DYNEMICINS O, P AND Q: NOVEL ANTIBIOTICS RELATED TO DYNEMICIN A

ISOLATION, CHARACTERIZATION AND BIOLOGICAL ACTIVITY

MEGUMI MIYOSHI-SAITOH, NAOKO MORISAKI, YOSHIYUKI TOKIWA and SHIGEO IWASAKI*

Institute of Applied Microbiology, The University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113, Japan

MASATAKA KONISHI, KYOICHIRO SAITOH and TOSHIKAZU OKI

Bristol-Myers Squibb Research Institute, 2-9-3 Shimo-meguro, Meguro-ku, Tokyo 153, Japan

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New antibiotics, dynemicins O (**3**), P (**4**) and Q (**5**), were isolated from the culture broth of *Micromonospora chersina* M956-1, the dynemicin A (**1**)-producing strain.

An ethyl acetate extract of a tank culture was roughly separated by a column of Diaion SP-800 and was further purified by combining a variety of column chromatographies to afford a few mg of compounds **3**, **4** and **5**. Their structures, analyzed from the spectral data, had a bridging phenylene group, a vic-diol and an oxo group in place of the respective 1,5-diyn-3-ene bridge, the epoxide ring and the carboxyl group in dynemicin A. Their biological activities were evaluated against bacteria and tumor cells.

Dynemicin A (**1**), an enediyne class of antibiotic, is a potent antibacterial and antitumor agent recently isolated from *Micromonospora chersina*[1,2] and from *Micromonospora globosa*[3]. It is a hybrid containing enediyne and anthraquinone substructures. Isolation of dynemicins L, M (**2**) and N, homologues of dynemicin A, has also been reported[2]. This paper deals with the isolation and the structures of compounds **3**, **4** and **5**, new members of the dynemicin group compounds designated as dynemicins O, P and Q, respectively.

Isolation and Purification

From the ethyl acetate extract of a 1,000-liter culture of the *M. chersina* M956-1 strain grown in a medium containing corn starch 1%, Pharmamedia 0.5%, $CuSO_4 \cdot 5H_2O$ 0.005%, $CaCO_3$ 0.1% and NaI

Fig. 1. Structures of dynemicins A (**1**) and M (**2**).

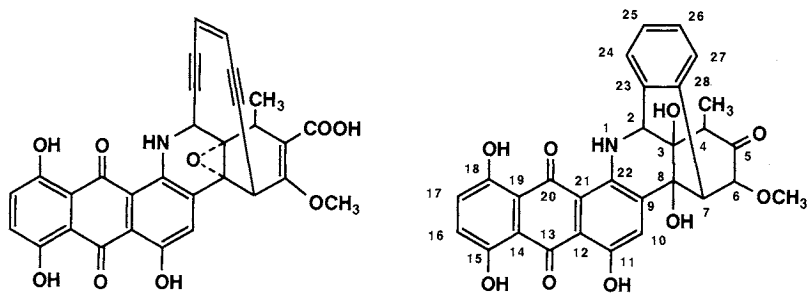
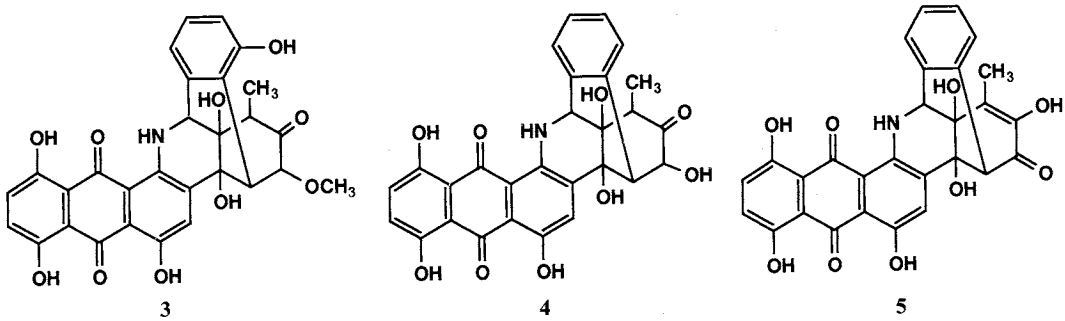


Fig. 2. Structures of dynemicins O (**3**), P (**4**) and Q (**5**).



0.00005%, most of dynemicin A was first separated by a Diaion SP-800 column. On HPLC analysis monitored by a photodiode array UV-VIS detector, the residual mixture (*ca.* 1.6 kg), composed mostly of the fermentation oil, showed four components which have the absorption spectra characteristic of dynemicin class compounds. One of them was identified as dynemicin M (**2**) as reported previously[2].

The isolation procedure of the other three compounds, dynemicins O (**3**), P (**4**) and Q (**5**), is illustrated in Fig. 3. The oily mixture was resolved by Diaion SP-800 column into three fractions A (*ca.* 1.5 kg), B (*ca.* 85 g) and C (*ca.* 40 g) containing compounds **3**, **4** and **5**, respectively. Each fraction was separated by successive chromatographies as shown in Fig. 3. The final purifications of the compounds were performed by HPLC using an ODS column to give **3** (3 mg), **4** (2 mg) and **5** (1 mg).

Properties

Compounds **3**, **4** and **5** were obtained as blue powders and showed blue spots on silica gel TLC plates. They are all soluble in various organic solvents but their solubilities are generally poor in such solvents except dimethyl sulfoxide. The physico-chemical properties of these compounds are shown in Table 1. The ^{13}C NMR data of **3** are shown in Table 2 in which the signal assignments were obtained from ^{1}H-^{13}C COSY and heteronuclear multiple-bond ^{1}H-^{13}C correlation spectroscopy (HMBC) (Fig. 4). The ^{1}H NMR data of compounds **2**, **3**, **4** and **5** are shown in Table 3. The signal assignments were made based on chemical shifts, ^{1}H-^{1}H decoupling and NOE experiments.

Structure

The structures of dynemicin O (**3**), P (**4**) and Q (**5**) were determined spectroscopically by comparison with the data of dynemicins A (**1**) and M (**2**)[1,2]. Both the UV absorption spectra and ^{1}H NMR data of position 10 to 18 of compounds **3**, **4** and **5** were essentially the same as those of dynemicin M (**2**) indicating the presence of 1,2,4,5,8-pentasubstituted anthraquinone (see Table 1 and ref 2).

Dynemicin O, a blue powder, gave a HRFAB-MS spectrum agreeing with the formula $C_{29}H_{23}NO_{10}$ (Table 1) which is one carbon less than that of dynemicin A ($C_{30}H_{19}NO_9$). The facts that a signal due to a ketone appeared at δ 204.6 in the ^{13}C NMR spectrum of **3** at the expense of a carboxyl carbon (δ 167.4) observed in the spectrum of **1** and that ^{1}H NMR signals due to the position 1 to 8 of **3** were very similar to those of **2** established the partial structure N-1 through C-8 of **3**. The stereochemistry at the 4 and 6 positions were elucidated as shown in Fig. 2 from the observed NOE enhancements of 4-CH_3 (δ 1.30) and 6-H (δ 5.05) signals on irradiation of 2-H and 4-H, respectively. This indicated a 1,3-diaxial relationship between 4-H and 6-H and the vicinity of 2-H and 4-CH_3 group and is compatible with the assigned stereochemistry of these positions.

Fig. 3. A diagram of the isolation procedure.

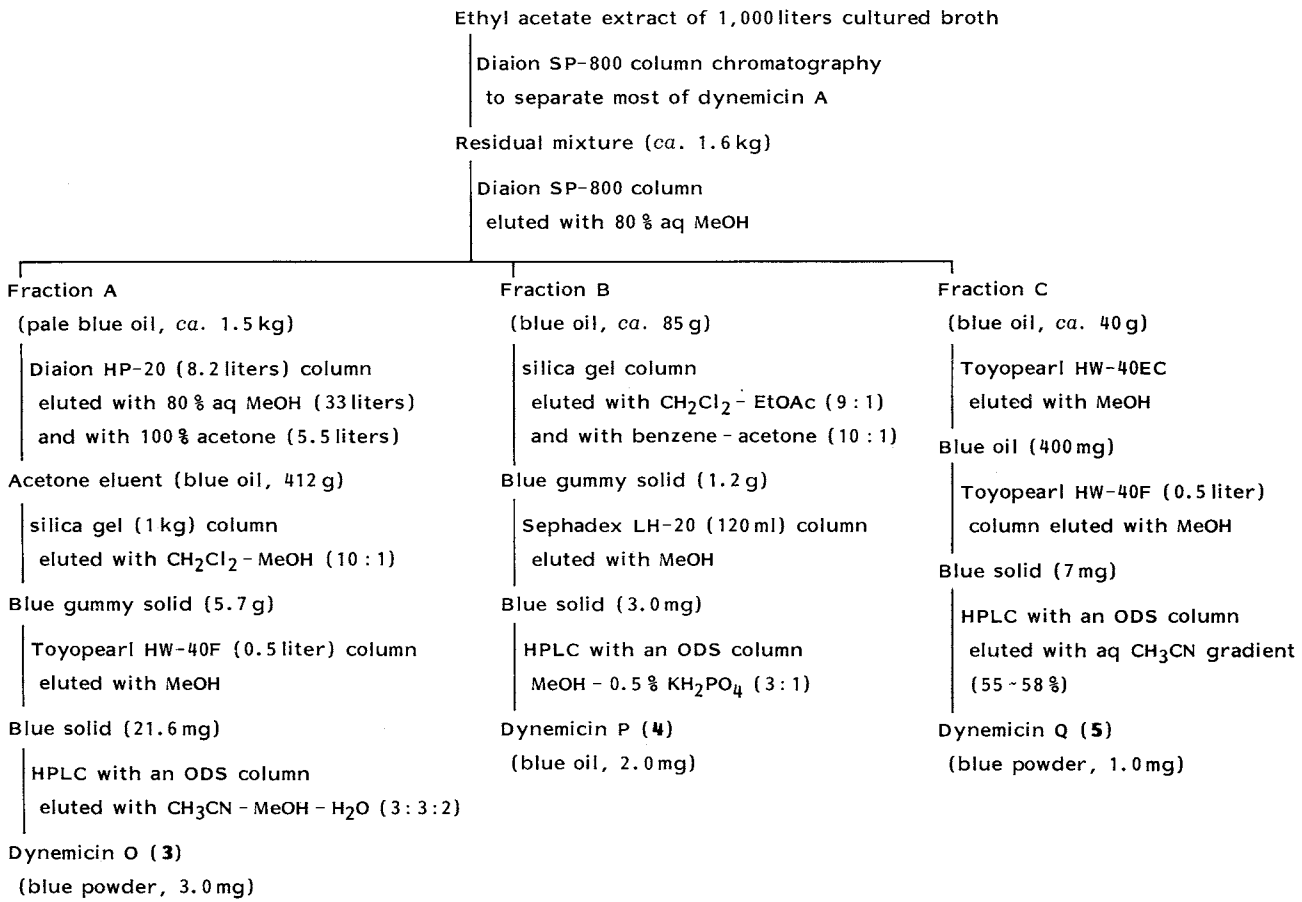


Table 1. Physico-chemical properties of dynemicins O, P and Q[a].

	Dynemicin O (3)	Dynemicin P (4)	Dynemicin Q (5)
Molecular formula	$C_{29}H_{23}NO_{10}$	$C_{28}H_{21}NO_9$	$C_{28}H_{19}NO_9$
Appearance	Blue powder	Blue powder	Blue powder
UV-VIS[b] λ_{max}^{MeOH} nm (ε)	212 (13,800), 241 (20,600), 283 (5,900), 335 (2,360), 589 (11,600), 633 (11,500)	210 (18,100), 241 (30,700), 333 (2,630), 586 (13,300), 632 (14,000)	217 (15,900), 243 (20,800), 335 (3,070), 588 (13,600), 634 (13,700)
HRFAB-MS[c] (m/z)			
M^+	545.1293 (545.1322)[d]	515.1266 (515.1216)[d]	513.1072 (513.1060)[d]
$(M+H)^+$	546.1334 (546.1400)[d]	516.1367 (516.1295)[d]	514.1145 (514.1138)[d]

[a] NMR and CD data are shown in Tables 2, 3 and Fig. 4.
[b] UV-VIS absorption spectra were measured on a Shimadzu apparatus (model UV-300).
[c] FAB-MS were measured on a Jeol JMS-HX110 mass spectrometer using *m*-nitrobenzyl alcohol as the matrix.
[d] Theoretical value for the molecular formula in parenthesis.

The spectra of **3** lacked both the characteristic four carbon signals of the conjugated acetylene (δ 88.9~99.4) and the two doublet protons of the alkene (δ 6.06 and 6.09) observed in **1**, and exhibited eighteen signals assignable to aromatic carbons. This fact clearly indicated that 1,5-diyn-3-ene had aromatized. Four ^{13}C-signals at δ 155.5, 155.5, 156.7 and 156.0 and four singlets of exchangeable protons appeared at δ 7.35, 12.16, 12.48 and 12.82 in the spectra of **3** were assigned to aromatic carbons bearing a hydroxy group and phenolic protons, respectively, suggesting that one of the newly formed aromatic carbons should be substituted by a hydroxy group. And this position was determined to be C-27 as follows: Three aromatic proton signals appearing at δ 6.85, 7.10 and 6.80 exhibited vicinal couplings to one another (Table 3) indicating that these hydrogens are attached to three sequential carbons. Among these, the signal at δ 6.85 was assigned to 24-H, since the signal could be correlated with 2-H (at δ 4.59) by NOE experiment. The carbon signal at δ 156.0 was, consequently, assigned to C-27.

Table 2. ^{13}C NMR data of dynemicin O (**3**) (in DMSO-d_6)[a].

Assignment[b]	Chemical shift (ppm)	Assignment[b]	Chemical shift (ppm)
2	56.4	15	155.5
3	73.6	16	126.0[e]
4	47.1	17	128.1[e]
4-CH_3	6.8	18	155.5
5	204.6	19	114.2[d]
6	84.2	20	187.9[e]
6-OCH_3	57.3	21	106.5
7	48.4	22	145.4
8	72.8	23	137.1
9	143.4	24	114.8
10	127.2	25	128.7
11	156.7	26	119.4
12	112.1	27	156.0
13	184.0[e]	28	118.9
14	113.3[d]		

[a] Data are recorded on a Jeol JNM GX-500 NMR spectrometer at 125.65 MHz. Chemical shifts are given relative to Me_4Si.
[b] The numbering system is shown in Fig. 1.
[c~e] Signal assignments were interchangeable and were made taking the assignment for dynemicin A into consideration[5].

Dynemicin P (**4**), a blue powder, gave a HRFAB-MS spectrum agreeing with the formula $C_{28}H_{21}NO_9$ which is one carbon less than dynemicin M (**2**, $C_{29}H_{23}NO_9$). The ^{1}H NMR spectrum of **4** indicated the presence of a OH group at C-6 instead of the OCH_3 group present at this position of **2** showing a broad doublet at δ 4.83 (J=3.8 Hz) coupled with 7-H (δ 3.19) and a broad singlet at δ 3.13 due to an exchangeable proton. The spectral data of **4** indicated that the structural difference between **4** and **2** lie only in this part and are fully consistent with the structure **4**, *O*-desmethyldynemicin M.

Dynemicin Q (**5**), a blue powder, gave a HRFAB-MS spectrum consistent with the formula $C_{28}H_{19}NO_9$

Fig. 4. ^{1}H-^{13}C correlation by HMBC experiment.

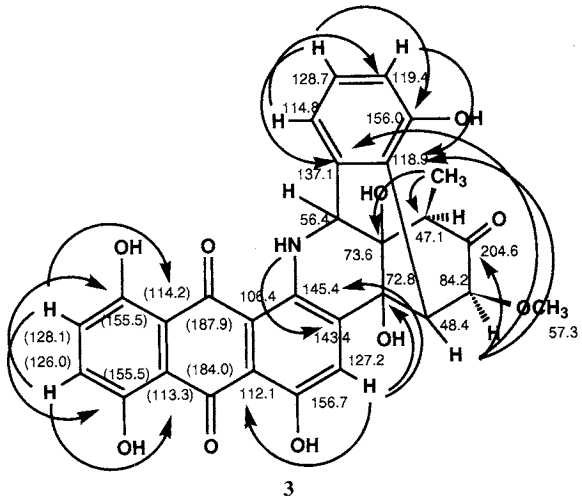


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Table 3. ^{1}H NMR data of dynemicins M, O, P and Q[a].

Position No.[b]	Proton	Chemical shift (δ, ppm), multiplicity and coupling constant (in Hz)			
		Dynemicin M (**2**)	Dynemicin O (**3**)	Dynemicin P (**4**)	Dynemicin Q (**5**)
1	1-NH	9.77 (1H, d, J=4.5)[c]	9.70 (1H, d, J=7.0)[c]	10.03 (1H, d, J=3.5)[c]	10.08 (1H, d, J=5.2)[c]
2	2-H	4.61 (1H, d, J=4.5)	4.59 (1H, d, J=7.0)	3.51 (1H, d, J=3.5)	4.73 (1H, d, J=5.2)
3	3-OH	3.97 (1H, br s)[d,f]	4.14 (1H, br s)[d,f]	4.09 (1H, br s)[f]	3.41 (1H, br s)[d,f]
4	4-H	3.29 (1H, q, J=7.0)	3.27 (1H, br q, J=7.5)[e]	4.12 (1H, br q, J=6.3)	
	4-CH_3	1.29 (3H, d, J=7.0)	1.30 (3H, d, J=7.5)	1.37 (3H, d, J=6.3)	2.01 (3H, s)
5	5-OH				5.79 (1H, s)[d]
6	6-H	4.83 (1H, d, J=4.5)	5.05 (1H, dd, J=4.0, 0.5)[e]	4.83 (1H, br d, J=3.8)	
	6-OCH_3	3.56 (3H, s)	3.65 (3H, s)		
	6-OH			3.13 (1H, br s)[c]	
7	7-H	3.67 (1H, d, J=4.5)	3.95 (1H, d, J=4.0)	3.19 (1H, d, J=3.8)	5.28 (1H, s)
8	8-OH	3.45 (1H, br s)[d,f]	3.51 (1H, br s)[d,f]	3.11 (1H, br s)[f]	3.20 (1H, br s)[d,f]
10	10-H	7.68 (1H, s)	7.63 (1H, s)	7.76 (1H, s)	7.74 (1H, s)
11	11-OH	12.82 (1H, s)[d]	12.74 (1H, s)[d]	12.99 (1H, s)	12.98 (1H, s)[d,i]
15	15-OH	12.48 (1H, s)[d]	12.40 (1H, s)[d]	12.67 (1H, s)	12.61 (1H, s)[d,i]
16	16-H	7.14 (1H, d, J=9.5)	7.13 (1H, d, J=9.0)	7.26 (1H, d, J=9.5)	7.20 (1H, d, J=9.5)
17	17-H	7.11 (1H, d, J=9.5)	7.09 (1H, d, J=9.0)	7.22 (1H, d, J=9.5)	7.23 (1H, d, J=9.5)
18	18-OH	12.16 (1H, s)[d]	12.12 (1H, s)[d]	12.30 (1H, s)	12.28 (1H, s)[d,i]
24	24-H	7.0~7.3 (4H, m)	6.85 (1H, dd, J=8.0, 0.5)	7.03 (1H, br d, J=8.0)[g]	7.1~7.3 (4H, m)
25	25-H		7.10 (1H, dd, J=8.0, 8.0)	7.22 (1H, dd J=8.0, 8.0)[h]	
26	26-H		6.80 (1H, dd, J=8.0, 0.5)	7.33 (1H, dd, J=8.0, 8.0)[h]	
27	27-H			7.46 (1H, br d, J=8.0)[g]	
	27-OH		7.35 (1H, s)[d]		

[a] Spectra were taken in $CDCl_3$ solution using a Jeol JMN-FX 500 spectrometer.
[b] The numbering system is shown in Fig. 1.
[c] Signal intensity decreased slowly by addition of D_2O.
[d] Signal disappeared by addition of D_2O.
[e] Coupling between 4-H and 6-H was confirmed by decoupling technique.
[f~i] Signal assignments were interchangeable.

which is only two hydrogen atoms less than that of **4**. Its ^{1}H NMR spectrum lacked both a proton quartet and the methyl doublet due to 4-H and 4-CH_3 in the spectra of **2**~**4**. Instead it exhibited a methyl singlet shifted to a lower field (δ 2.01). This suggested the introduction of a C(4)=C(5) double bond. The spectrum also showed the 7-H signal as a singlet at a lower field (δ 5.28) and a singlet of an exchangeable proton at δ 5.79 (C(5)-OH). Summarizing all the spectral data, structure **5**, an oxidized form of **4**, was proposed for dynemicin Q.

As shown above, compounds **3**, **4** and **5** have, like **2**, a bridging phenylene group, a vicinal diol and a keto function in place of a 1,5-diyn-3-one bridge, an epoxide and a carboxyl group in **1**, respectively. It is quite conceivable that these compounds were derived from the precursor(s) having an enediyne and an epoxy group *via* the hydrolytic opening of the epoxide followed by aromatization of the enediyne system. The possibility that compounds **2**~**5** may be artifacts should be eliminated, because, on the one hand, the compound **2** which is the most abundant component of these four compounds appeared in the ethyl acetate extract of the cultured broth, even at earlier stages of cultivation. On the other hand, the epoxy group in **1** was stable even under acidic and alkaline conditions that are much more severe than that in the cultivation and isolation processes.

CD Spectra of Dynemicins

The CD spectra of compounds **1**~**5** were drawn in Fig. 5. A distinct difference of the CD curves was observed at around 280 nm between **1** having an enediyne system and **2**~**5** in which the enediyne system had aromatized. The CD curve of **1** showed peaks at 280 and 293 nm, whereas the latter group of compounds exhibited a trough at this region. Since the 1,5-diyn-3-ene chromophore normally has UV absorption maxima at 265~290 nm, the positive Cotton effects shown in this region by dynemicin A should reflect the stereochemistry of its tricyclo core containing the enediyne moiety.

Biological Activities

The antimicrobial activity and *in vitro* cytotoxicity against murine and human tumor cells were

Fig. 5. CD spectra of dynemicins A (**1**), M (**2**), O (**3**), P (**4**) and Q (**5**).

CD spectra were taken in dioxane solutions on a Jasco J-20A recording spectro polarimeter. Respective CD curves are drawn as follows: —·—·—, A (**1**); ——, M (**2**); – – –, O (**3**); —·—·—, P (**4**); ------, Q (**5**).

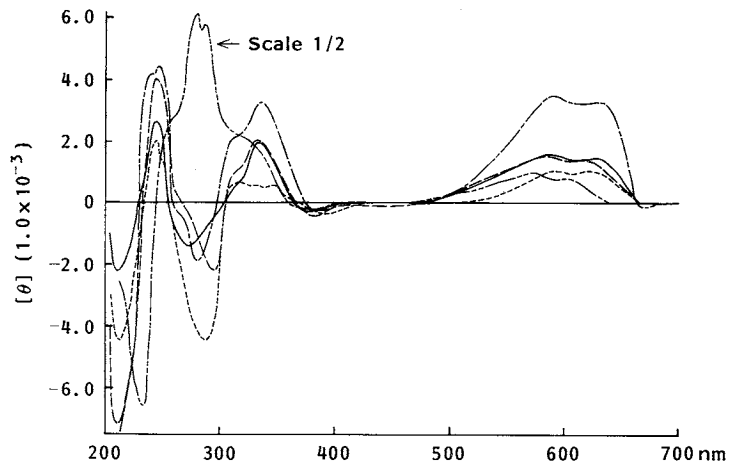

Table 4. Antibacterial activity of dynemicins O (**3**) and Q (**5**)[a] compared with that of dynemicin A (**1**).

Strain	MIC (μg/ml)		
	3	**5**	**1**
Staphylococcus aureus FDA 209P	0.08	0.08	<0.00008
S. aureus Smith	0.02	0.02	<0.00008
S. epidermidis 11-1168	0.01	0.01	<0.00008
S. epidermidis 11-1230	0.02	0.02	<0.00008
Enterococcus feacalis A9808	0.63	1.25	0.00016
E. faecium A24817	0.63	10	<0.00008
Micrococcus luteus PCI 1001	0.63	5	<0.00008
Bacillus subtilis PCI 219	0.0025	0.005	<0.00008
Escherichia coli Juhl A15119	>10	>10	0.01
Klebsiella pneumoniae PCI 602	>10	>10	0.01
Proteus mirabilis IFO 3849	>10	>10	0.005
P. vulgaris IPM-13	>10	>10	0.0013
P. rettgeri IPM-14	>10	>10	0.0013
Morganella morganii 1510	>10	>10	0.0025
Enterobacter cloacae IPM-12	>10	>10	0.005
Serratia marcescens IPM-15	>10	>10	0.01
Citrobacter freundii GN7391	>10	>10	0.005
Pseudomonas aeruginosa A9843A	>10	>10	0.005
P. cepacia No. 651	>10	>10	0.0025
Xanthomonas maltophilia GN12873	>10	>10	0.005
Comamonas terrigena IFO 12685	0.08	0.16	0.00016

[a] Activity of dynemicin P (**4**) could not be assayed because of the shortage of the material.

evaluated for the compounds **3**, **4** and **5**, and the results are shown in Tables 4 and 5. It should be pointed out that they showed potent, though much less so than dynemicin A, activities, especially against Gram-positive bacteria. It has been emphasized in the enediyne class of antibiotics that they act by metabolic rearrangement to 1,4-dihydrobenzene diradicals which abstract hydrogens from a deoxyribose unit in DNA, initiating the strand breakage. DNA breakage by dynemicin A has also been demonstrated[4]. It is, however, noteworthy that compounds **3**, **4** and **5** retained high activity even without enediyne moiety. This suggested that the DNA cleavage should not be the sole mechanism causing the biological activity of dynemicin A and that the anthraquinone nucleus can also cause the damage in DNA functions by intercalating into base pairs.

Table 5. Cytotoxicity of dynemicins O (**3**), P (**4**) and Q (**5**).

Cell[a]	IC_{50} (μg/ml)		
	Dynemicin O (**3**)	Dynemicin P (**4**)	Dynemicin Q (**5**)
B16F10	0.48	3.6	8.3
HCT116	2.8	5.3	9.3
P388	0.04	0.18	0.54
K562/S	0.13	0.45	1.71

[a] B16F10: Mouse melanoma, HCT116: human colon carcinoma, P388: mouse leukemia, K562/S: human chronic myelogenous leukemia.

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