STUDIES ON MACROCYCLIC LACTONE ANTIBIOTICS. VI[1]

SKELETAL STRUCTURE OF COPIAMYCIN

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Skeletal structure of copiamycin (**1**) ($C_{54}H_{95}N_3O_{17}$), a potent antifungal antibiotic, was determined from the physicochemical properties of this compound and of its degradation products. This compound consists of 32-membered polyhydroxy lactone ring, an α,β-unsaturated ester group, as well as a side chain with a disubstituted guanidine moiety as its terminal. One of the hydroxyl groups (presumably at C-19) forms a hemiketal ring with the keto group at C-15, and another (at either C-21 or C-23) forms a hemiester with a malonic acid moiety.

The antibiotic copiamycin (**1**) was discovered in 1965 by ARAI *et al.* as the product of *Streptomyces hygroscopicus* var. *crystallogenes*,[2] and its antimicrobial activities[3] as well as its physicochemical properties[2] are known to resemble those of azalomycin Fs.[1,4~6]

In the course of our studies on the structures of azalomycins F_{4a},[4~6] and F_{3a} and F_{5a}[1] we became interested also in the structure of copiamycin. This paper report the skeletal structure of copiamycin assigned as **1** from the physicochemical properties of this compound and of its chemical degradation products. The structure was characterized by 32-membered polyhydroxy lactone ring, a guanidine, intramolecular hemiketal ring and a hemiester of a malonic acid. These structural feature were shown to be largely common to those of azalomycin Fs.[1,4~6]

Physicochemical Properties of Copiamycin

Copiamycin obtained by the described procedure[2] was further purified for the measurement of its physicochemical properties by thin-layer chromatography (TLC) developed with 2-butanol - water (4: 1), followed by recrystallization from aqueous methanol.

The UV spectrum of copiamycin in methanol exhibited a maximum at 220 nm (ε 20,600). This suggested the presence of an α,β-unsaturated acid (or ester) group. The IR spectrum indicated the presence of multiple hydroxyl (ν_{max}^{KBr} 3600~

Fig. 1. The structure of copiamycin.

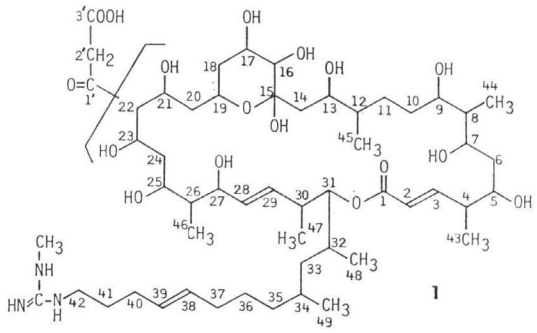

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Table 1. ^{13}C NMR data for copiamycin in $^{12}CD_3OD$.

Signal No.	Chemical shift (ppm)	Multiplicity	Assignment[a]	Signal No.	Chemical shift (ppm)	Multiplicity	Assignment[a]
1.	10.48	q		27.	44.67	d	
2.	11.26	q		28.	44.78	t	
3.	14.46	q		29.	45.46	d	
4.	15.03	q		30.	46.10	t	2′
5.	16.53	q	43	31.	65.64	d	
6.	17.69	q	47	32.	65.71	d	
7.	20.69	q		33.	68.81	d	
8.	27.73	t	40	34.	69.71	d	
9.	28.35	q	*N*–CH_3	35.	70.95	d	[b]
10.	29.89	t		36.	72.05	d	
11.	30.63	t+d		37.	72.52	d	
12.	30.75	t		38.	74.81	d	
13.	32.76	d		39.	75.41	d	
14.	33.42	t		40.	75.75	d	
15.	33.75	t		41.	77.02	d	16
16.	37.34	t		42.	80.08	d	31
17.	39.32	t		43.	99.73	s	15
18.	39.80	d	30	44.	123.22	d	2
19.	40.62	d		45.	129.97	d	39
20.	41.24	t	42	46.	132.79	d	38
21.	41.58	t		47.	134.80	d	28
22.	41.98	t		48.	134.89	d	29
23.	42.02	t		49.	152.52	d	3
24.	42.42	t		50.	158.29	s	[c]
25.	43.21	t		51.	168.22	s	1
26.	43.45	d	4	52.	171.59	s	1′
				53.	174.10	s	3′

[a] Assignments were made by ^1H-^{13}C selective decoupling experiments.
[b] The carbon bearing malonyl hemiester.
[c] Guanido carbon.

2700 cm^{-1}, broad strong) and carbonyl (1700~1500 cm^{-1}, broad strong) groups as demonstrated in the structures of azalomycin Fs.[1,4~6] The ^{13}C NMR (100 MHz) and ^1H NMR (400 MHz) signals of copiamycin are assigned from their chemical shifts as well as by extensive decoupling experiments, and are listed in Tables 1 and 2. These NMR data indicated that copiamycin is composed of 54 carbons including 8 methyl carbons, 23 methylene and methine carbons, 12 carbons bearing hydroxyl or acyloxy groups, a carbon forming a hemiketal group, 6 olefinic carbons, a guanido carbon and 3 acyl carbonyl carbons.

Partial Structures of Copiamycin

The following partial structures A to E were elucidated from the spectroscopic data of copiamycin.

Structure A (C-1 to C-5): A UV absorption maximum of copiamycin (**1**) appearing at 220 nm indicated the presence of an α,β-unsaturated acid (or ester) moiety. In its ^1H NMR spectrum signals at δ 5.87 and 6.92 (signals No. 2 and 1 in Table 2, respectively) also suggested the conjugation of the ole-

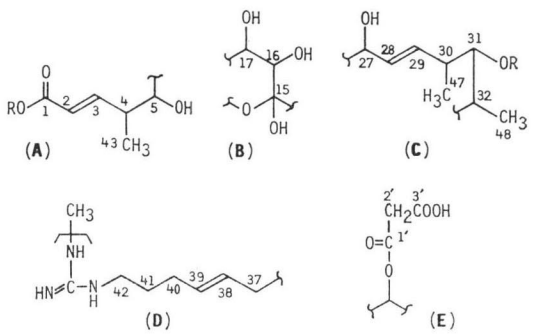
Table 2. Assigned ^1H NMR signals of copiamycin in CD_3OD.

Signal No.	Chemical shift (ppm)	Multiplicity and coupling constant (J in Hz)	Assignment[a]
1.	6.92	1H, dd, $J_{3,2}=15.6$, $J_{3,4}=9.0$ Hz	H-3
2.	5.87	1H, d, $J_{2,3}=15.6$ Hz	H-2
3.	5.53	1H, dd, $J_{29,28}=15.6$, $J_{29,30}=7.6$ Hz	H-29
4.	5.48	1H, m, $J_{38,39}=15.1$ Hz	H-38
5.	5.45	1H, m, $J_{28,29}=15.6$ Hz	H-28
6.	5.42	1H, m, $J_{39,38}=15.1$ Hz	H-39
7.	5.22	1H, m,	[b]
8.	4.75	1H, dd, $J_{31,30}=7.1$, $J_{31,32}=4.9$ Hz	H-31
9.	3.88	1H, m,	H-27
10.	3.86	1H, m, $J_{17,16}=9.3$ Hz	H-17
11.	3.75	1H, m,	H-5
12.	3.38	1H, d, $J_{16,17}=9.3$ Hz	H-16
13.	3.23	2H, s,	H_2-2′
14.	3.16	2H, t, $J_{42,41}=7.1$ Hz	H_2-42
15.	2.83	3H, s,	N-CH_3
16.	2.53	1H, m, $J_{30,29}=7.6$, $J_{30,31}=7.1$, $J_{30,47}=6.8$ Hz	H-30
17.	2.47	1H, m, $J_{4,3}=9.0$, $J_{4,43}=6.8$ Hz	H-4
18.	2.07	2H, m,	H_2-40
19.	1.97	2H, m,	H_2-37
20.	1.90	1H, m,	H-32
21.	1.65	2H, m, $J_{41,42}=7.1$ Hz	H_2-41
22.	1.12	3H, d, $J_{43,4}=6.8$ Hz	H_3-43
23.	0.99	3H, d, $J_{47,30}=6.8$ Hz	H_3-47
24.	0.87	3H, d,	H_3-48
25.	0.78	3H, d, $J=6.8$ Hz	

[a] Assignments of respective signals were made by decouplig experiments.
[b] The methine group bearing malonyl hemiester.

finic bond with an acyl carbonyl group. ^1H-^1H Spin couplings between vicinal protons of the system, C-2 to C-5 were determined by the decoupling experiments (see Table 2, signals No. 1, 2, 11, 17 and 22). An *E*-orientation of the C(2)=C(3) bond was evident from the large coupling constant (15.6 Hz) between H-2 and H-3.

Fig. 2. Partial structures of copiamycin.



Structure B (C-15 to C-17): The one proton signal at δ 3.38 could be assigned to H-16 which is on a carbon vicinal to the hemiketal position (C-15), by comparison with the H-18 signal (appeared at δ 3.34) of azalomycin F_{4a}.[4] The coupling between the signals at δ 3.38 and 3.86 (H-17) was also verified by the decoupling experiments.

Structure C (C-27 to C-32): In the ^1H NMR spectrum of copiamycin ^1H-^1H spin couplings between vicinal protons of the system C-29 to C-32 were determined by the decoupling technique (see Table 2,

signals No. 3, 8, 16, 20, 23 and 24). Since the signal of H-28 could not be resolved in the normal spectrum because of the overlapping of this signal with the signals of H-38 and H-39, the signal was analyzed by the use of decoupling difference spectra generated by subtraction of the control spectrum from the decoupling spectra irradiated at the resonance frequencies of H-27 and H-30 to determine spin couplings between H-27 and H-28, H-28 and H-29. The *E*-orientation of the C(28)=C(29) bond was elucidated from the large coupling constant (15.6 Hz) between H-28 and H-29.

Structure D (C-37 to C-42): The facts that the 3.89% nitrogen content observed on elemental analysis of copiamycin (**1**) corresponds to 3 nitrogen atoms on the basis of its molecular weight (1,057) and that the ^{13}C NMR spectrum exhibited a signal due to a guanido carbon (δ 158.29) indicated the presence of a guanidine moiety. Signals due to *N*-methyl and *N*-methylene groups were also observed in the ^1H NMR spectrum (δ 2.83, signal No. 15 and δ 3.16, signal No. 14, respectively, in Table 2). Signals due to H-38 (signal No. 4 in Table 2) and H-39 (signal No. 6) could be resolved by decoupling difference spectra between the control spectrum and the decoupled spectra irradiated at the resonance frequencies of H-37 and H-40, respectively. The *E*-orientation of the C(38)=C(39) bond was determined from the large coupling constant (15.1 Hz) between H-38 and H-39.

Structure E (malonyl hemiester moiety): A methylene proton signal appearing at δ 3.23 was found to be very easily deuterated in CD_3OD as in the case of the methylene group of azalomycin F_{4a} (its ^1H NMR signal appears at δ 3.23).[4] This observation taken together with the spectral evidences for three acyl carbonyls (see Table 1, signals No. 51, 52 and 53) and two acyloxy methine groups (see Table 2, signals No. 7 and 8) suggested the presence of the partial structure E, which was further supported by comparison of this evidence with the spectral data of such a moiety present in the structure of azalomycin F_{4a}.[4]

Analysis of ^{13}C and ^1H NMR data for copiamycin revealed that the following functional groups, in addition to the partial structures A to E, are present in the molecule; $6\times CH$–OH, $4\times CH$–CH_3 and $11\times CH_2$. From all these spectral and analytical data, the molecular formula of this compound was determined as $C_{54}H_{95}N_3O_{17}$.

Results of Degradation Experiments

Copiamycin (**1**) was found to have three olefinic bonds as well as 1,2 diol and 1,2 ketol moieties as shown in the partial structure B (C-15 to C-17). The antibiotic was subjected to ozonolyses and to periodate oxidations to obtain degradation products which facilitated the structural study of **1**.

Ozonolysis of Copiamycin

Copiamycin, on ozonization in methanol at −78°C followed by decomposition of the ozonide by sodium borohydride ($NaBH_4$) treatment for 1 hour, afforded a mixture of degradation products. Separation of the mixture by a column of Amberlite IR-120B gave a basic and a neutral fraction. From the former **2a** was obtained by acetylation of a basic product, and from the latter **3a** and **4a** were isolated by acetylation of the ethyl acetate extract of the fraction, and a crude mixture of **5a** and **6a** was also obtained after drying the aqueous phase remaining after the ethyl acetate extraction. Compound **5a** gave **6a** and malonic acid upon alkali treatment.

Compound **6a** was obtained as a mixture of two stereoisomers epimeric at C-15 and they were separated by TLC (developed with 1-butanol - acetic acid - water, 5: 1: 1). One of these epimers which showed a higher Rf value on TLC was designated as **6a**.

Decomposition of the ozonide with sodium borodeuteride ($NaBD_4$) treatment for 1 hour, with work

up and separation as in the case of the $NaBH_4$ treatment, afforded **2b**, **3b**, **4b**, **5b** and **6b** (1, 3, 2, 3 and 3 deuterium atoms incorporated, respectively). Compound **5b** was converted to **6b** and malonic acid upon alkali treatment.

Compound **6b** was also obtained as a mixture of a pair of epimers at C-15, and the epimer having a higher Rf value on TLC was designated **6b** as in the case of **6a**.

Periodate Oxidation of Compounds **6a** and **6b**

Compound **6a** was oxidized with sodium periodate in water at room temperature. The crude mixture of the oxidation products, on $NaBH_4$ reduction followed by acetylation, afforded **8a** and **9a** with

Fig. 3. The structures of degradation products.

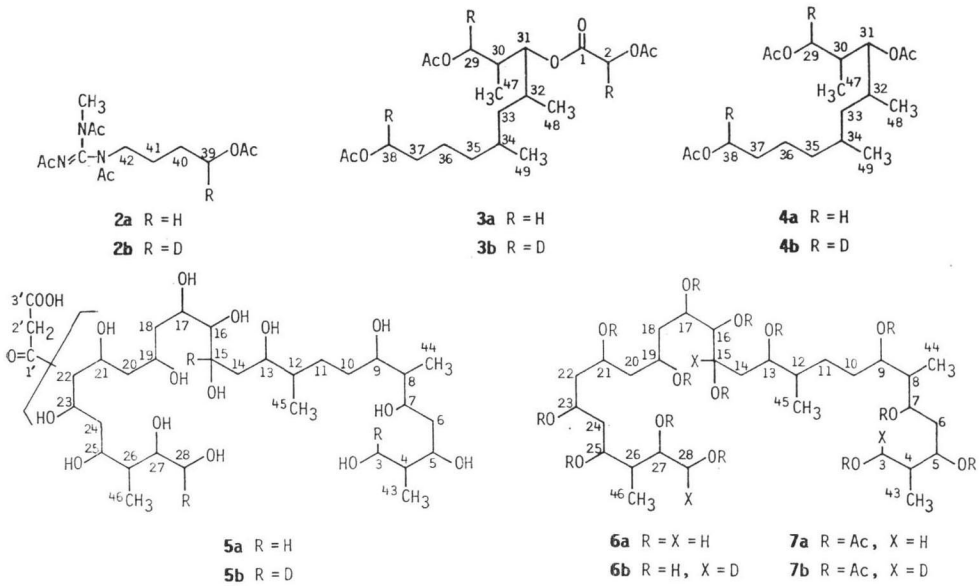
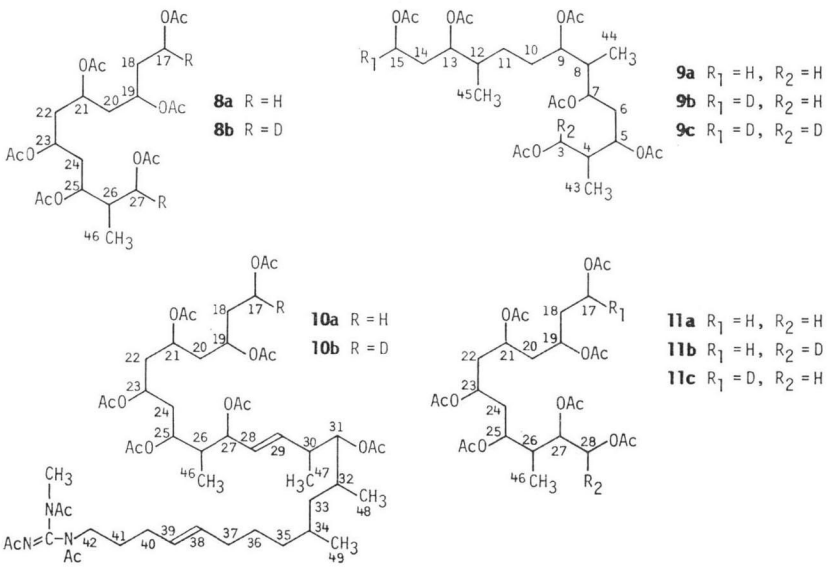


Fig. 4. The structures of degradation products.



loss of a $C_2H_5O_2$ unit in the structure of **6a**, and $NaBD_4$ reduction followed by acetylation yielded **8b** and **9b** (2 and 1 deuterium atoms incorporated, respectively).

The $NaIO_4$ degradation of **6b** using $NaBH_4$ as the reducing agent gave **8a** and **9c** (none and 2 deuterium atoms incorporated, respectively).

Periodate Oxidation of Copiamycin

The $NaIO_4$ oxidation of **1** in methanol - water (2: 1) at room temperature followed by $NaBH_4$ treatment gave a product mixture. On alkaline hydrolysis followed by acetylation it yielded a guanidine derivative **10a**, together with other products whose structures are not discussed in this paper.

The same $NaIO_4$ oxidation, but using $NaBD_4$ for the reduction of the oxidation products, gave **10b**.

Compounds **10a** and **10b** were unstable and were subjected to ozonolysis without further purification. Reduction of the ozonide with $NaBH_4$, reacetylation and chromatographic separation yielded **2a**, **11a** and **4a** from **10a**, and **2a**, **11c** (1 deuterium atom incorporated) and **4a** from **10b**, respectively. Reduction of the ozonide of **10a** with $NaBD_4$ gave **2b**, **11b** and **4b** (respectively 1, 1 and 2 atoms of deuterium incorporated).

Structures of Degradation Products

Structural assignments of the degradation products were essentially based on their spectral and analytical data.

Compounds **2a, b** and **9a ~ c** were identified spectroscopically and chromatographically with the same degradation products obtained from azalomycin F_{4a}[4~6] (compounds **6a, b** and **16a ~ c** in the reference 5).

The molecular formulas of **3a** and **4a** were determined to be $C_{21}H_{36}O_8$ and $C_{19}H_{34}O_6$, respectively, from their FD-MS and elemental analysis. In their ^1H NMR spectra all the signals of non-equivalent protons could be assigned by simple decoupling experiments for the systems C-29 to C-32 and C-37 to C-38, and by analysis of their decoupling difference spectra for the systems C-33 to C-36. Selective ^1H-^{13}C decoupling by irradiating at the resonance positions of all the proton signals permitted the complete assignment of their ^{13}C NMR signals. Their NMR data except for the acetyl groups are summarized in Tables 3 and 4.

These data demonstrated that **4a** consists of $2\times CH_2$–OAc, $1\times$CH–OAc, $3\times$CH, $4\times CH_2$ and $3\times CH_3$, and that compound **3a** consists of $2\times CH_2$–OAc, $1\times$CH–OCO–CH_2–OAc, $3\times$CH, $4\times CH_2$ and $3\times CH_3$. The functional groups of these two compounds differ only in their acyloxy methine moieties (at C-31) and this result suggested the overlapping of the carbon chains of these two compounds.

According to the spectral assignments for **3a** and **4a** deuterium incorporation at C-2, C-29 and C-38 was determined for **3b** from the integrations of the ^1H NMR signals at δ 4.60 (H_2-2), 4.00 and 3.92 (H_2-29), and 4.05 (H_2-38). Similarly one deuterium incorporated at C-29 and another at C-38 was determined for **4b**. These facts indicated that these carbons bearing deuterium were originally present in double bonds, and also that the hydroxyl function at C-31 should have formed the ester linkage in **1**.

Compound **6a** showed a molecular ion peak at *m/z* 646 in its FD-MS. The ^{13}C NMR spectrum of **6a** suggested that it is composed of $2\times CH_2$–OH, $12\times$CH–OH, $4\times$CH, $8\times CH_2$ and $4\times CH_3$. This composition was also supported by spectral data of its peracetylated derivative (**7a**) whose FD-MS showed a molecular ion peak at *m/z* 1,234, indicating the incorporation of 14 acetyl groups. These spectral data for **6a** and **7a** were in accord with the molecular formula of **6a**, $C_{30}H_{62}O_{14}$.

Table 3. ^{1}H and ^{13}C NMR data for compound **3a** in $CDCl_3$.[a]

Carbon No.	^{1}H Signals			^{13}C Signals	
1.				167.6	s
2.	4.60	2d,	$J_{2,2}=15.8$ Hz	60.6	t
29.	4.00	dd,	$J_{29,30}=4.2$, $J_{29,29}=11.0$ Hz	65.9	t
	3.92	dd,	$J_{29,30}=6.3$, $J_{29,29}=11.0$ Hz		
30.	2.13	m,	$J_{30,29}=4.2$, 6.3, $J_{30,47}=6.8$, $J_{30,31}=8.8$ Hz	34.4	d
31.	4.90	dd,	$J_{31,30}=8.8$, $J_{31,32}=3.2$ Hz	78.1	d
32.	1.89	m,	$J_{32,31}=3.2$, $J_{32,48}=6.6$ Hz	31.3	d
33.	1.29	m,		36.3	t
	0.93	m,			
34.	1.54	m,	$J_{34,49}=6.6$ Hz	29.5	d
35.	1.29	m,		41.1	t
	1.08	m,			
36.	1.29	m,		23.1	t
37.	1.59	m,	$J_{37,38}=6.8$ Hz	28.9	t
38.	4.05	t,	$J_{38,37}=6.8$ Hz	64.6	t
47.	0.98	d,	$J_{47,30}=6.8$ Hz	14.4	q
48.	0.87	d,	$J_{48,32}=6.6$ Hz	13.9	q
49.	0.86	d,	$J_{49,34}=6.6$ Hz	19.9	q

[a] Assignments of respective signals were made by decoupling experiments.

Table 4. ^{1}H and ^{13}C NMR data for compound **4a** in $CDCl_3$.[a]

Carbon No.	^{1}H Signals			^{13}C Signals	
29.	4.00	dd,	$J_{29,30}=4.2$, $J_{29,29}=11.0$ Hz	66.2	t
	3.92	dd,	$J_{29,30}=6.3$, $J_{29,29}=11.0$ Hz		
30.	2.09	m,	$J_{30,29}=4.2$, 6.3, $J_{30,47}=6.8$, $J_{30,31}=8.8$ Hz	34.5	d
31.	4.82	dd,	$J_{31,30}=8.8$, $J_{31,32}=3.2$ Hz	76.7	d
32.	1.86	m,	$J_{32,31}=3.2$, $J_{32,48}=6.6$ Hz	31.3	d
33.	1.29	m,		36.4	t
	0.93	m,			
34.	1.54	m,	$J_{34,49}=6.6$ Hz	29.6	d
35.	1.29	m,		41.3	t
	1.08	m,			
36.	1.29	m,		23.2	t
37.	1.59	m,	$J_{37,38}=6.8$ Hz	28.9	t
38.	4.05	t,	$J_{38,37}=6.8$ Hz	64.6	t
47.	0.98	d,	$J_{47,30}=6.8$ Hz	14.4	q
48.	0.88	d,	$J_{48,32}=6.6$ Hz	13.9	q
49.	0.86	d,	$J_{49,34}=6.6$ Hz	20.0	q

[a] Assignments of respective signals were made by decoupling experiments.

The structure of **6a** was clarified partly (systems C-3 to C-5, C-25 to C-26 and C-27 to C-28) by decoupling experiments in the ^{1}H NMR spectrum of **7a**, but was still too complex for complete spectroscopic analysis. It was, therefore, elucidated from the data for its periodate oxidation products, **8a**, **b** and **9a**~**c**, as well as for **11a**~**c**. The structure of **6a** will, therefore, be discussed later.

The FD-MS of **6b** exhibited a molecular ion peak at *m/z* 649 (3 deuterium atoms incorporated).

The ^1H NMR spectrum of its acetylation product (**7b**) revealed single deuterium incorporations at C-3 and C-28 with the integrations of the ^1H NMR signals appearing at δ 4.03 and 3.96 (H_2-3), and at δ 4.38 and 4.06 (H_2-28). A remaining deuterated position in **6b** was determined to be C-15 from the ^1H NMR spectrum of **9c** whose carbon chain is overlapping with that of **6b** in the system C-3 to C-15.

The molecular formula of **8a**, $C_{24}H_{38}O_{12}$, was determined from its FD-MS and elemental analysis. The NMR data for this compound are summarized in Table 5 except for the signals due to acetyl groups. Its ^1H NMR signals were assigned by ^1H-^1H and ^1H-^{13}C decoupling experiments, and also by referring to the chemical shifts of the corresponding ^{13}C NMR signals. The ^{13}C NMR spectrum of **8a** showed

Table 5. ^1H and ^{13}C NMR data for compound **8a** in $CDCl_3$.[a]

Carbon No.	^1H Signals			^{13}C Signals	
17.	4.07	m,	$J_{17,18}$=9.0, $J_{17,17}$=11.0 Hz	60.6	t
	4.09				
18.	1.89	m,	$J_{18,17}$=9.0 Hz	33.6	t
19.	4.99	m,		66.7	d
20.	1.82	m,		39.2	t
21.	4.99	m,		67.4	d
22.	1.82	m,		39.5	t
23.	4.92	m,		67.0	d
24.	1.82	m,		36.1	t
25.	4.99	m,		70.1	d
26.	2.06	m,	$J_{26,27}$=6.6, 6.6, $J_{26,46}$=7.0 Hz	36.3	d
27.	4.01	dd,	$J_{27,26}$=6.6, $J_{27,27}$=11.0 Hz	65.7	t
	3.87	dd,	$J_{27,26}$=6.6, $J_{27,27}$=11.0 Hz		
46.	0.96	d,	$J_{46,26}$=7.0 Hz	11.7	q

[a] Assignments of respective signals were made by decoupling experiments.

Table 6. ^1H and ^{13}C NMR data for compound **11a** in $CDCl_3$.[a]

Carbon No.	^1H Signals			^{13}C Signals	
17.	4.07	m,	$J_{17,18}$=9.0, $J_{17,17}$=11.0 Hz	60.6	t
	4.09				
18.	1.89	m,	$J_{18,17}$=9.0 Hz	33.6	t
19.	4.99	m,		66.7	d
20.	1.82	m,		39.2	t
21.	4.99	m,		67.4	d
22.	1.82	m,		39.4	t
23.	4.85	m,		67.3	d
24.	1.85	m,		36.8	t
	1.77	m,			
25.	5.15	m,		68.4	d
26.	2.01	m,	$J_{26,46}$=7.0 Hz	37.2	d
27.	4.82	m,	$J_{27,28}$=2.5, 5.4 Hz	71.9	d
28.	4.38	dd,	$J_{28,27}$=2.5, $J_{28,28}$=12.4 Hz	63.5	t
	4.06	dd,	$J_{28,27}$=5.4, $J_{28,28}$=12.4 Hz		
46.	0.98	d,	$J_{46,26}$=7.0 Hz	9.9	q

[a] Assignments of respective signals were made by decoupling experiments.

that it consists of 12 carbons in addition to those of acetyl groups, composed of $2\times CH_2$–OAc, $4\times$CH–OAc, $1\times CH$, $4\times CH_2$ and $1\times CH_3$. Since the decoupling experiments in its ^1H NMR spectrum proved the structures of both terminal moieties, C-17 to C-18 and C-26 to C-27, and since **8a** was the product of a periodate oxidation of **6a** there should be no 1,2-glycol moiety on its carbon chain. Hence, the arrangement of the remaining functional groups, $3\times$CH–OAc and $3\times CH_2$, in the system C-19~C-25 was determined unambiguously.

The incorporation of a single atom of deuterium at C-17 and C-27 in **8b** was apparent from its ^1H NMR spectrum (see experimental part).

The determination of the molecular formula of **11a**, $C_{27}H_{42}O_{14}$, was based on its mass spectroscopic and analytical data. All the signals in its ^1H NMR spectrum were assigned by decoupling of vicinal protons, and the ^{13}C NMR signals were assigned by ^1H-^{13}C selective decoupling (see Table 6).

Comparison of the NMR data for **8a** (Table 5) and for **11a** (Table 6) clearly indicated the overlapping of the system C-17 to C-27 in these two compounds, indicating that the carbon chain of **11a** is one carbon longer than that of **8a**.

The ^1H NMR spectrum of **11b** showed deuterium incorporation at C-28 by the integrations of the signals at δ 4.38 and 4.06 (H_2-28). Similarly deuterium incorporation at C-17 in **11c** was apparent from the integration of the signal at δ 4.08 (H_2-17).

As described previously, **5a** gave **6a** and malonic acid on alkaline hydrolysis, and **6a** gave rise to **8a** and **9a** (after acetylation) on $NaIO_4$ oxidation with the loss of a $C_2H_5O_2$ unit contained in its structure.

It was observed that **6a**, on $NaIO_4$ oxidation followed by $NaBD_4$ reduction, gave **8b** and **9b** (after acetylation), the former bears a deuterium atom on each terminal carbon (at C-17 and C-27), and the latter was deuterated only on a single terminal carbon of the carbon chain (at C-15).

Likewise, the $NaIO_4$ oxidation of the deuterated **6b** followed by $NaBH_4$ reduction gave **8a** and **9c** (after acetylation), of which the former was not deuterated, while the latter retained a deuterium atom at each terminal position (at C-3 and C-15). This indicated that one of the 3 deuterium atoms incorporated in **6b** was located at C-15.

These results clearly indicated that one of the terminal carbons of **6a** (C-3) constituted a terminal carbon of **9a**, and that the other terminal moiety of **6a** was lost in the oxidation process yielding the fragment **8a** (after acetylation).

On the other hand, it was shown that the remaining terminal moiety, CH_2–OH, of **6a** (C-28) was retained in **11a** whose structure was correlated spectroscopically with that of **8a**. One deuterium atom incorporated at C-28 of **11b** also provided evidence for that this carbon (C-28) was originally present in an olefinic linkage in **10a** (consequently also in **1**).

These facts suggested that the remaining CH–OH unit which is not retained in the products of $NaIO_4$ oxidation of **6a** should be located at a position between the fragments **8a** and **9a**. This carbon is the C-16 unit in **6a** (consequently in **1**). The presence of such a moiety in **1** was already pointed out previously in the partial structure B. The structure of **6a** was thus established to be as shown in Fig. 3.

Skeletal Structure of Copiamycin

It was described previously that ozonolysis of **1** ($C_{54}H_{95}N_3O_{17}$) followed by $NaBH_4$ treatment gave rise to a mixture of degradation products which were isolated as **5a** ($C_{33}H_{64}O_{17}$), as **2a** ($C_6H_{15}N_3O$-tetraacetate) and as **3a** ($C_{15}H_{30}O_5$-triacetate) after acetylation.

Since the sum of the carbon atom numbers (excluding acetyl carbons), methyl groups, acyl carbonyl

groups (excluding acetyl carbonyls) and carbons of the methylguanidine group represented by these products coincide with those of **1**, and since such ozonolysis products should, of course, be derived from the cleavage of three C-C double bonds present in **1**, elucidation of the skeletal structure of **1** should be possible by determining the mode of connection of these three fragments.

The ^{13}C NMR spectrum of **1** indicated the presence of a carbonyl function forming a hemiketal (signal at δ 99.73, see Table 1). The location of this functional group was defined as C-15 from the fact that a deuterium atom was incorporated at this position in **6b** (determined from the structure of **9c**).

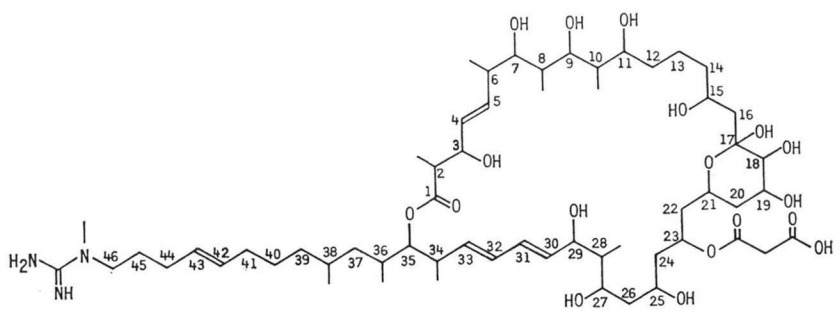
Hemiester formation of malonic acid with a hydroxyl group in the partial structure **6a** was indicated by the fact that both **5a** and **1** liberated malonic acid on alkaline hydrolyses, as well as by NMR spectroscopic evidence of an additional ester linkage present in **1** other than the lactone linkage between C-1 and C-31 (see Tables 1 and 2). The position of this hemiester group could not be determined, and will be discussed later.

Inspection of the partial structure discussed above taken together with the structures of the degradation products **2a** and **3a** as well as with the partial structures A, C and D, suggested strongly that the skeletal structure of **1** should be depicted as shown in Fig. 1.

The position of the malonyl hemiester group remained undetermined, but the location of the hemiester group was deduced to be probably at either C-21 or C-23 on the basis of the following argument: As summarized in Table 2, extensive studies of the ^1H NMR spectra of **1** revealed that the signal due to the proton on the carbon bearing the hemiester group appears at δ 5.22, and that the signals due to H-5, H-16, H-17 and H-27 appear at δ 3.75, 3.38, 3.86 and 3.88, respectively. These positions (at C-5, C-16, C-17 and C-27) were, therefore, excluded as possible positions of the hemiester forming hydroxyl group. Analysis of the decoupling difference spectrum between the control spectrum and the decoupled spectrum irradiated at δ 5.22 (the proton on the carbon attached to the hemiester linkage) showed a signal change at δ 1.80~1.60. Irradiations of this field divided at 10 Hz intervals gave no decoupled signal among the 7 methyl doublets, indicating that the protons which is vicinal to that giving the δ 5.22 signal are not on the carbons bearing these methyl groups. This result demonstrates that H-4, H-8, H-12 and H-26 (H-32 and H-34, as well) are not on the carbons next to the hemiester-bearing carbon. The hydroxyl groups at C-7, C-9, C-13 and C-25 were, hence, also excluded as possible hemiester-forming hydroxyl groups. Of the remaining hydroxyl groups the position C-19 should presumably be involved in the 6-membered hemiketal ring,[7~10] consequently the position of the malonyl hemiester linkage was deduced at either C-21 or C-23.

In conclusion, it has been shown that copiamycin (**1**) is a 32-membered macrocyclic lactone anti-

Fig. 5. The structure of scopafungin.



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biotic, possessing a *N*-methylguanidine group as a terminal moiety of its side chain, an intramolecular hemiketal ring involving the keto group at C-15 and a hydroxyl group presumably at C-19, and a malonyl hemiester group at either C-21 or C-23. The structural features of **1** resemble those of azalomycins F_{3a}, F_{4a} and F_{5a},[1,4~6] scopafungin (**12**),[11] niphimycin[12] and especially of niphithricins[13,14]*.

Experimental

General

Melting points were taken using Yamato MP-1 apparatus and are uncorrected. UV spectra were measured on a Shimadzu apparatus (model UV-300), the maxima are given in nm (extinction ε). IR spectra were measured on a Japan Spectroscopic Co. apparatus (model IR-S) and are recorded in cm^{-1}. ^1H NMR spectra and ^{13}C NMR spectra were measured on a JEOL apparatus, JNM FX-400 (^1H: 400.5 MHz, ^{13}C: 100.7 MHz) machine, chemical shifts are given in ppm (in δ) relative to TMS (0 ppm) as an internal standard and coupling constants are recorded in Hz (J). Mass spectra were measured on a JEOL JMS-OlSG-2 apparatus (FD-MS) or on a JEOL JMS-DX-300 apparatus (FAB-MS). Optical rotation were measured on a Japan Spectroscopic Co. apparatus (model DIP-181).

Thin-layer chromatography (TLC) was carried out on Merck DC-Fertigplatten (Kieselgel 60 F-254), and gas chromatography was performed on a Shimadzu GC-4A, GC-4APF or GC-4BPFT machine.

Purification of Copiamycin (1)

Copiamycin isolated by the previously described procedure[2] was used for its degradation reactions. For the measurements of physicochemical properties it was further purified as follows: this preparation (10~15 mg) was applied to a TLC plate (20×20 cm, 0.25 mm thick) which was developed with a solvent system composed of 2-butanol - water (4 : 1) for 16~20 hours. During the developing time the top of plate was exposed to air to allow the solvents to evaporate. The copiamycin fraction, obtained by extraction of the plate silica gel, was filtered to remove insoluble materials. Copiamycin thus obtained was recrystallized from aqueous methanol to give colorless platelets; mp 147~149°C (Lit. 144°C)[2] (dec.), $[\alpha]_D^{25}$ +14.4° (*c* 4.15, methanol), UV λ_{max}^{MeOH} nm (ε): 220 (20,600), 256 (sh). IR (KBr) cm^{-1}: 3380 (br. s), 2960 (s), 1730 (m), 1680 (br. s), 1595 (m), 1465 (m), 1385 (m), 1365 (m), 1330 (m), 1275 (m), 1245 (m), 1200 (m), 1170 (m), 1145 (m), 1075 (m), 985 (m). FD-MS: *m/z* 1,057 (M^+) or 1,058 (MH^+), FAB-MS: *m/z* 1,058 (MH^+). ^{13}C NMR: see Table 1, ^1H NMR: see Table 2.

Anal. Calcd. for $C_{54}H_{95}N_3O_{17}$: C 61.28, H 9.05, N 3.97, O 25.70.
Found: C 61.59, H 9.15, N 3.89, O 25.44.

Ozonolysis of Copiamycin

1) Copiamycin (520 mg) was ozonized in methanol (40 ml) at −78°C, and the ozonide was decomposed by 1 hour's treatment with $NaBH_4$ (1.02 g in methanol, 10 ml) at 0°C. The reaction solution was passed through a column of Amberlite IR-120B (40 ml) to adsorb a basic product, and the column was washed with methanol (100 ml) and water (50 ml) successively. The washings were combined with the effluent from the column (fr. 1). The column was eluted with aqueous 1 N HCl (200 ml) and the eluate was neutralized and concentrated to dryness (fr. 2). Fr. 2 was extracted with methanol to separate an ozonolysis product from salt, and the extract was again concentrated. This procedure was repeated several times. The basic product, thus obtained, was acetylated with acetic anhydride in pyridine, and was purified by TLC to afford 32 mg of **2a**.

Fr. 1 was concentrated to *ca.* 20 ml, and was extracted with ethyl acetate. The extract was concentrated (125 mg of oil) and the residue was acetylated (170 mg, a mixture containing **3a** and small amount of **4a**) and separated by GLC (10% OV-1, 1.5 m, 220°C) to give 120 mg of **3a** and 10 mg of **4a**.

The water layer after extraction of fr. 1, a mixture of **5a** and small amount of **6a**, was further treated with $NaBH_4$ (510 mg, in water, 5 ml) at room temperature for 26 hours. The reaction mixture was passed through a column of Amberlite IR-120B followed by a column of IRA-410, and was concentrated

* Prof. KELLER-SCHIERLEIN informed that the skeletal structure of niphithricin A was identical with that of copiamycin[14].

to dryness to afford 205 mg of **6a** (a mixture of two stereoisomers at C-5).

2) Copiamycin (610 mg) was ozonized in methanol (40 ml) at −78°C, and the ozonide was decomposed with $NaBD_4$ (1.06 g in methanol, 10 ml) at 0°C for 1 hour. Work up and separation as in the case of 1) gave rise to **2b** (38 mg), **3b** (165 mg), **4b** (16 mg) and **6b** (245 mg; a mixture of two stereoisomers at C-15).

2a ($C_{14}H_{23}N_3O_5$) ^1H NMR (in $CDCl_3$): δ 4.08 (2H, t, $J_{39,40}$=6.1 Hz, H_2-39), 3.58 (2H, t, $J_{42,41}$= 7.5 Hz, H_2-42), 3.12 (3H, s, *N*-methyl), 2.26, 2.23 and 2.20 (9H, 3s, *N*-acetyl methyls), 2.05 (3H, s, *O*-acetyl methyl), 1.80~1.55 (4H, m, H_2-40 and 41).

2b ($C_{14}H_{23}DN_3O_5$) ^1H NMR (in $CDCl_3$): Integration of the signal at δ 4.08 gave 1H.

3a ($C_{21}H_{36}O_8$) ^1H NMR (in $CDCl_3$) and ^{13}C NMR (in $CDCl_3$): see Table 3. FD-MS: *m/z* 417 (MH^+). IR (CCl_4) cm^{-1}: 3000 (m), 2900 (w), 1750 (br. s), 1370 (m), 1360 (m), 1240 (br. s), 1195 (s), 1090 (m), 1040 (m). $[\alpha]_D^{25}$ −3.0° (*c* 2.6, methanol).

Anal. Calcd. for $C_{21}H_{36}O_8$: C 60.55, H 8.71.
Found: C 60.70, H 8.74.

3b ($C_{21}H_{33}D_3O_8$) FI-MS: *m/z* 420 (MH^+). ^1H NMR (in $CDCl_3$): Integration of the signals at δ 4.60 and 4.05 gave 1H each, and integration of the signals at δ 4.00 and 3.92 gave 0.5H each.

4a ($C_{19}H_{34}O_6$) ^1H NMR (in $CDCl_3$) and ^{13}C NMR (in $CDCl_3$): see Table 4. FI-MS: *m/z* 359 (MH^+). $[\alpha]_D^{25}$ −0.4° (*c* 0.5, methanol).

Anal. Calcd. for $C_{19}H_{34}O_6$: C 63.66, H 9.56.
Found: C 63.51, H 9.39.

4b ($C_{19}H_{32}D_2O_6$) FI-MS: *m/z* 361 (MH^+). ^1H NMR (in $CDCl_3$): Integration of the signal at δ 4.05 gave 1H, and integration of the signals at δ 4.00 and 3.92 gave 0.5H each.

Separation of Two Stereoisomers of **6a** and **6b**

Compound **6a** (or **6b**) (10~15 mg) was applied to a TLC plate (20×20 cm, 0.25 mm thick) and was developed with 1-butanol - acetic acid - water (5: 1: 1) as in the case of the purification of copiamycin. Two stereoisomers, obtained by extractions of the plate silica gel, were passed through a column of Amberlite IR-120B and of IRA-410, and then filtered, respectively.

One of these stereoisomers which showed a higher Rf value on TLC was designated as **6a** (or **6b**). Compound **6a** and **6b** were acetylated with acetic anhydride in pyridine to give **7a** and **7b**, respectively.

6a ($C_{30}H_{62}O_{14}$) FD-MS: *m/z* 646 (M^+). ^{13}C NMR (in CD_3OD+D_2O): δ 77.8 (d), 75.8 (d), 75.1 (d), 75.0 (d), 74.3 (d), 72.3 (d), 72.2 (d), 68.7 (d), 67.9 (d), 66.2 (d), 66.0 (d), 65.9 (d), 65.0 (t), 64.8 (t), 46.2 (t+t), 43.4 (d), 43.2 (t), 42.3 (t), 41.7 (d), 41.5 (d), 39.2 (d), 38.0 (t), 36.6 (t), 32.9 (t), 28.7 (t), 15.6 (q), 13.6 (q), 10.2 (q), 10.0 (q).

6b ($C_{30}H_{59}D_3O_{14}$) FD-MS: *m/z* 649 (M^+). ^{13}C NMR (in CD_3OD+D_2O): The signals at δ 72.3 (d), 65.0 (t) and 64.8 (t) were not observed in the spectrum.

7a ($C_{58}H_{90}O_{28}$) FD-MS: *m/z* 1,234 (M^+). IR (CCl_4) cm^{-1}: 3000 (w), 1750 (br. s), 1375 (m), 1240 (br. s), 1025 (m), 910 (m). ^1H NMR (in $CDCl_3$): δ 5.23 (1H, m), 5.15 (1H, m, H-25), 5.04~4.74 (10H, m)*, 4.38 (1H, dd, $J_{28,27}$=2.5, $J_{28,28}$=12.4 Hz, H-28), 4.06 (1H, dd, $J_{28,27}$=5.4, $J_{28,28}$=12.4 Hz, H-28), 4.03 (1H, dd, $J_{3,4}$=5.9, $J_{3,3}$=11.2 Hz, H-3), 3.96 (1H, dd, $J_{3,4}$=6.3, $J_{3,3}$=11.2 Hz, H-3), 2.11 (1H, m, $J_{4,3}$= 5.9, 6.3, $J_{4,43}$=6.8 Hz, H-4), 2.06~2.00 (42H, 14s, acetyl methyls), 1.99 (1H, m, $J_{26,46}$=7.0 Hz, H-26), 1.92~1.58 (15H, m), 1.31 (2H, m), 1.03 (1H, m), 0.98 (3H, d, $J_{46,26}$=7.0 Hz, H_3-46), 0.95 (6H, 2d, $J_{43,4}$= 6.8 Hz, $J_{44,8}$=6.8 Hz, H_3-43 and 44), 0.85 (3H, d, $J_{45,12}$=6.8 Hz, H_3-45). ^{13}C NMR (in $CDCl_3$): δ 170.8~ 169.9 (14s, acetyl carbonyls), 74.2 (d), 73.7 (d), 72.3 (d), 71.9 (3d), 68.7 (d), 68.4 (d), 67.3 (d), 67.0 (d), 66.8 (d), 66.6 (d), 65.3 (t), 63.5 (t), 39.5 (t+d), 39.4 (t), 37.2 (d), 36.9 (t), 36.3 (d), 36.0 (d), 35.9 (t), 33.5 (t), 31.3 (t), 29.9 (t), 27.6 (t), 21.1~20.7 (14q, acetyl methyls), 14.9 (q), 13.7 (q), 10.0 (q), 9.9 (q).

7b ($C_{58}H_{87}D_3O_{28}$) FD-MS: *m/z* 1,237 (M^+). ^1H NMR (in $CDCl_3$): Integration of the multiplet appearing at δ 5.04~4.74 gave 9H, and integration of the signals at δ 4.38, 4.06, 4.03 and 3.96 gave 0.5 H, respectively. ^{13}C NMR (in $CDCl_3$): The signals at δ 68.7 (d), 65.3 (t) and 63.5 (t) were not observed in the spectrum.

* 4.93 (1H, m, H-5), 4.80 (1H, m, $J_{27,28}$=2.5, 5.4 Hz, H-27).

Alkaline Hydrolysis of **5a** and **5b**

Compound **5a** (5 mg, a mixture of the stereoisomers at C-15), which was separated by TLC from a mixture containing **5a** and a small amount of **6a**, was dissolved in 1 N KOH (5 ml) under a nitrogen atmosphere. The solution was stirred overnight at room temperature. The reaction solution was treated with Amberlite IR-120B to remove KOH. Removal of the solvent by evaporation yielded a mixture, whose ether extract afforded crude malonic acid, and the residue gave **6a** (a mixture of the stereoisomers at C-15) by purification with TLC (*n*-butanol - acetic acid - water, 5: 1: 1).

The same treatment of **5b** gave **6b** and malonic acid.

Periodate Oxidation of **6a** and **6b**

A solution of **6a** (105 mg) and $NaIO_4$ (120 mg) in water (15 ml) were stirred for 4 hours at room temperature. Excess $NaIO_4$ was decomposed with ethylene glycol. Water was evaporated *in vacuo*, and the residue was extracted with methanol. The extract was concentrated to dryness, and was treated with $NaBH_4$ (110 mg) in water (20 ml) for 1 hour under ice cooling. The reaction solution was then stirred with Amberlite IR-120B and with IRA-410, successively. Evaporation of water yielded oily products mixture (70 mg). Acetylation of the products followed by a silica gel column chromatography afforded 38 mg of **8a** and 42 mg of **9a**.

Similar $NaIO_4$ oxidation of **6a** (85 mg) followed by $NaBD_4$ (95 mg) treatment gave **8b** (47 mg) and **9b** (14 mg).

Similar $NaIO_4$ oxidation of **6b** (98 mg) followed by $NaBH_4$ treatment yielded **8a** (43 mg) and **9c** (29 mg).

8a ($C_{24}H_{38}O_{12}$) ^1H NMR (in $CDCl_3$) and ^{13}C NMR (in $CDCl_3$): see Table 5. FD-MS: *m/z* 519 (MH^+). IR (CCl_4) cm^{-1}: 3010 (m), 2890 (w), 1750 (br. s), 1385 (s), 1240 (br. s), 1120 (m), 1040 (s), 1020 (s). $[\alpha]_D^{25}+3.6°$ (*c* 3.9, methanol).

Anal. Calcd. for $C_{24}H_{38}O_{12}$: C 55.59, H 7.38.
Found: C 55.58, H 7.31.

8b ($C_{24}H_{36}D_2O_{12}$) FD-MS: *m/z* 521 (MH^+). ^1H NMR (in $CDCl_3$): Integration of the signal at δ 4.08 gave 1H, and integration of the signals at δ 4.01 and 3.87 gave 0.5H each.

9a ($C_{28}H_{46}O_{12}$) FD-MS: *m/z* 575 (MH^+). $[\alpha]_D^{25}$ +3.5° (*c* 0.7, methanol). ^1H NMR (in $CDCl_3$) δ 5.00 (1H, ddd, J=8.1, 5.5, 2.8 Hz, H-9), 4.94 (1H, ddd, $J_{5,4}$=4.9, $J_{5,6}$=5.1, 6.9 Hz, H-5), 4.89 (1H, ddd, J=5.0, 8.0, 5.0 Hz, H-13), 4.80 (1H, ddd, J=7.8, 7.1, 3.9 Hz, H-7), 4.07 (2H, m, H_2-15), 4.02 (1H, dd, $J_{3,3}$=11.2, $J_{3,4}$=5.9 Hz, H-3), 3.98 (1H, dd, $J_{3,3}$=11.2, $J_{3,4}$=6.3 Hz, H-3), 2.11 (1H, m, $J_{4,3}$=5.9, 6.3, $J_{4,5}$=4.9, $J_{4,43}$=7.0 Hz, H-4), 2.16~2.10 (18H, 6s, acetyl methyls), 2.00 (1H, m, H-6), 1.83 (4H, m, H_2-14, H-8 and 6), 1.72 (2H, m, H-12 and 10), 1.40 (1H, m, H-10), 1.32 (1H, m, H-11), 1.10 (1H, m, H-11), 0.97 (3H, d, $J_{43,4}$=7.0 Hz, H_3-43), 0.97 (3H, d, $J_{44,8}$=7.0 Hz, H_3-44), 0.89 (3H, d, $J_{45,12}$=6.8 Hz, H_3-45). ^{13}C NMR (in $CDCl_3$): δ 170.9~170.3 (6s, acetyl carbonyls), 74.1 (d, C-13), 72.4 (d, C-5), 72.1 (d, C-9), 72.0 (d, C-7), 65.3 (t, C-3), 61.1 (t, C-15), 39.3 (d, C-8), 36.3 (d, C-12), 36.1 (d, C-4), 33.5 (t, C-6), 29.9 (t, C-10), 29.4 (t, C-14), 28.0 (t, C-11), 21.1~20.8 (6q, acetyl methyls), 14.8 (q, C-45), 13.6 (q, C-43), 9.8 (q, C-44).

9b ($C_{28}H_{45}DO_{12}$) FI-MS: *m/z* 576 (MH^+). ^1H NMR (in $CDCl_3$): Integration of the signal at δ 4.07 gave 1H.

9c ($C_{28}H_{44}D_2O_{12}$) FI-MS: *m/z* 577 (MH^+). ^1H NMR (in $CDCl_3$): Integration of the signal at δ 4.07 gave 1H, and integration of the signals at δ 4.02 and 3.98 gave 0.5 H each.

Periodate Oxidation of Copiamycin

To copiamycin (610 mg) in methanol (50 ml) was added $NaIO_4$ (430 mg) in water (15 ml) under a nitrogen atmosphere, and the whole was stirred for 20 hours at room temperature. Excess $NaIO_4$ was decomposed with ethylene glycol. The solvent was evaporated off, and the residue was extracted with methanol to remove inorganic salts. The extract, after evaporation of methanol, was again dissolved in methanol (30 ml) and was treated with $NaBH_4$ (640 mg in water, 10 ml) for 1 hour under ice cooling. The reaction solution was neutralized with 2 N HCl, and was concentrated to dryness. The products were extracted with methanol from the residue to remove salt. The products mixture obtained was stirred overnight with 1 N KOH (methanol - water, 3: 1; 40 ml) at room temperature under a nitrogen atmo-

sphere. The solution, after neutralization, was concentrated, and the residue was extracted with methanol to separate the products from salt. The product mixture was suspended in dioxane and acetylated with acetic anhydride-pyridine in the presence of 4-dimethylaminopyridine. The acetate mixture was treated with diazomethane to give a mixture (810 mg) which was separated by silica gel column chromatography to afford **10a** (270 mg).

Similar $NaIO_4$ oxidation of copiamycin (310 mg) followed by $NaBD_4$ (320 mg) treatment yielded 125 mg of **10b**.

Ozonolysis of **10a** and **10b**

Compound **10a** (110 mg) was ozonized in methanol (20 ml) and treated with $NaBH_4$ (260 mg) as in the case of copiamycin (ozonolysis 1). The product mixture, after treatment with Amberlite IR-120B and IRA-410, was acetylated and was separated by silica gel column chromatography to give **11a** (13 mg), **4a** (5 mg) and **2a**.

Similar ozonolysis of **10a** (140 mg) followed by $NaBD_4$ (280 mg) treatment gave **11b** (16 mg), **4b** (3 mg) and **2b**.

Similar ozonolysis of **10b** (120 mg) followed by $NaBH_4$ treatment yielded **11c** (20 mg), **4a** (7 mg) and **2a**.

11a ($C_{27}H_{42}O_{14}$) ^1H NMR (in $CDCl_3$) and ^{13}C NMR (in $CDCl_3$): see Table 6. FD-MS: *m/z* 591 (MH^+). IR (CCl_4) cm^{-1}: 2990 (w), 1750 (s), 1375 (m), 1240 (br. s), 1050 (m), 1020 (m). $[\alpha]_D^{25}$ +1.1° (*c* 0.5, methanol).

Anal. Calcd. for $C_{27}H_{42}O_{14}$: C 54.90, H 7.17.
Found: C 54.86, H 7.26.

11b ($C_{27}H_{41}DO_{14}$) FD-MS: *m/z* 592 (MH^+). ^1H NMR (in $CDCl_3$): Integration of the signals at δ 4.38 and 4.06 gave 0.5H each.

11c ($C_{27}H_{41}DO_{14}$) FD-MS: *m/z* 592 (MH^+). ^1H NMR (in $CDCl_3$): Integration of the signal at δ 4.08 gave 1H.

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