indicated that **II** had the same 27 carbons as **I** and **III** had 28 (Fig. 1), and that **II** possessed ethyl carbons (δ 7.4 and 35.4) instead of acetyl carbons (δ 24.8 and 212.0) in **I**. **III** had one more methylene carbon (δ 54.1) than **I**. The peaks assigned for the sugar moiety were same throughout.

Fig. 1. CMR spectra of daunomycin (I), feudomycin A (II) and feudomycin B (III).

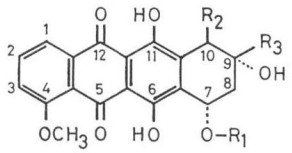


Acid hydrolysis of **II** and **III** with 0.1 N HCl gave red aglycones, named as feudomycinones A and B respectively. The sugar moiety obtained from the aqueous layer of the acid hydrolysates was determined as daunosamine by a direct comparison with the authentic sample on TLC.

Feudomycinone A (**IV**) melted at 201~206°C and had a molecular formula $C_{21}H_{20}O_7$ as established by elemental analysis and MS spectrum. The PMR spectrum of **IV** showed the presence of ethyl protons (δ 1.08 and 1.6~1.95) instead of acetyl protons (δ 2.44) in daunomycinone (**IX**), and its IR spectrum also indicated absence of the acetyl carbonyl group. In the CMR spectrum of **IV**, the shift of carbon at C-9 to higher field and the presence of additional ethyl carbons (δ 7.4 and 35.3) were observed in comparison with **IX**. These data supported **IV** as 13-deoxodaunomycinone and **II** was thus identical to 13-deoxodaunomycin, which had been chemically synthesized from ε-rhodomycinone[4].

Feudomycinone B (**V**) from **III** had a molecular formula of $C_{22}H_{20}O_8$ and melting point of 184~189°C. IR and PMR spectra revealed the presence of an acetyl group in **V**. In the PMR spectrum of **V**, the acetyl protons were shifted to higher field (δ 2.30) than those of **IX** (δ 2.44) and the broad singlet peak at δ 2.84 was assigned to the isolated methylene protons. Moreover, the off-resonance CMR spectrum of **V** showed the triplet peak at δ 53.6, to be assigned to the methylene carbon, which was shifted to low field. On the contrary, the carbon at C-9 (δ 69.0) and the acetyl carbonyl (δ 207.9) were shifted to higher field than those of **IX** (δ 76.9 and 212.0). These spectral data suggested the presence of one methylene group between the carbon at C-9 and the acetyl ketonic carbon. Further structural confirmation of **V** was provided by the spectral analysis of **VIII** which was obtained by hydrogenation of **V** with sodium borohydride. **VIII** had a molecular weight of 414 by mass spectral analysis, which showed that two hydrogen atoms were introduced into **V**. The IR spectrum showed the absence of the carbonyl absorption peak in **VIII**. The PMR spectrum of **VIII** indicated the loss of both peaks of acetyl and methylene in the low field and the appearance of two peaks splitting as doublets at δ 1.15 (3H, J=6.5) and δ 1.66 (2H, J=6.2) which were assigned as methyl protons at C-15

Fig. 2. Structure of daunomycin and feudomycins.



		R_1	R_2	R_3
I	(Daunomycin)		H	$COCH_3$
II	(Feudomycin A)		H	CH_2CH_3
III	(Feudomycin B)		H	CH_2COCH_3
IV	(Feudomycinone a=13-deoxodaunomycinone)	H	H	CH_2CH_3
V	(Feudomycinone B)	H	H	CH_2COCH_3
VI	(Feudomycinone C)	H	H	CH_3
VII	(Feudomycinone D)	H	OH	CH_3
VIII	(Dihydrofeudomycinone B)	H	H	$CH_2CHOHCH_3$
IX	(Daunomycinone)	H	H	$COCH_3$

and methylene protons at C-13, respectively. **V** is proposed as a new aglycone (feudomycinone B) in which the acetyl at C-9 of **IX** is replaced by acetonyl, and thus **III** is 9-acetonyldaunomycin (feudomycin B), as shown in Fig. 2.

New Aglycones, Feudomycinones C and D

Feudomycinones C (**VI**) and D (**VII**) were minor components and isolated from the acid hydrolysate of crude extract from the cultured broth of a mutant strain 4N-140 culture.

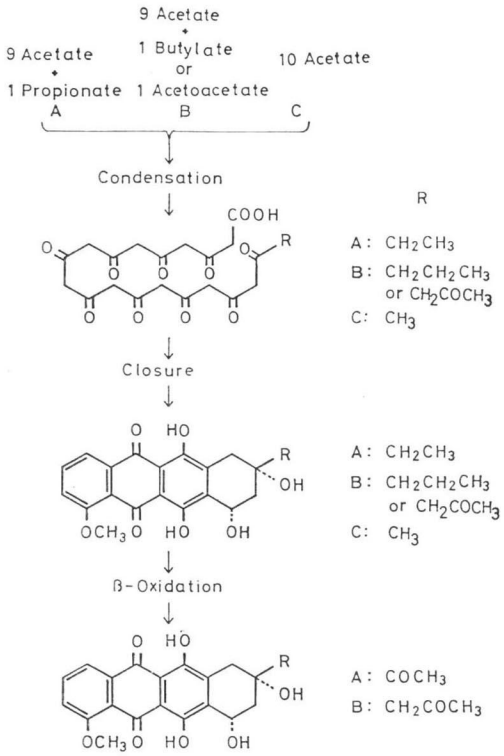
The melting points of **VI** and **VII** were 218~219°C and 132~135°C, respectively. The molecular formulae of **VI** and **VII** were $C_{20}H_{18}O_7$ and $C_{20}H_{18}O_8$, respectively. The absence of the acetyl group at C-9 in both aglycones was observed in the IR and PMR spectra. PMR spectrum indicated that the isolated methyl group (δ 1.47) with a singlet peak was present in **VI**. Other peaks were superimposable on those, except for the acetyl protons, of **IX**. Thus, **VI** was identified as 9-methyldaunomycinone (feudomycinone C). On the other hand, the PMR spectrum of **VII** also showed isolated methyl protons with a singlet peak at δ 1.30. The proton at C-10 appeared as a doublet peak at lower field (δ 4.50) than that of **IX** (δ 2.5~3.4). This proton was coupled with the hydroxyl group splitting as a doublet at δ 5.48 with a coupling constant of 6.2 Hz which was exchanged with D_2O. From the results, **VII** was identified as 9-methyl-10-hydroxydaunomycinone (feudomycinone D) as shown in Fig. 2.

Discussion

S. coeruleorubidus ME130-A4 produces baumycins in which daunomycin was substituted at C-4′ of daunosamine with an acetal residue. It is of great interest that baumycins and 4-hydroxybaumycins were also accumulated in the culture of *S. peucetius* known as an adriamycin producer (unpublished data) and *Actinomadura* strain D326[5]. BLUMAUEROVA *et al.* have reported the isolation of blocked mutants from *S. coeruleorubidus* which accumulated non-glycosidated aglycones such as ε-rhodomycinone or aklavinone[6]; we isolated ε-rhodomycinone-producers and other unidentified aglycone producers from *S. coeruleorubidus* ME130-A4. We demonstrated that ε-rhodomycinone is a precursor aglycone in the biosynthesis of daunomycinone[3], and proposed that daunomycin is biosynthesized *via*

glycosidation of ε-rhodomycinone followed by 10-decarbomethoxylation and further β-oxidation of ethyl at C-9. Feudomycin A (13-deoxodaunomycin) was found to be an intermediate in the daunomycin biosynthesis as deduced from the proposed biosynthetic pathway[7]. It seems likely that other daunomycinone-related aglycones with a side chain of methyl or acetonyl at C-9 position arise from irregularity in the formation of the polyketide. It has been reported that daunomycinone was basically formed by the condensation of nine units of acetate and one propionate[8]. Thus, it is deduced that the above aglycones were built from ten units of acetate in the methyl analog and 11 acetate units or nine units of acetate and one butylate or one acetoacetate unit in the acetonyl analog, as shown in Fig. 3.

Fig. 3. Proposed biosynthetic pathway of feudomycinones.



The production of new aglycones having methyl and acetonyl group at C-9 position of aklavinone, named as auramycinone and sulfurmycinone, respectively, was recently reported with *S. galilaeus* by FUJIWARA *et al.*[9]. We also recently found an additional 13-methylaklavinone in the aclacinomycin broth as a minor component[10]. This may be formed from nine units of acetate and one isobutylate. These results showed that the starter carbon unit in the formation of polyketide leading to anthracyclinone is usually propionate, but variable under cultural conditions where the propionate level was suppressed by any reason, so that new anthracyclinone aglycones with different side chains at C-9 position could be produced.

Experimental

Microorganisms

The original strain ME130-A4 and mutant strains of *Streptomyces coeruleorubidus* were maintained on YS agar (0.3% yeast extract, 1% soluble starch, 1.5% agar, pH 7.2). Anthracycline compounds produced by strain ME130-A4 were previously described[7,11].

Mutation

Spore cells (5×10^8 cells/ml) of *S. coeruleorubidus* ME130-A4 from the culture grown for one week on YS agar were exposed to 1 mg/ml of *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG) in 0.2 M tris-HCl (pH 8.5) with shaking at 28°C in the dark, or the spore suspension (5×10^9 cells/10 ml of saline) in a Petri dish were irradiated by ultraviolet light (UV). Both treatments were performed to give about 5~0.5% survivals. The treated spore cells were plated on YS agar after dilution with saline and incubated for 5 days at 28°C. About 200~500 colonies were taken on YS agar slant and tested for the production of anthracycline compounds as described below.

Analyticals

Thin-layer (TLC) and preparative layer (PLC) chromatographies were carried out on silica gel $60F_{254}$ and $60PF_{254}$ plates (E. Merck & Co.) using the following solvent systems: S1; $CHCl_3$ - MeOH - ammonia water (90: 10: 0.2, v/v/v), S2; $CHCl_3$ - MeOH - acetic acid (80: 20: 4, v/v/v), S3; $CHCl_3$ - MeOH (40: 1, v/v), S4; $CHCl_3$ - MeOH (20: 1, v/v), S5; benzene - acetic acid - formic acid (100: 30: 1,

v/v/v) and S6; *n*-BuOH - acetic acid - water (4: 1: 1, v/v/v). The extraction of anthracycline pigment from the removed silica gel was done by a solvent mixture of $CHCl_3$ - MeOH - ammonia water (100: 15: 0.2, v/v/v) (S7). Wakogel C-200 (silica gel, 74~149 μ, Wako Junyaku) and Sephadex LH-20 (Pharmacia Fine Chemicals) were employed for the column chromatography.

The detection of sugars was done by spraying the TLC plate with a mixture of 5% *p*-anisaldehyde and 5% sulfuric acid in ethanol and heating at 90°C for color development.

Detection of Anthracycline Compounds

Cultures on YS agar were inoculated in YS medium (4 ml/test tube), incubated on a reciprocal shaker at 28°C for 2 days, and added to a 250-ml Erlenmeyer flask containing 30 ml of the fermentation medium as described in a previous paper[7]. The fermentation took place by cultivation at 28°C for 3 days on a rotary shaker (220 rpm). Five ml of the culture broth was sampled and centrifuged, and the pigments in the mycelial pellet were extracted with 5 ml of acetone. The acetone extract was concentrated *in vacuo* and re-extracted with 2 ml of chloroform. The extract was separated into two parts and evaporated to dryness. The pigment residues of one part were hydrolyzed in 1 ml of 0.1 N HCl at 85°C for 30 minutes to obtain aglycones and extracted with 1 ml of chloroform. Pigments in the hydrolysate were chromatographed on a silica gel plate using solvent system of $CHCl_3$ - MeOH (10: 1 or 20: 1) and compared with authentic sample on TLC.

Fermentation of Strain 4N-140

The seed culture of strain 4N-140 was prepared by cultivation at 28°C for 2 days on a rotary shaker in a 500 ml Erlenmeyer flask containing 100 ml of the following medium: 1.5% soluble starch, 0.1% yeast extract, 0.1% K_2HPO_4, 0.1% $MgSO_4 \cdot 7H_2O$ and 0.3% NaCl, pH 7.4, one ml of which was added to a 500-ml Erlenmeyer flask containing 50 ml of the fermentation medium as described in a previous paper[11]. Cultivation was performed for 5 days at 28°C on a rotary shaker (220 rpm). The culture broth (20 liters) thus obtained was centrifuged and the pigments were extracted from the mycelia with acetone and the supernatant fluid with chloroform, and the combined extract was evaporated to oily crude pigments.

Isolation of Feudomycins A and B

The crude pigment extract from strain 4N-140 was dissolved in methanol and the insoluble material was centrifuged. The supernatant was subjected to a Sephadex LH-20 column ($\phi 5 \times 45$ cm) and eluted with a $CHCl_3$ - MeOH - ammonia water (125: 250: 1, v/v/v) mixture. The eluate containing anthracycline glycosides was pooled and concentrated *in vacuo* to dryness. The residue was dissolved in 200 ml of toluene and extracted three times with 200 ml of 0.1 M acetate buffer (pH 3.0). The aqueous layer was extracted twice with 200 ml of $CHCl_3$ after adjusting to pH 7 with saturated $NaHCO_3$ solution. The extract was dried over anhydrous Na_2SO_4, filtered and evaporated to dryness. The residue (425 mg) was chromatographed on PLC using the solvent system S1. Three major red bands appeared at Rf values of 0.15 (feudomycin A), 0.13 (feudomycin B), and 0.02 (dihydrodaunomycin). The regions of these bands were scratched and extracted with solvent S7. Further purification was done by rechromatography on PLC using solvent S2. Each component thus obtained was dissolved in 20 ml of 0.1 M acetate buffer (pH 3.0) and the aqueous solution was washed with 10 ml of toluene and re-extracted twice with 20 ml of $CHCl_3$ after adjusting to pH 7.0 with 10% $NaHCO_3$ solution. The $CHCl_3$ layer was dried over anhydrous Na_2SO_4 and concentrated to a small volume and the pure pigments were obtained as red powder by precipitating with excess *n*-hexane; 57.9 mg of feudomycin A; 31.8 mg of feudomycin B; 52 mg of dihydrodaunomycin.

Feudomycin A: Red amorphous powder, m.p. 158~163°C, $[\alpha]_D^{23}$ +243° (*c* 0.044, MeOH), λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 235 (688), 253 (510), 290 (170), 475 (227), 497 (235), 530 (135), ν_{max}^{KBr} cm^{-1}: 1620, 1580, 1120, PMR ($CDCl_3$) δ in ppm: 1.07 (3H, t, J=7.5, H-14), 1.34 (3H, d, J=6.5, H-6′), 1.6~1.8 (4H, m, H-13 and 2′), 1.7~2.4 (2H, m, H-8), 2.4~3.3 (3H, m, H-10 and 3′), 3.47 (1H, bs, H-4′), 4.04 (3H, s, OMe), 4.0~4.2 (1H, q, J=6.5, H-5′), 5.16 (1H, bs, H-7), 5.46 (1H, bs, H-1′), 7.33 (1H, dd, J=1.5 and 8, H-3), 7.72 (1H, t, J=8.0, H-2), 7.92 (1H, dd, J=1.5 and 8.0, H-1). (Found: C 62.41, H 5.95, N 2.77, Calcd. for $C_{27}H_{31}NO_9$: C 63.15, H 6.08, N 2.73%).

Feudomycin B: Red amorphous powder, m.p. 148~150°C, $[\alpha]_D^{23}$ +146° (*c* 0.04, MeOH), λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 235 (631), 253 (461), 290 (170), 475 (214), 498 (224), 532 (130), ν_{max}^{KBr} cm^{-1}: 1700, 1615, 1580, 1010, PMR ($CDCl_3$) δ in ppm: 1.36 (3H, d, J=6.5, H-6'), 1.65~1.8 (2H, m, H-2'), 1.85~2.75 (2H, m, H-8), 2.28 (3H, s, Ac), 2.5~3.5 (2H, m, H-10), 2.85 (2H, bs, H-13), 3.0~3.2 (1H, m, H-3'), 3.5 (1H, bs, H-4'), 4.02 (3H, s, OMe), 3.95~4.2 (1H, q, J=6.5, H-5'), 5.10 (1H, bs, H-7), 5.46 (1H, bs, H-1'), 7.31 (1H, dd, J=1.5 and 8.0, H-3), 7.7 (1H, t, J=8.0, H-2), 7.94 (1H, dd, J=1.5 and 8.0, H-1). (Found: C 61.98, H 5.77, N 2.34, Calcd. for $C_{28}H_{31}NO_{10}$: C 62.10, H 5.77, N 2.59%).

Isolation of Feudomycinones A, B, C and D

The crude pigment extract from 20 liters of cultured broth of strain 4N-140 was dissolved in 30 ml of acetone and added 200 ml of 0.3 N HCl. The mixture was heated at 85°C for 60 minutes, extracted twice with 200 ml of $CHCl_3$ and evaporated *in vacuo* to dryness. The oily residue was chromatographed on a silica gel column (ϕ 4×20 cm) using $CHCl_3$ - MeOH (100: 2 and 100: 5). Feudomycinone A was eluted with concomitant daunomycinone. Further purification was done by PLC using solvent S5 and feudomycinone A was crystallized from acetone to yield 48.3 mg. The fractions containing feudomycinone B, C or D were respectively purified by further twice PLC in which solvent S5 and subsequent S4 were used. The pure aglycones were crystallized from acetone. Their yields were; feudomycinone B, 26.7 mg; feudomycinone C, 11.9 mg; feudomycinone D, 15.8 mg.

Feudomycinone A: Red crystals, m.p. 201~206°C, $[\alpha]_D^{23}$ +181° (*c* 0.02, MeOH), λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 235 (920), 253 (706), 290 (220), 475 (312), 497 (325), 530 (183), ν_{max}^{KBr} cm^{-1}: 1620, 1580, PMR ($CDCl_3$) δ in ppm: 1.08 (3H, t, J=7.5, H-14), 1.6~1.95 (4H, m, H-13 and 8), 2.25~3.25 (2H, m, H-10), 3.47 (1H, s, OH-9 exchanged with D_2O), 3.7 (1H, d, J=5.5, OH-7 exchanged with D_2O), 4.06 (3H, s, OMe), 5.26 (1H, bs, H-7), 7.36 (1H, dd, H-3), 7.75 (1H, t, H-2), 8.00 (1H, dd, H-1), 13.24 and 13.90 (2H, s×2, phenolic OH exchanged with D_2O), MS: m/z 384 (M^+). (Found: C 65.55, H 5.27, Calcd. for $C_{21}H_{20}O_7$: C 65.62, H 5.24%).

Feudomycinone B: Red crystals, m.p. 184~189°C, $[\alpha]_D^{23}$ +158° (*c* 0.02, MeOH), λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 236 (922), 253 (676), 292 (214), 473 (308), 497 (317), 532 (176), ν_{max}^{KBr} cm^{-1}: 1700, 1610, 1580, PMR ($CDCl_3$) δ in ppm: 1.85~2.35 (2H, m, H-8), 2.30 (3H, s, Ac), 2.84 (2H, bs, H-13), 2.5~3.45 (2H, m, H-10), 3.97 (1H, d, J=6.0, OH-7 exchanged with D_2O), 4.06 (3H, s, OMe), 4.46 (1H, s, OH-9, exchanged with D_2O), 5.16 (1H, bs, H-7), 7.36 (1H, dd, H-3), 7.75 (1H, t, H-2), 7.98 (1H, dd, H-1), 13.24 and 13.91 (2H, s×2, phenolic OH exchanged with D_2O). MS: m/z 412(M^+) (Found: C 63.60, H 5.05, Calcd. for $C_{22}H_{20}O_8$: C 64.08, H 4.89%).

Feudomycinone C: Red crystals, m.p. 218~219°C, $[\alpha]_D^{23}$ +160° (*c* 0.02, MeOH), λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 235 (902), 252 (688), 290 (215), 472 (305), 495 (317), 530 (176), ν_{max}^{KBr} cm^{-1}: 1610, 1580, PMR ($CDCl_3$ - CD_3OD, 5: 1) δ in ppm: 1.47 (3H, s, H-13), 1.8~2.5 (2H, m, H-8), 2.4~3.32 (2H, m, H-10), 4.06 (3H, s, OMe), 5.21 (1H, bs, H-7), 7.40 (1H, dd, H-3), 7.75 (1H, t, H-2), 7.96 (1H, dd, H-1), 13.28 and 13.92 (2H, s×2, phenolic OH exchanged with D_2O). MS: m/z 370 (M^+). (Found: C 64.26, H 4.89, Calcd. for $C_{20}H_{18}O_7$: C 64.86, H 4.90%).

Feudomycinone D: Red crystals, m.p. 132~135°C, λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 235 (403), 252 (315), 293 (74), 502 (121), 537 (143), 579 (116), ν_{max}^{KBr} cm^{-1}: 1615, 1585, PMR (DMSO-d_6) δ in ppm: 1.30 (3H, s, H-13), 1.7~2.2 (2H, m, H-8), 3.90 (3H, s, OMe), 4.50 (1H, d, J=6.2, H-10), 4.75~5.10 (2H, m, H-7 overlapped with OH-7 exchanged with D_2O), 5.20 (1H, s, OH-9 exchanged with D_2O), 5.48 (1H, d, J=6.2, OH-10 exchanged with D_2O), 7.5 (1H, dd, H-3), 7.8~ (2H, m, H-1 and 2), 12.43 and 12.95 (2H, s×2, phenolic OH exchanged with D_2O).

Reduction of Feudomycinone B

Sodium borohydride (5 mg) was added to a solution of feudomycinone B (25 mg) in a mixture of $CHCl_3$ (25 ml) and ethanol (2.5 ml), which was stirred at room temperature for 30 minutes. 5×10^{-3} M EDTA solution (20 ml) and $CHCl_3$ (10 ml) were then added and the mixture was vigorously shaken. The chloroform layer was washed with distilled water, dried over anhydrous Na_2SO_4 and evaporated *in vacuo* to dryness (18 mg). The residue was purified by PLC with solvent S4 and crystallized from acetone to yield 5 mg of 14-dihydrofeudomycinone B: Red needle crystals, m.p. 188~193°C, $[\alpha]_D^{23}$ +183°

(*c* 0.02, MeOH), λ_{max}^{MeOH} nm ($E_{1cm}^{1\%}$): 236 (850), 250 (685), 290 (197), 475 (281), 497 (290), 530 (165), PMR (DMSO-d_6) δ in ppm: 1.15 (3H, d, J=6.5, H-15), 1.66 (2H, J=6.5, H-13), 1.8~2.4 (2H, m, H-8), 2.6~3.2 (2H, m, H-10), 4.02 (3H, s, OMe), 4.7~ (1H, m, H-14), 5.02~ (1H, m, H-7), 7.68 (1H, dd, H-3), 7.93 (2H, m, H-1 and 2), MS: m/z 414 (M^+).

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