REVEROMYCINS, NEW INHIBITORS OF EUKARYOTIC CELL GROWTH

III. STRUCTURES OF REVEROMYCINS A†, B, C AND D

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Reveromycins A, B, C and D are new group inhibitors of the mitogenic activity of epidermal growth factor (EGF), produced by *Streptomyces* sp. Reveromycins are novel polyketide type antibiotics which have two terminal carboxylic groups, a spiroketal, a succinate and a varied side chain in the molecule. Determination of their structures by chemical and spectroscopic methods, in particular NMR studies, is described.

Reveromycins A (**1**), B (**2**), C (**3**) and D (**4**) are novel antibiotics produced by *Streptomyces* sp. Reveromycins inhibit the mitogenic activity of epidermal growth factor (EGF). The isolation, physico-chemical properties and biological activities are described in preceding papers[1,2]. In this paper, we report the structural determination of reveromycins A, B, C and D.

Reveromycin A (**1**), $[\alpha]_D^{20}$ $-115°$, is a white amorphous powder with mp 95°C. It has the molecular formula $C_{36}H_{52}O_{11}$ which was confirmed by elemental analysis and HRFAB-MS *m/z* 683.3496 $(M+Na)^+$; calcd 683.3407. The UV spectrum showed the presence of conjugated diene chromophores, λ_{max} (in MeOH) 238 and 260 (sh) nm. In the IR spectrum, broad bands at 3600~3200 and 1690 cm^{-1} were observed and suggested the presence of carboxyl groups. In the SI-MS spectrum, characteristic fragment ions at *m/z* 643 (MH^+-H_2O) and *m/z* 525 $(643-118(C_4H_6O_4))$ suggested the presence of one hydroxyl group and a succinate moiety. The ^{13}C NMR spectrum (Table 1) exhibited the signals of four carbonyl, ten olefinic carbons, two quaternary carbons, three oxygenated methine, two ordinary methine, ten methylene, and five methyl carbons. The ^{13}C and ^1H NMR (Table 2) assignments and partial structures (Fig. 1) were established by ^1H-^1H COSY, ^{13}C-^1H COSY and heteronuclear multiple-bond correlation (HMBC)[3] spectra (Fig. 2). The positions and assignments of four carbonyl carbons and two sp^2 carbons (C-8 and C-22) were confirmed by the HMBC spectrum. ^1H-^{13}C long range couplings from H-19 (δ 4.51) to C-15 (δ 94.9), C-17 (δ 23.7) and C-18 (δ 82.0) were also observed in HMBC spectrum. The quaternary carbon at δ 94.9 (C-15) suggested the presence of a six-membered spiroketal moiety[4]. The stereochemistry of three disubstituted double bonds were determined as *E* configuration from the large coupling constants (J=15.9 or 15.6 Hz). To determine the stereochemistry of two trisubstituted double bonds, NOESY experiments was carried out on trimethyl ester (**1a**). Observed NOEs (Fig. 3) between H-7 and H-9, H-10a (δ 2.38) and Me-8, and H-21 and H-23 suggested these double bonds were all *E* configuration. In NOESY

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Table 1. ^{13}C NMR data of reveromycins A (**1**), B (**2**) and C (**3**).

Carbons	Chemical shifts (in ppm)				Carbons	Chemical shifts (in ppm)			
	1[a]	**1**[b]	**2**[b]	**3**[b]		**1**[a]	**1**[b]	**2**[b]	**3**[b]
C-1	167.2	170.0[c]	171.1[c]	170.3	C-20	132.1	134.2	132.1	134.1
C-2	121.5	122.5	122.6	122.7	C-21	137.5	139.2	136.4	139.4
C-3	150.9	152.9	152.2	152.8	C-22	149.2	152.5	151.4	152.4
C-4	42.0	44.1	44.2	44.2	C-23	121.6	121.5	123.6	121.8
C-5	73.9	76.9	77.4	76.9	C-24	167.8	170.1[c]	171.6[c]	170.3
C-6	128.2	128.0	127.6	128.0	C-25	33.3	35.2	35.8[d]	33.4
C-7	134.7	137.9	138.7	137.9	C-26	23.2	25.1	26.8	31.8
C-8	133.7	135.2	135.5	135.5	C-27	22.1	23.8	24.5	29.4
C-9	127.2	129.2	131.1	129.5	C-28	13.5	14.2	14.2	23.1
C-10	31.1[c]	33.0	33.1[d]	33.0	4-Me	14.3	15.4	15.4	15.3
C-11	74.0	76.2	78.6	76.3	8-Me	12.5	13.0	13.0	13.0
C-12	32.7	34.8	35.9	34.8	12-Me	17.3	18.1	18.4	18.0
C-13	27.1	28.6	30.9[d]	28.8	22-Me	13.5	14.7	14.7	14.7
C-14	35.4	36.9	39.7[d]	37.0	27-Me	—	—	—	22.7
C-15	94.9	97.0	108.8	97.1	C-1′	171.3	173.1	173.4	173.4
C-16	31.0[c]	32.8	35.5[d]	32.8	C-2′	28.8	29.9	30.6[d]	29.9
C-17	23.7	25.4	33.1[d]	25.4	C-3′	29.7	31.2	31.2[d]	31.2
C-18	82.0	84.2	89.0	84.2	C-4′	173.3	176.0	177.8	176.0
C-19	77.7	79.7	80.6	80.0					

[a] DMSO-d_6 as solvent.
[b] CD_3OD as solvent.
[c,d] Assignments may be interchanged in each column.

Table 2. ^1H NMR data of reveromycins A (**1**), B (**2**), C (**3**) and D (**4**)[a,b].

Protons	**1**[c]	**2**[d]	**3**[d]	**4**[d]
H-2	5.72 (dd, 15.9, 0.9)	5.80 (dd, 15.6, 1.2)	5.81 (dd, 15.9, 1.2)	5.81 (dd, 15.9, 1.2)
H-3	6.86 (dd, 15.9, 7.3)	6.94 (dd, 15.6, 7.6)	6.97 (dd, 15.9, 7.6)	6.92 (dd, 15.9, 7.6)
H-4	2.40 (m)	2.48 (m)	2.51 (m)	2.49 (m)
H-5	3.99 (dd, 6.4, 5.5)	4.07 (dd, 7.3, 5.5)	4.06 (dd, 7.3, 5.5)	4.05 (dd, 7.0, 5.8)
H-6	5.47 (dd, 15.6, 6.4)	5.48 (dd, 15.6, 7.3)	5.53 (dd, 15.6, 7.3)	5.53 (dd, 15.6, 7.0)
H-7	6.18 (d, 15.6)	6.38 (d, 15.6)	6.24 (d, 15.6)	6.24 (d, 15.6)
H-9	5.54 (dd, 6.9, 6.9)	5.76 (dd, 6.7, 6.7)	5.58 (dd, 6.7, 6.7)	5.58 (dd, 6.9, 6.9)
H-10	2.25 (m), 2.40 (m)	2.14～2.28 (m)	2.35 (m), 2.40 (m)	2.35 (m), 2.40 (m)
H-11	3.36 (m)	3.45 (m)	3.44 (m)	3.45 (m)
H-19	4.51 (d, 9.8)	5.56 (d, 4.6)	4.57 (d, 7.6)	4.60 (d, 8.0)
H-20	6.36 (dd, 15.6, 9.8)	6.21 (dd, 15.9, 4.6)	6.43 (m)	6.40 (m)
H-21	6.46 (d, 15.6)	6.27 (d, 15.9)	6.43 (m)	6.40 (m)
H-23	5.86 (s)	5.79 (s)	5.87 (br s)	5.88 (br s)
H-28	0.78 (t, 6.7)	0.91 (t, 6.9)	0.83 (d, 6.4)	—
H-29	—	—	—	0.85 (t, 7.1)
Me-4	0.97 (d, 6.7)	1.01 (d, 6.7)	1.07 (d, 6.7)	1.07 (d, 6.7)
Me-8	1.68 (s)	1.74 (s)	1.75 (s)	1.75 (s)
Me-12	0.73 (d, 6.1)	0.89 (d, 6.4)	0.78 (d, 6.4)	0.77 (d, 6.4)
Me-22	2.20 (s)	2.21 (d, 1.2)	2.25 (d, 0.9)	2.23 (d, 1.2)
Me-27	—	—	0.79 (d, 6.7)	—
H-2′,3′	2.45 (m), 2.50 (m)	2.51～2.70 (m)	2.55～2.65 (m)	2.55～2.65 (m)

[a] Chemical shifts are given in ppm from TMS as internal standard.
[b] Coupling constants (J in Hz) are given in parentheses.
[c] DMSO-d_6 as solvent.
[d] CD_3OD as solvent.

Fig. 1. Partial structures and assignments of proton signals of reveromycin A (**1**).

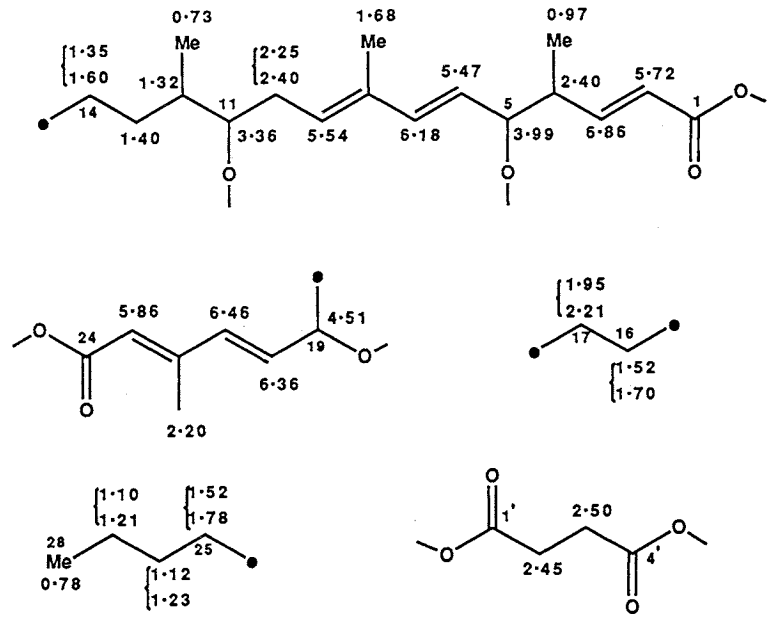


Fig. 2. Long range C–H coupling observed in HMBC spectrum of reveromycin A (**1**).

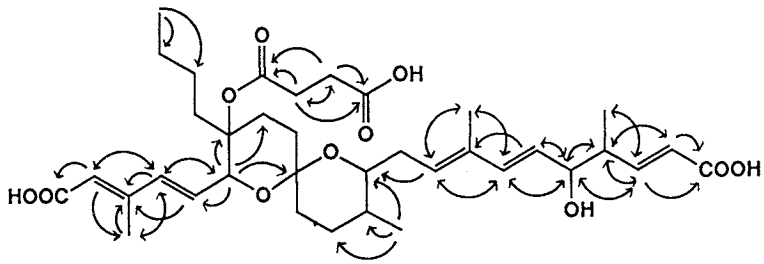
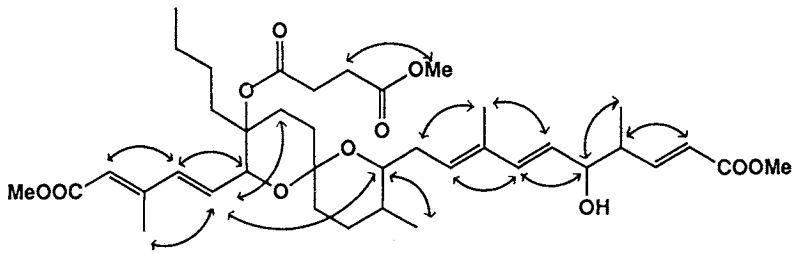


Fig. 3. NOE data of trimethyl ester (**1a**) of reveromycin A.



spectrum, NOEs between H-11 and H-20, and H-17$_{ax}$ and H-20 were also observed. The data supported the presence of 1,7-dioxaspiro[5.5]undecane system.

Presence of the one ester and three carboxyl groups in **1** was confirmed by methylation. Treatment of **1** with diazomethane gave trimethyl ester (**1a**). Subsequent acetylation of **1a** gave monoacetate (**1b**). In the ^{1}H NMR spectra (Table 3), the methine proton (H-5) was shifted down field from δ 4.11 ppm for **1a**

Table 3. ^{1}H NMR data of methyl ester derivatives of reveromycins[a~c].

Protons	1a	1b	2a	3a	4a
H-2	5.88 (dd, 15.9, 1.2)	5.85 (dd, 15.9, 1.2)	5.86 (dd, 15.6, 1.2)	5.88 (dd, 16.0, 1.5)	5.89 (dd, 15.9, 1.2)
H-3	7.01 (dd, 15.9, 7.3)	6.95 (dd, 15.9, 8.5)	7.03 (dd, 15.6, 7.6)	7.01 (dd, 16.0, 7.6)	7.01 (dd, 15.9, 7.6)
H-4	2.55~2.70 (m)	2.57~2.72 (m)	2.54 (m)	2.58 (m)	2.58 (m)
H-5	4.11 (dd, 7.3, 6.3)	5.27 (dd, 7.9, 5.2)	4.12 (m)	4.11 (m)	4.11 (m)
H-6	5.51 (dd, 15.6, 7.3)	5.38 (dd, 15.6, 7.9)	5.49 (dd, 15.6, 6.7)	5.51 (dd, 15.3, 6.9)	5.51 (dd, 15.9, 6.7)
H-7	6.22 (d, 15.6)	6.29 (d, 15.6)	6.37 (d, 15.6)	6.22 (d, 15.3)	6.22 (d, 15.9)
H-9	5.53 (dd, 7.3, 7.3)	5.64 (dd, 7.0, 7.0)	5.70 (dd, 7.0, 7.0)	5.53 (dd, 7.0, 7.0)	5.53 (dd, 7.0, 7.0)
H-10	2.22~2.32 (m),	2.22~2.32 (m),	2.13~2.33 (m)	2.24~2.34 (m), 2.38 (m)	2.23~2.33 (m), 2.38 (m)
	2.38 (ddd, 16.7, 7.3, 3.6)	2.41 (ddd, 15.6, 7.0, 4.1)	—	—	—
H-11	3.41 (ddd, 10.1, 4.6, 3.6)	3.43 (ddd, 10.4, 4.1, 4.1)	3.38 (ddd, 9.2, 9.2, 3.0)	3.44 (ddd, 9.2, 3.8, 3.8)	3.41 (m)
H-19	4.62 (d, 8.0)	4.63 (d, 8.5)	5.42 (d, 3.1)	4.58 (d, 8.8)	4.61 (d, 8.2)
H-20	6.37 (dd, 15.6, 8.0)	6.38 (dd, 15.9, 8.5)	6.17 (m)	6.38 (dd, 15.3, 8.8)	6.37 (dd, 15.6, 8.2)
H-21	6.33 (d, 15.6)	6.32 (d, 15.9)	6.17 (m)	6.33 (d, 15.3)	6.33 (d, 15.6)
H-23	5.83 (br s)	5.83 (br s)	5.77 (br s)	5.83 (s)	5.83 (s)
H-28	0.82 (t, 7.3)	0.83 (t, 7.3)	0.90 (t, 6.9)	0.81 (d, 6.9)	—
H-29	—	—	—	—	0.84 (t, 7.0)
Me-4	1.09 (d, 6.7)	1.07 (d, 7.0)	1.05 (d, 7.0)	1.09 (d, 6.9)	1.09 (d, 6.7)
Me-8	1.72 (s)	1.70 (s)	1.72 (s)	1.72 (s)	1.72 (s)
Me-12	0.76 (d, 6.4)	0.75 (d, 6.4)	0.87 (d, 6.4)	0.76 (d, 6.1)	0.76 (d, 6.1)
Me-22	2.24 (d, 0.9)	2.27 (d, 0.9)	2.28 (d, 1.2)	2.24 (s)	2.24 (s)
Me-27	—	—	—	0.77 (d, 6.9)	—
H-2',3'	2.55~2.70 (m)	2.57~2.72 (m)	2.55~2.71 (m)	2.58~2.68 (m)	2.58~2.68 (m)
OMe-1	3.73 (s)	3.74 (s)	3.71 (s)	3.73 (s)	3.73 (s)
OMe-24	3.72 (s)	3.72 (s)	3.72 (s)	3.73 (s)	3.72 (s)
OMe-4'	3.69 (s)	3.69 (s)	3.69 (s)	3.70 (s)	3.69 (s)
OAc	—	2.07 (s)	—	—	—

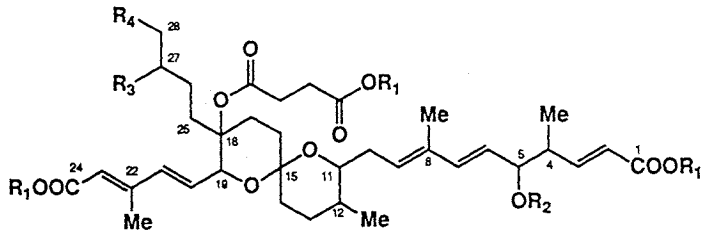
[a] Chemical shifts are given in ppm from TMS as internal standard.
[b] Coupling constants (J in Hz) are given in parentheses.
[c] $CDCl_3$ as solvent.

Table 4. ^{1}H NMR data of desuccinyl derivatives[a~c].

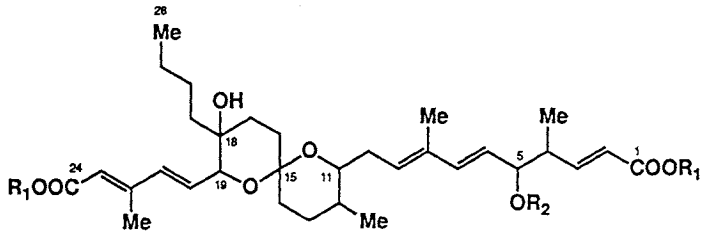
Protons	5	5a	5b	6a	6b
H-2	5.86 (dd, 15.9, 1.5)	5.88 (dd, 15.9, 1.2)	5.84 (br d, 15.6)	5.87 (dd, 15.9, 1.2)	5.82 (dd, 15.9, 1.2)
H-3	7.11 (dd, 15.9, 7.3)	7.01 (dd, 15.9, 7.6)	6.95 (dd, 15.6, 7.3)	7.00 (dd, 15.9, 7.3)	6.95 (dd, 15.9, 7.3)
H-4	2.59 (m)	2.57 (m)	2.69 (m)	2.57 (m)	2.67 (m)
H-5	4.14 (dd, 7.3, 4.9)	4.11 (m)	5.27 (dd, 7.9, 5.5)	4.14 (m)	5.29 (dd, 7.6, 5.2)
H-6	5.49 (dd, 15.6, 7.6)	5.51 (dd, 15.6, 7.3)	5.38 (dd, 15.6, 7.9)	5.51 (dd, 15.6, 7.3)	5.35 (dd, 15.1, 7.6)
H-7	6.18 (d, 15.6)	6.22 (d, 15.6)	6.29 (d, 15.6)	6.43 (d, 15.6)	6.39 (d, 15.1)
H-9	5.44 (dd, 7.3, 7.3)	5.53 (dd, 7.3, 7.3)	5.63 (dd, 6.9, 6.9)	5.61 (dd, 7.3, 7.3)	5.76 (dd, 6.7, 6.7)
H-10	2.24~2.36 (m)	2.25~2.40 (m)	2.18~2.36 (m), 2.41 (m)	2.24~2.41 (m)	2.25~2.35 (m), 2.46 (m)
H-11	3.39 (m)	3.44 (ddd, 10.1, 4.3, 4.0)	3.46 (m)	3.56 (m)	3.47 (m)
H-19	4.00 (d, 9.2)	3.97 (d, 9.2)	3.97 (d, 8.9)	4.21 (br d, 6.1)	5.48 (d, 4.3)
H-20	6.44 (dd, 15.6, 9.2)	6.40 (dd, 15.6, 9.2)	6.40 (dd, 15.6, 8.9)	6.02 (dd, 15.9, 6.1)	6.20 (dd, 15.9, 4.3)
H-21	6.35 (d, 15.6)	6.31 (d, 15.6)	6.31 (d, 15.6)	6.28 (d, 15.6)	6.16 (d, 15.9)
H-23	5.85 (br s)	5.83 (br s)	5.83 (s)	5.80 (br s)	5.75 (br s)
H-28	0.86 (t, 7.0)	0.86 (t, 6.9)	0.86 (t, 7.0)	0.90 (t, 7.3)	0.88 (t, 7.0)
Me-4	1.10 (d, 7.0)	1.09 (d, 6.7)	1.07 (d, 6.7)	1.08 (d, 6.7)	1.03 (d, 7.0)
Me-8	1.71 (s)	1.72 (s)	1.70 (s)	1.72 (s)	1.70 (s)
Me-12	0.78 (d, 6.4)	0.77 (d, 6.4)	0.75 (d, 6.4)	0.86 (d, 7.1)	0.84 (d, 6.7)
Me-22	2.20 (d, 1.2)	2.25 (d, 0.9)	2.28 (s)	2.27 (d, 1.2)	2.28 (d, 1.6)
OMe-1	—	3.73 (s)	3.74 (s)	3.71 (s)	3.71 (s)
OMe-24	—	3.72 (s)	3.72 (s)	3.74 (s)	3.73 (s)
OAc-5	—	—	2.07 (s)	—	2.06 (s)
OAc-19	—	—	—	—	2.10 (s)

[a] Chemical shifts are given in ppm from TMS as internal standard.
[b] Coupling constants (J in Hz) are given in parentheses.
[c] $CDCl_3$ as solvent.

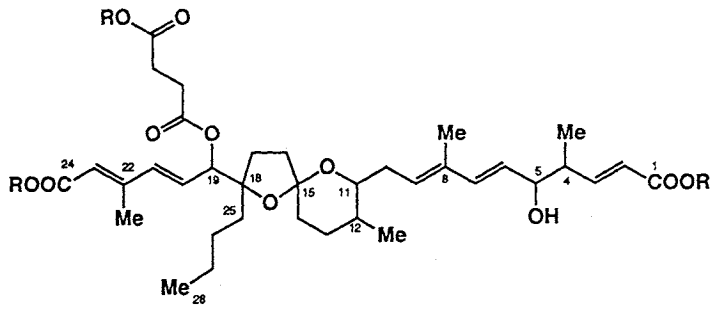
Fig. 4. Structures.



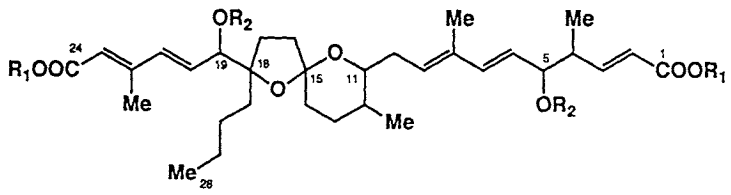
1 $R_1=R_2=R_3=R_4=H$
1a $R_1=Me$, $R_2=R_3=R_4=H$
1b $R_1=Me$, $R_2=Ac$, $R_3=R_4=H$
3 $R_1=R_2=R_4=H$, $R_3=Me$
3a $R_1=R_3=Me$, $R_2=R_4=H$
4 $R_1=R_2=R_3=H$, $R_4=Me$
4a $R_1=R_4=Me$, $R_2=R_3=H$



5 $R_1=R_2=H$
5a $R_1=Me$, $R_2=H$
5b $R_1=Me$, $R_2=Ac$



2 $R=H$
2a $R=Me$



6 $R_1=R_2=H$
6a $R_1=Me$, $R_2=H$
6b $R_1=Me$, $R_2=Ac$

to δ 5.27 ppm for **1b** by acetylation, indicating the presence of the hydroxyl group at C-5 in **1**. Alkaline hydrolysis of **1** gave desuccinated compound **5**, and the following methylation with diazomethane gave dimethyl ester (**5a**), and supported the presence of succinate moiety. Position of the succinate moiety was determined as the quaternary carbon C-18, because high chemical shifts of two oxygenated methine protons, H-11 (δ 3.36) and allylic H-19 (δ 4.51) in **1**, indicated that the oxygens are not acylated. Acetylation of **5a** gave monoacetate (**5b**) and supported the succinate position. Position of *n*-butyl side chain must be C-18, which is only possible quaternary carbon. Based on chemical and spectroscopic evidences described above, the structure of reveromycin A was determined as **1**.

Reveromycin B (**2**) has the same molecular formula $C_{36}H_{52}O_{11}$ as that of reveromycin A (**1**), determined by HRFAB-MS *m/z* 683.3433 $(M+Na)^+$. The IR, UV and SI-MS spectra of **2** are similar to those of **1**. Comparison of the ^{13}C NMR (Table 1) data between **1** and **2** revealed that **2** possessed the same partial structures to those of **1** especially in C-1 ~ C-11, C-19 ~ C-24 and succinate portions. In the ^{13}C NMR of **2**, the signal of spiroketal quaternary carbon C-15 was observed at δ 108.8 ppm. The data suggested that **2** possessed 1,6-dioxaspiro[4.5]decane system[5] instead of 1,7-dioxaspiro[5.5]undecane system in **1**. The ^1H NMR spectrum of **2** (Table 2) analyzed by spin decoupling experiments and ^1H-^1H COSY confirmed the presence of the partial structures speculated from ^{13}C NMR data. In the ^1H NMR of **2**, the oxygenated methine proton H-19 was observed at δ 5.56 (d, J=4.6 Hz), indicating that succinate was situated on C-19 position. Methylation with diazomethane of **2** gave trimethyl ester (**2a**). Alkaline hydrolysis of **2** gave **6**, and subsequently methylated with diazomethane gave dimethyl ester (**6a**). In the ^1H NMR spectrum (Table 4) of **6a**, the methine proton H-19 was shifted upfield by deacylation and observed at δ 4.21 ppm. Acetylation of **6a** gave diacetate (**6b**). Based on these data, the structure of reveromycin B was determined as **2**. Carbon skeleton of **2** is the same as that of **1**. The spiroketal system and the position of succinate are different in structures between **1** and **2**.

Reveromycin C (**3**), was formulated to be $C_{37}H_{54}O_{11}$ by HRFAB-MS *m/z* 697.3574 $(M+Na)^+$; calcd 697.3564. The IR and UV spectra of **3** resembled to those of **1**. In the SI-MS spectrum of **3**, fragmentation ions *m/z* 657 (MH^+-H_2O), 579 $(M^++Na-118)$ and 539 (MH^+-H_2O-118) were observed and fragmentation patterns were similar to those of **1**. The IR and UV spectra of **3** showed similar absorptions to those of **1**. In the ^1H NMR spectrum of **3** (Table 2), four doublet signals of methyl groups were observed at δ 1.07 (Me-4), 0.78 (Me-12), 0.79 (Me-27) and 0.83 ppm (H-28), and triplet methyl signal for H-28 in **1** was absent in **3**. Other proton signals of **3** coincided with those of **1**. In the ^{13}C NMR spectrum (Table 1), some differences were observed at the side chain portion (C-25 ~ C-28) between **1** and **3**. Carbon signals of C-27, C-26 and C-28 of **3** were shifted to low field and C-25 was shifted to high field by α-, β- and γ-effects of the C-27 methyl group. Based on these spectroscopic data, the structure of reveromycin C was determined as **3**, 27-methyl reveromycin A.

Reveromycin D (**4**), has the same molecular formula $C_{37}H_{54}O_{11}$ to that of **3**. The IR, UV and SI-MS spectra were similar to those of other reveromycins (**1** ~ **3**). In the ^1H NMR spectrum of **4** (Table 2), five signals of methyl groups were observed at δ 0.77 (d), 0.85 (t), 1.07 (d), 1.75 (s) and 2.23 (s) ppm. Together with methyl signals, eight olefinic signals, three oxygenated methine signals and signals of succinate moiety were indistinguishable between **1** and **4**. Trimethyl ester (**4a**) of reveromycin D also showed similar ^1H NMR spectrum to that of **1a**. Little chemical shift difference, 0.02 ppm, was observed on triplet methyl signals at 0.84 ppm (H-29 of **4a**) and 0.82 ppm (H-28 of **1a**). These data indicated that only difference in structures between **1** and **4** was the side chain at C-18, and consequently the structure of reveromycin D

was determined as **4**, *n*-pentyl side chain analogue of reveromycin A.

Reveromycins have a unique polyketide like carbon skeleton with two terminal carboxyl groups, a spiroketal, a succinate moiety and a varied side chain. Structural feature of reveromycin B is different from other reveromycins in spiroketal portion and position of succinate. Reveromycin B showed relatively weak biological activities than reveromycins A, C and D[2]. Structure of spiroketal portion or directions of side chains at C-11, C-18 and C-19 and succinate moiety may be important to biological activity. Stereochemical studies are now in progress.

Experimental

Methylation

Reveromycin A (**1**, 10 mg) was dissolved in MeOH (1 ml). Excess diazomethane in ether was added to the solution and stirred for 4 hours at room temperature. The reaction mixture was evaporated to give trimethyl ester (**1a**) in quantitative yield. Other reveromycin derivatives were methylated by the same manner. Methyl esters of reveromycins were also prepared from the complex of reveromycins. The complex (30 mg) was methylated with diazomethane. The mixture of methyl esters were separated by HPLC to yield **1a** (10.0 mg), **2a** (1.1 mg), **3a** (1.1 mg) and **4a** (0.7 mg). HPLC condition was as follows; column: Capcell Pak C_{18} (20 i.d. × 250 mm, Shiseido, Tokyo), solvent system: MeOH - H_2O (9 : 1), flow rate: 4.0 ml/minute, UV detection at 240 nm, retention time: 30, 38, 33 and 41 minutes for **1a**, **2a**, **3a** and **4a**, respectively.

Alkaline Hydrolysis

Reveromycin A (**1**, 15 mg) was dissolved in 3 ml 1 N LiOH solution and stirred for 10 hours at room temperature. The reaction mixture was acidified by 6 N HCl at 0°C to pH 3, and extracted with EtOAc. The extract was washed by aqueous saturated NaCl solution and concentrated *in vacuo* to give **5** (13 mg). Reveromycin B was hydrolyzed by the same procedure.

Acetylation

1a (3 mg) was acetylated with Ac_2O (0.3 ml) - pyridine (0.3 ml) at room temperature for 7 hours. The reaction mixture was concentrated *in vacuo* to give acetate (**1b**) quantitatively. Other reveromycin derivatives were acetylated by similar procedure.

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