DEMALONYLCOPIAMYCIN, A NEW ANTIBIOTIC PRODUCED BY *STREPTOMYCES HYGROSCOPICUS* VAR. *CRYSTALLOGENES*, THE COPIAMYCIN SOURCE

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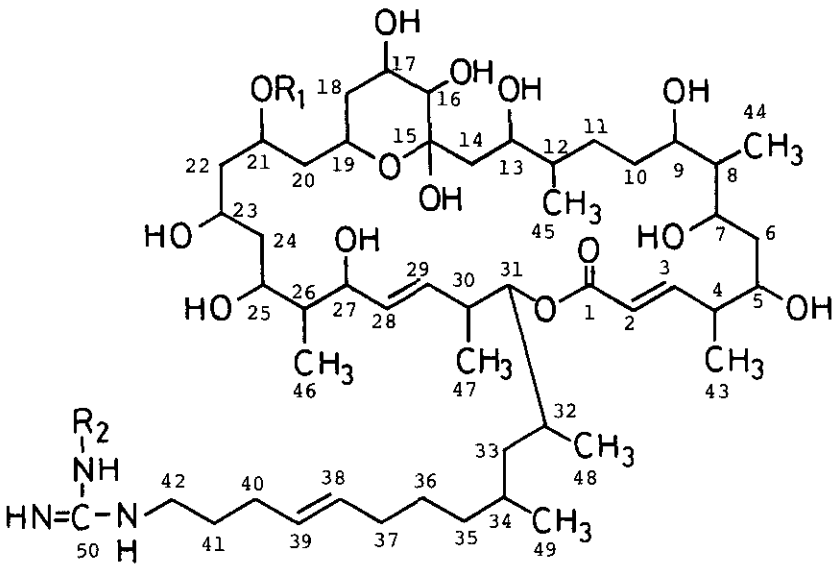
Abstract ——— Demalonylcopiamycin (**1**) was isolated from the mycelial cake of *Streptomyces hygroscopicus* var. *crystallogenes*. The structure was determined by the identification of **1** with alkaline hydrolysis product of copiamycin (**2**). The new antibiotic (**1**) was found to be more active against Gram-positive bacteria than copiamycin (**2**), while slightly more active against fungi.

In the previous papers,[1-5] it was reported that *Streptomyces hygroscopicus* var. *crystallogenes*, produces several minor components in addition to copiamycin (**2**), a macrocyclic lactone antifungal antibiotic, and one of the minor components was designated as neocopiamycin A (**3**). Copiamycin and neocopiamycin A were found to be synergistic with chlorinated imidazole antimycotics.[6] This paper describes the isolation, and the structure determination of demalonylcopiamycin (**1**) as well as its antibacterial and antifungal spectra. The wet mycelial cake (8 Kg)[5] was extracted with methanol and the extract was evaporated under reduced pressure to give a solid (900 g). The solid (100 g) was chromatographed on silica gel, benzene-methanol as an eluent, and each fraction was checked by tlc. The fraction eluted with benzene containing 50% methanol was fractionated sequentially by Sephadex LH-20 column chromatography with methanol and by preparative tlc (silica gel, developing solvent mixture, 2-butanol:H_2O=5:1), and gave crude demalonyl-copiamycin (18 mg). This crude compound was purified by Sephadex LH-20 column chromatography (acetone:methanol=1:1) to give **1** (5 mg).



1: $R_1 = H$, $R_2 = CH_3$

2: $R_1 = \overset{1'}{C}O\overset{2'}{C}H_2\overset{3'}{C}OOH$, $R_2 = \overset{51}{C}H_3$

3: $R_1 = COCH_2COOH$, $R_2 = H$

Demalonylcopiamycin (**1**) was obtained as colorless powder, gave the FAB-MS showing the protonated molecular ion peak, MH^+, at m/z 972, which is smaller by 86 mass units than that of copiamycin (**2**). The ^{13}C nmr spectrum of **1** indicated the presence of 51 carbon atoms, and the compound (**1**) was positive to Sakaguchi reaction. These results suggest the composition of demalonylcopiamycin to be $C_{51}H_{93}O_{14}N_3$. The compound (**1**) showed the following spectra; ir ν_{max}^{KBr} cm^{-1}: 3400 (br), 1710, 1660 (sh), 1640, 1600 (sh); uv λ_{max}^{EtOH} nm (log ε): 222 (3.24), 320 (infl. 2.53); FAB-MS m/z: 972 (M+1), 954, 448, 408, 360, 348, 320, 294, 282, 252, 224, 210, 182, 168, 154. In the FAB-MS of **1**, the fragmentation pattern was similar to that of copiamycin (**2**).[4,5] The ^{13}C nmr spectrum (100 MHz) of **1** was analysed by off-resonance decoupling technique as well as by comparison of the ^{13}C nmr spectra of **2**[4], neocopiamycin A (**3**),[5] and azalomycin F_{4a} (**4**).[7] In the spectrum, it was indicated that the 51 carbon atoms were composed of 8 methyl carbons, 22 methylene and methine carbons, 12 carbons bearing hydroxyl, ether, or ester groups, a carbon forming a hemiketal group, 6 olefinic carbons, a guanido carbon, and a acyl carbonyl carbon (Table 1). The ^1H nmr spectrum of **1** (400 MHZ) was analysed by

Table 1. ^{13}C-NMR data of demalonylcopiamycin (1)[a] and copiamycin (2)[b] in CD_3OD

Signal No.	1 Chemical shift (ppm)	Assign-ment	2 Chemical shift (ppm)	Signal No.	1 Chemical shift (ppm)	Assign-ment	2 Chemical shift (ppm)
1	10.15 (q)		10.48 (q)	27	44.63 (d)		44.67 (d)
2	10.92 (q)		11.26 (q)	28	45.31 (d)		45.67 (d)
3	14.35 (br)		14.46 (q)	29	46.63 (br t)		44.78 (t)
4	14.61 (q)		15.03 (q)	30	———	2'	46.10 (t)
5	16.37 (q)	43	16.53 (q)	31	65.70 (d)		65.64 (d)
6	17.47 (q)	47	17.69 (q)	32	65.80 (d)		65.71 (d)
7	20.41 (q)		20.69 (q)	33	66.21 (d)	21	70.95 (d)
8	27.73 (t)	40	27.73 (t)	34	68.27 (d)		68.81 (d)
9	28.22 (q)	51	28.35 (q)	35	69.41 (d)		69.71 (d)
10	29.73 (t)		29.89 (t)	36	72.21 (d)		72.05 (d)
11	30.44 (t+d)		30.63 (t+d)	37	72.21 (d)		72.52 (d)
12	31.00 (br t)		30.75 (t)	38	74.81 (d)		74.81 (d)
13	32.36 (d)		32.76 (d)	39	75.15 (br d)		75.41 (d)
14	33.40 (t)		33.42 (t)	40	75.34 (br d)		75.75 (d)
15	33.61 (t)		33.75 (t)	41	76.70 (d)	16	77.02 (d)
16	37.43 (t)		37.34 (t)	42	79.49 (d)	31	80.08 (d)
17	38.76 (br)		39.32 (t)	43	99.51 (s)	15	99.73 (s)
18	40.00 (d)	30	39.80 (d)	44	122.77 (d)	2	123.22 (d)
19	40.47 (d)		40.62 (d)	45	129.24 (d)	39	129.97 (d)
20	41.17 (t)	(42)	41.24 (t)	46	132.27 (d)	38	132.79 (d)
21	41.35 (br)		41.58 (t)	47	134.23 (d)	28	134.80 (d)
22	41.85 (t)		41.98 (t)	48	134.84 (d)	29	134.89 (d)
23	41.85 (t)		42.02 (t)	49	151.90 (d)	3	152.52 (d)
24	42.44 (t)		42.42 (t)	50	157.56 (s)	50	158.29 (s)
25	43.45 (t)		43.21 (t)	51	167.56 (s)	1	168.22 (s)
26	43.77 (d)	4	43.45 (d)	52	———	1'	171.59 (s)
				53	———	3'	174.10 (s)

a): Digital resolution = 0.0073 ppm

b): Data from Fukushima *et al.* (ref. 4)

Table 2. ^{1}H-NMR data of demalonylcopiamycin (1)[a] and copiamycin (2)[4] in CD_3OD at 40 °C

	1			2
Signal No.	Chemical shift (ppm)	multiplicity and coupling constant	Assignment	Chemical shift (ppm)
1	6.88	1H,dd,$J_{3,2}$=15.7,$J_{3,4}$=9.2 Hz	H-3	6.93
2	5.87	1H,d,$J_{2,3}$=15.7 Hz	H-2	5.88
3	5.50	1H,dd,$J_{29,28}$=16.0,$J_{29,30}$=5 Hz	H-29	5.53
4	5.48	1H,m,$J_{38,39}$=15.5 Hz	H-38	5.48
5	5.45	1H,dd,$J_{28,29}$=16.0,$J_{28,27}$=7 Hz	H-28	5.45
6	5.41	1H,m,$J_{39,38}$=15.5 Hz	H-39	5.42
7	———		(H-21)	5.23
8	4.77	1H,dd,$J_{31,30}$=6.0,$J_{31,32}$=3.0 Hz	H-31	4.75
9	4.22	1H,d like,J=10 Hz		4.15 (d like)
10	4.17	1H,t like,J=12 Hz	H-19	4.09 (t like)
11	4.04	1H,m	H-21	———
12	3.85	1H,m	H-25	
13	3.87	1H,m	H-27	3.88
14	3.89	1H,m,J=9.2 Hz	H-17	3.86
15	3.85	4H,m		3.84 (3H,m)
16				3.74 (1H,m)
17	3.70	1H,m	H-5	3.75
18	3.38	1H,d,$J_{16,17}$=9.2 Hz	H-16	3.37
19	———		H_2-2'	3.23
20	3.17	2H,t,$J_{42,41}$=7.1 Hz	H_2-42	3.17
21	2.84	3H,s	N-CH_3	2.84
22	2.49	1H,m,$J_{30,47}$=6.8 Hz	H-30	2.53
23	2.42	1H,m,$J_{4,3}$=9.2,$J_{4,43}$=6.8 Hz	H-4	2.47
24	2.07	2H,m	H_2-40	2.08
25	1.97	2H,m	H_2-37	1.98
26	1.93	1H,m	H-32	1.91
27	1.92	1H,m	H-18	
28	1.63	2H,m,$J_{41,42}$=7.1 Hz	H_2-41	1.65
29	1.42	1H,m	H-26	
30	1.38	1H,m	H-18	
31	1.09	3H,d,$J_{43,4}$=6.8 Hz	H_3-43	1.12
32	1.00	3H,d,$J_{47,30}$=6.8 Hz	H_3-47	1.00
33	0.91	3H,d(combined with the signal at δ 0.90)		0.91
34	0.90	3H,d(combined with the signal at δ 0.91)		0.87-0.89
35	0.87	3H,d(combined with the signal at δ 0.85)		(2Me)
36	0.85	3H,d(combined with the signal at δ 0.87)		0.87
37	0.77	3H,d,J=7.0 Hz	H_3-46	0.79

a): Digital resolution = 0.18 Hz

Table 3. Antibacterial spectra of demalonylcopiamycin (1) and related compounds (MIC (μg/ml))

Test organism	1	2[a)]	3[a)]
Bacillus subtilis PCI 219 IFM 2060	3.13	>100.0	12.5
Micrococcus luteus IFM 2066	1.56	100.0	6.25
Staphylococcus aureus 209P IFM 2014	3.13	>100.0	12.5
Staphylococcus aureus 67	3.13		
Staphylococcus aureus 74	3.13		
Staphylococcus aureus Smith IFM 2018	3.13		
Staphylococcus aureus Rosa IFM 2019	3.13		
Staphylococcus aureus Yamaguch	3.13		
Staphylococcus albus IFM 2013	6.25	>100.0	12.5
Staphylococcus citreus IFM 2025	3.13	>100.0	12.5
Streptococcus faecalis IFM 2001	6.25	>100.0	100.0
Mycobacterium smegmatis IFM 2051	100.0		
Streptomyces paraguayensis IFM 1148	12.5	>100.0	50.0
Escherichia coli F_1 IFM 3002	>100.0	>100.0	>100.0
Klebsiella pneumoniae IFM 3008	6.25	>100.0	25.0
Proteus vulgaris IFM 3014	>100.0	>100.0	>100.0
Pseudomonas aeruginosa IFM 3011	>100.0	>100.0	>100.0
Salmonella typhimurium IFM 3023	>100.0	>100.0	>100.0
Serratia marcescens IFM 3027	>100.0	>100.0	>100.0

a) Data from T. Arai et al. (ref. 5).

Table 4. Antifungal spectra of demalonylcopiamycin (1) and related compounds (MIC (μg/ml))[a)]

Test organism	1	2[b)]	3[b)]
Candida albicans IFM 40001	3.13	12.5	1.56
Candida albicans IFM 40002	3.13	25.0	3.13
Candida albicans IFM 40003	6.25	25.0	3.13
Candida albicans IFM 40004	12.5	100.0	6.25
Candida albicans IFM 40005	6.25	25.0	1.56
Candida albicans IFM 40007	3.13	12.5	3.13
Candida albicans IFM 40008	6.25	12.5	1.56
Candida albicans 7N IFM 40009	12.5	100.0	6.25
Candida guilliermondii IFM 40017	6.25	100.0	3.13
Candida tropicalis IFM 40018	100.0	100.0	3.13
Candida krusei IFM 40019	12.5	100.0	3.13
Candida parapsilosis IFM 40020	100.0	100.0	3.13
Candida stellatoidea IFM 40021	6.25	100.0	3.13
Candida utilis IFM 40099	12.5	>100.0	12.5
Cryptococcus neoformans IFM 40037	1.56	1.56	< 0.78
Cryptococcus neoformans IFM 40038	3.13	1.56	< 0.78
Cryptococcus neoformans IFM 40047	3.13	6.25	1.56
Torulopsis glabrata IFM 40065	25.0	100.0	6.25
Geotrichum candidum IMF 40068	25.0	1.56	1.56

a) Agar dilution method, medium: Yeast morphology agar, incubation: 48 h to 4 days at 27°C, depending on test strain. b) Data from T. Arai et al. (ref. 5).

Table 5. Antifungal spectra of demalonylcopiamycin (**1**) and related compounds (MIC (μg/ml))[a)]

Test organism	**1**	**2**[b)]	**3**[b)]
Aspergillus nidulans 21	12.5	100.0	6.25
Aspergillus flavus 23	100.0	100.0	6.25
Aspergillus fumigatus 25	12.5	>100.0	25.0
Aspergillus niger IFM 40606	3.13		
Aspergillus oryzae IFM 40607	100.0	>100.0	6.25
Aspergillus versicolor 26	6.25	>100.0	12.5
Penicillium expansum IFM 40619	3.13	100.0	3.13
Microsporum gypseum IFM 40727	3.13	6.25	1.56
Microsporum canis IFM 40729	3.13	3.13	1.56
Trichophyton rubrum IFM 40732	1.56	3.13	0.39
Trichophyton rubrum IFM 40733	3.13		
Trichophyton mentagrophytes IFM 40734	6.25		
Trichophyton mentagrophytes IFM 40735	3.13		
Trichophyton mentagrophytes IFM 40737	3.13	3.13	1.56
Trichophyton mentagrophytes Kamiyama	3.13	3.13	1.56
Epidermophyton floccosum IFM 40747	1.56	0.39	0.78
Sporothrix schenckii IFM 40750	3.13	50.0	6.25
Sporothrix schenckii IFM 40751	3.13		
Fonsecaea pedrosoi IFM 40756	12.5	100.0	3.13

a) Agar dilution method, medium: Sabouraud dextrose (2%) agar, incubation: 48 h to 5 days at 27°C, depending on test strain. b) Data from T. Arai et al. (ref. 5).

comparing it with those of **2**,[4] **3**,[5] and **4**,[7] as well as by the following techniques: ^{1}H 2D nmr spectroscopy, double INDOR difference spectroscopy,[8] and double spin-tickling difference spectroscopy.[9] The results are listed in Table 2. The above results suggest that **1** is demalonylcopiamycin. The compound (**1**) was derived from copiamycin (**2**)[4,10] by the following procedures: A mixture of **2** (196 mg), 0.01 N sodium hydroxide solution (50 ml), and methanol (50 ml) was kept at room temperature for 24 h, and the ammonium chloride (30 mg) was added. The product was purified by preparative tlc (silica gel, 2-butanol:H_2O=5:1) to give **1** (110 mg) and **2** (38 mg). The ^{1}H nmr, ^{13}C nmr, and ir spectra, and tlc of **1**, thus obtained, were identical with those of demalonylcopiamycin (**1**).

The minimum inhibitory concentration (MIC) of demalonylcopiamycin (**1**) and related compounds on bacteria, yeast and fungi were determined by the methods described in the previous paper,[5] and the antimicrobial spectra are given in Tables 3, 4, and 5. The tables show that demalonylcopiamycin (**1**) is active against a wide range of yeast, fungi and Gram-positive bacteria but Gram-negative bacteria. The compound (**1**) was also found to be more active against Gram-positive bacteria than copiamycin (**2**), while slightly more active against fungi.

Further evaluation of **1** is now in progress both *in vitro* and *in vivo*.

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