GUANIDOLIDE A, A NOVEL ANTIBIOTIC PRODUCED BY STREPTOMYCES HYGROSCOPICUS VAR. CRYSTALLOGENES, THE COPIAMYCIN SOURCE

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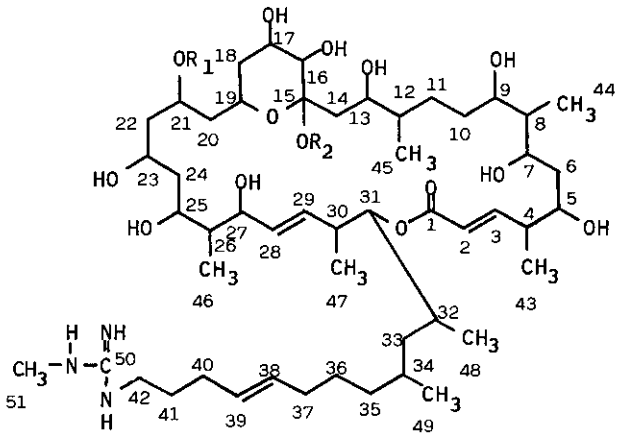
Abstract——— A new antibiotic, guanidolide A (**2**) was isolated, as well as demalonylmethylcopiamycin (**1**), from the mycelial cake of Streptomyces hygroscopicus var. crystallogenes. The structure of the compound (**2**) was determined by spectroscopic evidence.

In the previous paper,[1] we reported the structure determination of demalonylcopiamycin (**3**), a 32-membered polyhydroxy lactone antifungal antibiotic, obtained from the mycelial cake of Streptomyces hygroscopicus var. crystallogenes. Further extensive fractionation of minor components of the same strain led us to the isolation of a new antifungal antibiotic, guanidolide A (**2**), together with a known antifungal antibiotic, demalonylmethylcopiamycin (**1**).[2] We report herein the structure elucidation of the novel antibiotic (**2**).

Copiamycin complex has been separated from condensed methanol extract of the wet mycelial cake.[3] Mother liquor (440 g) of the copiamycin complex was extracted with $CHCl_3$-MeOH (1:1), and the extract (240 g) was chromatographed on silica gel with $CHCl_3$-MeOH as an eluent. The fraction eluted with $CHCl_3$ containing 10% MeOH (12.6 g) was fractionated sequentially by preparative tlc (silica gel, $CHCl_3$:MeOH = 1:1), silica-gel column chromatography (benzene-MeOH), preparative tlc (silica gel, $CHCl_3$:MeOH=3:1), and Sephadex LH-20 column chromatography with acetone-MeOH (1:4) to give demalonylmethylcopiamycin (**1**, 65 mg) and guanidolide A (**2**, 250 mg).

Demalonylmethylcopiamycin (**1**), mp 140-143 °C (colorless prisms from MeOH-H_2O), $[\alpha]_D$ +11° (MeOH), gave FAB-MS showing protonated molecular ion peak $(M+H)^+$, at m/z 986. The FAB-MS of **1** showed a series of characteristic fragment ion peaks of a side



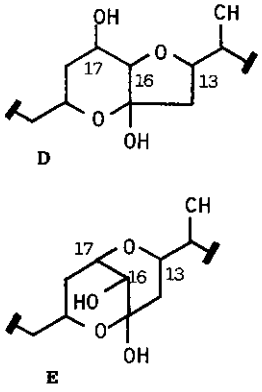
1: R_1=H, R_2= CH_3

3: R_1=R_2=H

4: R_1=$COCH_2COOH$, R_2= H

1'2' 3'

Fig. 1



D

E

Fig. 2

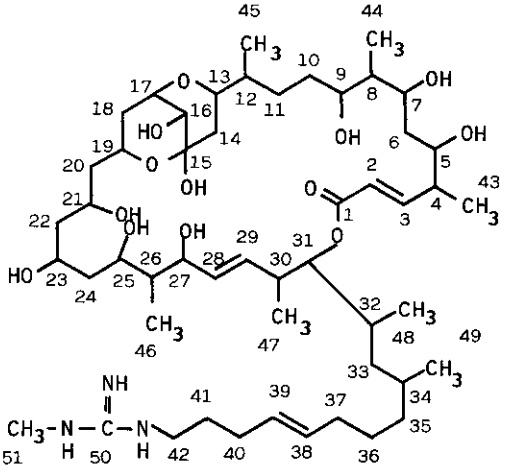


Fig. 3 2

Table 3. Antifungal[a)] spectra of 1, 2, 3 and 4 (MIC μg/ml)

test organism	1	2	3	4
Aspergillus nidulans 21	12.5	50.0	12.5	100.0
Penicillium expansum IFM 40619	6.25	100.0	3.12	100.0
Trichophyton mentagrophytes IFM 40734	0.78	12.5	6.25	3.12
Microsporum gypseum IFM 40727	0.78	25.0	3.12	6.25
Epidermophyton floccosum IFM 40747	0.78	12.5	1.56	0.39

a): agar dilution method (ref. 1)

Table 1. ^{1}H nmr (400 MHz) data of **1**, **2** and **3** in CD_3OD

(ppm) **3**[a]	**1**[b]	(ppm) **2**[b]	
6.86(1H,dd,$J_{3,4}$=9.0 Hz,H-3)	6.86	6.99 (1H,dd,$J_{3,4}$=8.6 Hz)	H-3
5.87(1H,d,$J_{2,3}$=15.7,H-2)	5.87	5.85 (1H,d,$J_{2,3}$=15.7)	H-2
5.48(1H,dd, $J_{29,28}$=16,H-29)	5.47	5.51 (1H, AA' type)	H-29
5.42(1H,dd,H-28)	5.42	5.49 (1H,AA' type,$J_{28,27}$=3.5)	H-28
5.48(1H,m,$J_{38,39}$=16,H-38)	5.48	5.48 (1H,td)	H-38
5.41(1H,m, H-39)	5.41	5.41 (1H,td,$J_{39,38}$=15.4,$J_{39,40}$=6.2)	H-39
4.77(1H,dd,$J_{31,30}$=9,$J_{31,32}$=3,H-31)	4.77	4.81 (1H,dd,$J_{31,32}$=3.0)	H-31
4.16(1H,t like,J=12,H-19)	4.12	4.24 (1H,br t,J=ca.10)	H-19
4.23(1H,br d,J=10,(td like),H-13)	4.27	4.13 (1H,br d,$J_{13,14}$=2 and 9)	H-13
		4.08*(1H,m, $J_{25,24}$=9 and 5)	H-25
4.04(2H,m,H-21)	4.05(1H)	4.05*(1H,m)	H-21
	3.92(2H)	3.95*(1H,m)	H-23
3.84(3H,m,H-27)	3.82(3H)	3.93 (1H,ddd,$J_{27,26}$=8.5,$J_{27,29}$=1.5)	H-27
3.75(1H,td like,J=ca.8 and 3)	3.77(1H)	3.79 (1H,m, $J_{9,10}$= $J_{9,8}$=3)	H-9
3.89(1H,m,H-17)	3.67(1H)	3.80 (1H,m, $J_{17,18}$=5)	H-17
3.68(1H,ddd,$J_{5,6}$=ca.4 and 10,H-5)	3.26(OMe)	3.72 (1H,brtd,$J_{5,4}$=4,$J_{5,6}$=4 and 9)	H-5
		3.66 (1H,ddd,$J_{7,6}$=2.5 and 8,$J_{7,8}$=7)	H-7
3.16(2H,t,$J_{42,41}$=7,H_2-42)	3.17	3.17 (2H,t,$J_{42,41}$=7.2)	H_2-42
3.38(1H,d,$J_{16,17}$=9.2,H-16)	3.49	2.99 (1H,d,$J_{16,17}$=9.3)	H-16
2.84(3H,s, H_3-51)	2.85	2.85 (3H,s)	H_3-51
2.49(1H,m,$J_{30,47}$=6.8,H-30)	2.49	2.52 (1H,m,$J_{30,31}$=8.7)	H-30
2.42(1H,m,$J_{4,43}$=6.8,H-4)	2.40	2.45 (1H,m)	H-4
1.62(m, $J_{14,13}$=10,H-14)		2.30 (1H,dd,$J_{14,14}$=12.9,$J_{14,13}$=9)	H-14
2.07(2H,td, J=ca.8 and 6,H_2-40)	2.08	2.08 (2H,td)	H_2-40
	1.98(2H)	1.97 (2H,brtd,$J_{37,38}$=6.2,$J_{37,36}$=7)	H_2-37
1.96(3H,m,H_2-37,H-32)	1.93(2H)	1.96 (1H,m)	H-32
1.89(1H,ddd like,$J_{18,19}$=ca.2,H-18)	1.88(2H)	1.91 (1H,ddd, $J_{18,18}$=12)	H-18
		1.86 (1H,brdd,$J_{11,10}$=3)	H-11
		1.74*(1H,m, $J_{24,25}$=5)	H-24
1.83(2H,m,H-6)		1.73 (1H,m,$J_{6,6}$=15,$J_{6,5}$=4,$J_{6,7}$=2.5)	H-6
		1.73 (1H,m,$J_{10,11}$=$J_{10,9}$=3)	H-10
		1.69 (1H,m,$J_{11,11}$=11)	H-11
1.63(2H,m,$J_{41,42}$=7.1,H_2-41)		1.65 (2H,m,$J_{41,40}$=7.3)	H_2-41
		1.59 (1H,m,J_{24}=9)	H-24
[1.20-1.65(br m, many signals combined)]		1.56 (1H,m,$J_{34,35}$=5)	H-34
		1.53 (1H,m,$J_{6,7}$=8,$J_{6,5}$=9)	H-6
1.42(m,H-6 and -12)		1.53 (1H,m,$J_{12,13}$=2)	H-12
		1.51 (1H,m,$J_{26,27}$=8.5)	H-26
		1.46*(1H,m,$J_{26,25}$=2)	H-26
		1.47 (1H,m,$J_{8,9}$=3)	H-8
1.53($J_{20,19}$=ca.10,H-20)		1.40-1.70* (4H,m)	H_4-20 and 22
		1.33 (2H,m,$J_{36,35}$=5, $J_{36,37}$=7)	H_2-36
1.36(m,$J_{14,13}$=2,$J_{18,19}$=ca.10, H-14 and -18)		1.31 (1H,m,$J_{14,14}$=12.9,$J_{14,13}$=2)	H-14
		1.31 (1H,m,$J_{18,19}$=2)	H-18
		1.24 (1H,dt,$J_{35,34}$=$J_{35,36}$=5)	H-35
		1.26 (1H,m)	H-33
		1.14 (1H,brd,$J_{10,10}$=14)	H-10
1.08(3H,d,H_3-43)	1.08	1.11 (3H,d, $J_{43,4}$=6.8)	H_3-43
1.00(3H,d,$J_{47,30}$=6.8,H_3-47)	1.01	1.02 (3H,d,$J_{47,30}$=6.8)	H_3-47
		1.09 (1H,m,$J_{35,35}$=15)	H-35
0.90(3H,d,J=ca.7)	0.93(6H)	0.97 (3H,d,$J_{45,12}$=7)	H_3-45
0.89(3H,d,J=6.8)		0.96 (3H,d,$J_{44,8}$=7)	H_3-44
0.87(3H,d,J=6.4)	0.88	0.93 (3H,d,$J_{48,32}$=6.8)	H_3-48
		0.90 (1H,m)	H-33
0.85(3H,d,J=7.0)	0.87	0.87 (3H,d,$J_{49,34}$=6.6)	H_3-49
0.77(3H,d,$J_{46,26}$=7.0,H_3-46)	0.77	0.81 (3H,d,$J_{46,26}$=7)	H_3-46

*: measured at 23 °C, the chemical shift at 40 °C: see Table 2, a): measured at 30 °C, the chemical shift at 40 °C: see ref. (1), b): measured at 40 °C.

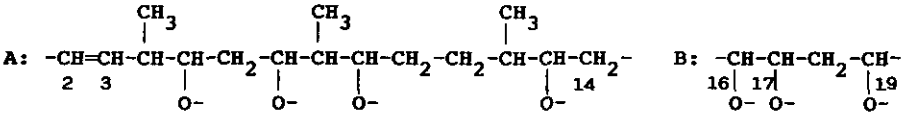
Table 2. ^{13}C nmr data of **1**, **2**, **3** and **4**, and ^{1}H nmr data of **2** in CD_3OD at 40°C

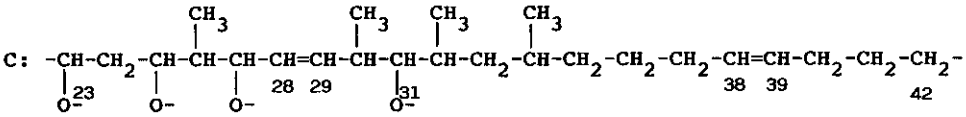
Signal No.	Assignment[a]		**4**	**3**	**1**	**2**	(^{1}H nmr)*	2(OH/OD, ppm)[b]
1		q	10.48	10.15	10.02	11.37	(0.96,C44)	0.05
2		q	11.25	10.92(C46)	10.74	11.66	(0.81,C46)	0
3		q	14.47	14.35	13.92	12.24	(0.97,C45)	0
4		q	15.01	14.61	14.52	14.59	(0.93,C48)	0
5	43	q	16.53	16.37(C43)	16.30	17.03	(1.11,C43)	0.06
6	47	q	17.63	17.47(C47)	17.37	17.54	(1.02,C47)	0.02
7		q	20.61	20.41	20.37	20.37	(0.87,C49)	0
8	40	t	27.60	27.73	27.73	27.68	(1.33,C36)	0.10
9	$N-CH_3$		28.23	28.22(NCH_3)	28.29	28.29	(2.85, NCH_3)	0.19
10		t	29.76	29.73(C41)	29.77	29.72	(1.65,C41)	0.11
11		t	30.54	30.44(C40)	30.43	30.39	(2.08,C40)	0
12		d	30.54	30.44	30.56	30.49	(1.56,C34)	0
13		t	30.57	31.00	31.10	24.49	(1.14, 1.73,C10)	0.08
14		d	32.68	32.36	32.37	32.43	(1.96,C32)	0.09
15		t	33.27	33.40	33.61	32.26	(1.69,1.86,C11)	
16		t	33.62	33.61(C37)	34.99	33.56	(1.97,C37)	0.07
17		t	37.14	37.43	37.56	37.51	(1.09, 1.24,C35)	0.09
18		t	39.23	38.76	38.65	40.34	(1.53, 1.73,C6)	0
19	30	d	39.58	40.00	40.09	39.79	(2.52,C30)	0.08
20		d	40.44	40.47	40.71	40.34	(1.47,C8)	0.10
21	42	t	41.08	41.17	41.19	41.91	(3.17, C42)	0.19
22		t	41.44	41.35	41.19	40.58	(1.31, 2.30, C14)	0.10
23		t	41.78	41.85(C42)	41.94	41.09	(1.31, 1.91,C18)	0.12
24		t	41.85	41.85	41.94	42.11	(1.59, 1.76,C24)	0.19
25		t	42.20	42.44	42.60	42.44	(0.90, 1.26,C33)	0
26		t	43.01	43.45	43.75	44.33	(1.40-1.67,C20 or C22)	0.14
27	4	d	43.23	43.77(C4)	44.01	43.72	(2.45,C4)	0.08
28		d	44.45	44.63	45.09	44.58	(1.53,C12)	
29		t	44.58	46.63	45.09	45.53	(1.62, 1.71,C20 or C22)	0.20
30		d	45.24	45.31	45.46	44.72	(1.51,C26)	0.09
31	2'	t	46.10	*****	*****	*****		
32	OMe		*****	*****	47.74	*****		
33		d	65.42	65.70	65.64	65.69	(4.24,C19)	0
34		d	65.42	65.80	66.14	67.21	(4.00,C23)	c)
35		d	68.61	68.27	67.92	69.63	(3.79,C9)	0.19
36		d	69.41	69.41(3.85)*	69.15	69.87	(4.10,C25)	0.16
37	21	d	70.69	66.21	66.14	66.98	(4.05,C21)	c)
38		d	71.77	72.21(3.85)*	71.92	73.91	(3.66,C7)	0.18
39		d	72.20	72.21(3.85)*	71.92	77.52	(4.13,C13)	0.10
40		d	74.42	74.81(3.75)*	75.08	78.22	(3.80,C17)	0.08
41		d	74.99	75.15(3.85)*	75.08	75.47	(3.93,C27)	0.24
42		d	75.45	75.34(C5)	75.76	75.71	(3.72,C5)	0.30
43	16	d	76.73	76.70(C16)	76.41	79.20	(2.99, C16)	0.28
44	31	d	79.82	79.49(C31)	79.54	79.65	(4.81, C31)	0
45	15	s	99.29	99.51	102.18	99.17	(C15)	0.18
46	2	d	122.66	122.77	122.86	122.42	(5.85, C2)	0
47	39	d	129.37	129.24	129.25	129.23	(5.41,C39)	0
48	38	d	132.20	132.27	132.34	132.21	(5.48,C38)	0.10
49	28	d	134.14	134.23	134.43	133.36	(5.49,C28)	0
50	29	d	134.19	134.84	135.12	133.99	(5.51,C29)	0
51	3	d	151.83	151.90	152.05	151.92	(6.99, C3)	0.09
52	50	s	157.47	157.56	157.66	157.59	(C50)	0.24
53	1	s	167.47	167.56	167.66	167.55	(C1)	0.12
54	1'	s	170.80	*****	*****	*****		
55	3'	s	173.32	*****	*****	*****		

a): Assignments of carbon signals of **4** with Fukushima et al. (ref. 11), b): deuterium-induced shift (in CH_3OH(δ 50.08)/CD_3OD(δ 48.80), ref. 10); digital resolution: 0.006 ppm, c): the shifted signal may be overlapping with the other signal, *: Cross-peak with proton signal (ppm) on ^{1}H-^{13}C COSY spectrum.

chain located at the C-31 position (C32 - guanidyl group) as follows: m/z 252, 224, 210, 182, 168 and 154. These fragment ions were also observed in the case of **3** and copiamycin (**4**).[1,3] The ^{1}H nmr spectrum indicated the presence of methoxyl group (δ 3.26, Table 1). The ^{13}C nmr spectrum (100 MHz) showed the signals of 52 carbons analyzed by off-resonance decoupling technique as well as by comparison of the spectrum with those of **3** and **4** (Table 2). In the ^{13}C nmr spectrum, the chemical shifts of the carbons of **1** were similar to those of the relevant carbons of **3** except C-15 signal. Downfield shift of the C-15 signal of **1** suggested that the carbon must be ketal carbon. These data indicated that the compound (**1**) is 15-O-methyldemalonylcopiamycin. The compound, named as demalonylmethylcopiamycin, was derived from copiamycin (**4**) by Takesako et al.[2] The identity of **1** with demalonylmethylcopiamycin was carried out by direct comparison with the authentic specimen.[4]

Guanidolide A (**2**) is colorless amorphous powder, $[\alpha]_D$ +16° (MeOH). The molecular formula of **2** was determined to be $C_{51}H_{91}O_{13}N_3$ from the high-resolution FAB-MS (m/z: 954.6617 $[M+H]^+$). This molecular formula corresponds to dehydrogenated demalonylcopiamycin. The compound (**2**) showed the following spectra; ir ν_{max}^{KBr} cm^{-1}: 3375 (br), 1715 (sh), 1690 (sh), 1650; uv λ_{max}^{MeOH} nm (log ε): 211 (4.23) and 225 (infl. 3.95). The ^{13}C nmr spectrum indicated the presence of 51 carbons: 8 methyl carbons (H-$\overset{|}{C}$-CH_3 x 7, -$\overset{|}{N}$-CH_3 x 1), 7 methine carbons, 15 methylene carbons, 12 carbons bearing hydroxy, ether, or ester group, a carbon forming a hemiketal group, 6 olefinic carbons, a guanidyl carbon and an acyl carbonyl carbon (Table 2). The following partial structures A (C2 - C14), B (C16 - C19) and C (C23 - C42) were determined by comparing the ^{1}H and ^{13}C nmr spectra of **2** with those of **3** and **4**, and by using the following ^{1}H nmr technique: 2D nmr spectroscopy, double pseudo-INDOL difference spectroscopy, double spin-tickling difference spectroscopy, double spin-tickling-decoupling (triple resonance) difference spectroscopy,[5] etc. (Table 1).





The characteristic proton and carbon signals in the above partial structures as well as fragment ions in FAB-MS are described below.

Structure A: In the ^{1}H nmr of **2**, the chemical shifts of the olefinic proton signals at δ 5.85 and 6.99 ppm assignable to H-2 and H-3, respectively, suggest the presence of a double bond conjugated with acyl carbonyl group. An E-orientation of the double bond is evident from the large coupling constants (15.7 Hz) between H-2 and H-3. One proton double doublet signal at δ 2.30 ppm (9 and 12.9 Hz, observed clearly) and signal at δ 1.31 ppm (combined with other signals) can be assigned to H_2-14 which is on a carbon vicinal to the hemiketal position (C-15).

Structure B: One proton doublet signal at δ 2.99 ppm can be assigned to H-16 which is on a carbon vicinal to the hemiketal carbon by comparing the spectrum with those of **3** and **4**.

Structure C: The presence of dialkylguanidyl group in **2** is indicated by ^{13}C nmr spectrum (δ 157.59 ppm, guanidyl carbon) and negative Sakaguchi test.[6] Signals due to N-methyl and N-methylene group (42-H_2) were also observed at δ 2.85 and 3.17 ppm in the ^{1}H-nmr spectrum, respectively. In FAB-MS, a series of fragment ion peaks of a side chain,[7] located at C-31, indicates that the side chain of **2** is in the same structure as of **1**. An E-orientation of the double bond (≻C(38)=C(39)≺) is concluded from large coupling constant (15.4 Hz) between H-38 and H-39. An E-orientation of the double bond (≻C(28)=C(29)≺) is also concluded from the coupling constant (15 Hz) between H-28 (δ 5.52 ppm, dd, in CD_3OD) and H-29 (δ 5.66 ppm, dd) of its peracetate (**2a**, FAB-MS m/z 1332 $[M+H]^+$). The chemical shift of H-31 (δ 4.81 ppm) indicates that the oxygen atom at C-31 is formed as ester linkage.

Remaining part (-CH_2- x 2, -O-$\overset{|}{C}$-H x 1) was elucidated as -CH_2-CH(O)-CH_2- by triple resonance experiments, and it is supported by the experimental result indicating that the part is located between the partial structures B and C (C20 - C22).

From these data, it was confirmed that the positions of functional groups (oxygen, methyl group and bouble bond) of **2** are the same as of **3** (except stereochemistry), and that **2** must be dehydrogenated demalonylcopiamycin which has an ether linkage

in the structure.

The position of the ether linkage is suggested from the following nmr data. One of the proton signals at C-14 and the H-16 signal were caused downfield (- 0.68 ppm) and upfield shift (+ 0.39 ppm), respectively, compared with the corresponding signals of **3** (Table 1). The ^{13}C nmr spectrum of **2** being compared with that of **3**, the C-13, C-16 and C-17 signals of **2** were shifted to downfield[8] (more than 2.5 ppm,[9] Table 2). These data indicate that the ether linkage is located between C-13 and C-16 or C-13 and C-17 positions to form the partial structures D or E, respectively (Fig. 2).

The evidence for the partial structure E was given by deuterium-induced shift on the ^{13}C nmr spectrum.[10] The shift was clearly observed at C-16, while the shifts of C-13 and C-17 were small (Table 2).

From the above result, the structure of guanidolide A is elucidated as structure **2** (Fig. 3).

Antifungal activities of the compounds were shown in Table 3. The details of bioactivity of these macrolide antibiotics will be presented in a subsequent paper.

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REFERENCES AND FOOTNOTES

1. T. Fukai, T. Nomura, J. Uno, and T. Arai, Heterocycles, 1986, **24**, 3351.
2. K. Takesako, T. Beppu, T. Nakamura, and A. Obayashi, J. Antibiotics, 1985, **38**, 1363.
3. T. Arai, J. Uno, I. Horimi, and K. Fukushima, J. Antibiotics, 1984, **37**, 103.
4. It was indicated from ^{1}H nmr spectrum that Takesako's sample includes ca. 40% impurity. All the signals of **1** were detected on ^{1}H and ^{13}C nmr spectrum of the authentic sample.
5. T. Fukai, C. Takahashi, Y. Hano, T. Nomura, J. Uno, and T. Arai, Symposium Papers of 29th Symposium on the Chemistry of Natural Products, 1987, p. 608, Sapporo.
6. J.D. Mold, J.M. Ladino, and E.J. Schantz, J. Am. Chem. Soc., 1953, **75**, 6321.
7. FAB-MS of **2**; m/z 252, 224, 210, 182, 168 and 154.

8. Assignments of all the carbon signals except the two carbons (C-20 and C-22) of **2** were performed by the carbon-proton chemical shift correlated 2D nmr (^{1}H-^{13}C COSY) spectrum.

9. It was shown on ^{1}H-^{13}C COSY spectrum that the C-17 signal of **3** is assignable at 69.41, 72.21 or 75.15 ppm. The C-13 signal of **3** may be assigned at 65.70, 65.80, 66.21 or 68.27 ppm.

10. P.E. Pfeffer, K.M. Valentine, and F.W. Parrish, J. Am. Chem. Soc., 1979, **101**, 1265.

11. K. Fukushima, T. Arai, S. Iwasaki, M. Namikoshi, and S. Okuda, J. Antibiotics, 1982, **35**, 1480.

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