45. The Structures of Some Products from the Photodegradation of the Pluramycin Antibiotics Hedamycin and Kidamycin

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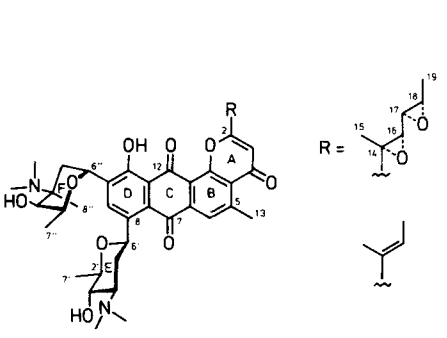
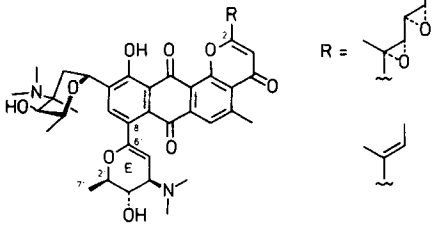
The photolability of the antitumor antibiotic hedamycin (**1**) was investigated by irradiation in different solvents in the presence or in the absence of oxygen. The products formed were separated chromatographically and their structures determined by NMR spectroscopy. Photolysis of **1** in the presence of oxygen gave only one isolable product, photohedamycin A (**3**), where ring E of hedamycin had been transformed into an enol ether. The reaction in the absence of oxygen yielded the photohedamycins B, C, and D (**5**, **6**, and **7**, respectively). In these compounds, one of the epoxides of hedamycin had been opened reductively, and in photohedamycin D (**7**) the substituent at C(8) – originally ring E of hedamycin – was now acyclic. In addition to these compounds, the photolyses yielded a large number of unstable minor products, which could not be isolated.

1. Introduction. – Some years ago, biochemists reported that solutions of the antitumor antibiotic hedamycin (**1**)[2]) lost their biological activity rapidly when allowed to stand



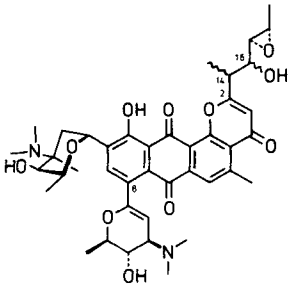
1 hedamycin

2 kidamycin



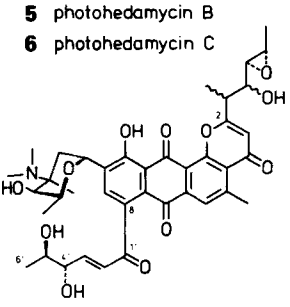
3 photohedamycin A

4 photokidamycin A



5 photohedamycin B

6 photohedamycin C



7 photohedamycin D

[1]) From the dissertation of *A. F.* [1].

[2]) The numbering in the formulae corresponds to that established earlier [2] [3].

in the daylight [4]. To shed light on this photodegradation, we recently undertook a study where model compounds with epoxy and diepoxy substituents related to the side chain at C(2) of **1** were irradiated and the photoproducts formed isolated and identified [5]. We now report on some products obtained from the photodegradation of **1** and of the related kidamycin (**2**).

Solutions of **1** in a number of solvents were irradiated with a high pressure Hg lamp using a *Pyrex* vessel. The glass thus acted as a filter cutting off any radiation below 300 nm [6]. The composition of the product mixtures obtained was determined chromatographically (see *Table 1*). In most cases, only one product could be isolated, the balance being many intractable minor products. Thus, for preparative attempts, the conditions were optimized for the isolation of one photoproduct at a time.

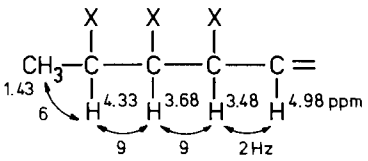
Table 1. *Products Obtained from the Photolysis of* **1**

Solvent	Period of irradiation [min][a])	Starting material **1** recovered[b])	Products[b])			
			3	**5**	**6**	**7**
Photolyses under oxygen						
Toluene	10	20	48			
Toluene	15	7	45			
CH_2Cl_2	15	15	35			
CH_3CN	20	18	21			
CH_3OH	10	7	33			
Photolyses in the absence of oxygen						
Toluene	40	5	12	7	2	6
CH_2Cl_2	15	36	21	2	3	[c])
CH_2Cl_2	30	19	16	2	3	[c])
CH_2Cl_2	42	9	14	2	3	3
CH_3CN	40	10	21	7	10	3
Acetone	20	11	5	6	5	5
Acetone	40	3	2	4	3	7
CH_3OH	40	2	7	5	21	< 1
aq. buffer[d])	40	2	18	2[e])		3
aq. buffer[d])	60	< 1	6	3[e])		3

[a]) Due to the different procedures used, the periods of irradiation applied in the presence of oxygen are different from those used in the absence of oxygen.
[b]) Yields in percent of the starting material subjected to photolysis.
[c]) Not determined.
[d]) 0.05M KHPhthalate buffer, pH 4.
[e]) Sum of the isomers **5** and **6**.

2. Structure Determinations. – The structures of the photoproducts were determined by comparison of the ^{1}H- and ^{13}C-NMR spectra with the corresponding spectra of hedamycin (**1**). The molecular weights of the compounds were confirmed by fast-atom-bombardment MS.

2.1. *Photohedamycin A* (**3**). A spectral comparison with **1** clearly showed that the constitution of **3** was the same as hedamycin, with the exception of ring E. The Me_2N group was still present, and the ^{1}H-NMR spectrum (see *Fig. 1* and *Table 2*) revealed the following structural fragment:



Assignments and coupling constants were corroborated by double-resonance experiments. Compound **3** thus lacked two H-atoms with respect to **1**. The ^{13}C-NMR spectrum of **3** (see *Table 3*) showed four resonances, whose chemical shifts corresponded rather well to those of C(2'), C(3'), C(4') and C(7') of **1**, indicating that this portion of ring E was still intact. Two further resonances at 156.7 and 95.2 ppm pointed to an enol structure, thus leading to the constitution **3**. The assignment of the ^{13}C-resonances was in general straightforward. The resonance of C(7a) was assigned assuming that the influence of the now unsaturated substituent at C(8) was more or less the same for C(9) and C(7a). The resonance of the protonated C(9) was easily detected and proved to have been shifted downfield by 3 ppm as compared with **1**. Thus, one of the lines around 129 ppm was assigned to C(7a), while the other one had to belong to C(8). The upfield shift observed for the latter as a consequence of the replacement of the saturated ring E in **1** by the enol structure in **3** is reasonable [7]. The configurations indicated for C(2'), C(3') and C(4') in formula **3** were not determined. We assume, however, that they were not affected by the photoreaction.

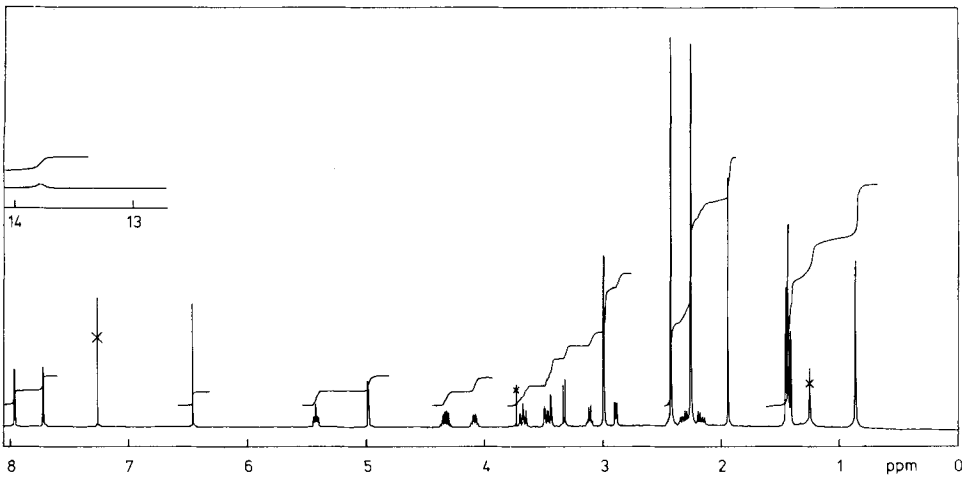
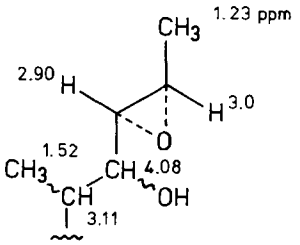


Fig. 1. *360-MHz ^{1}H-NMR Spectrum of* **3** *in $CDCl_3$.* The crossed peaks correspond to $CHCl_3$, $(CH_2Cl)_2$ and paraffin.

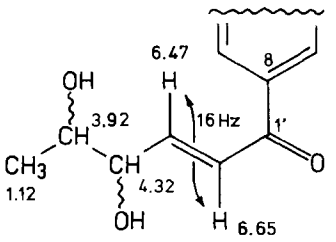
2.2. *Photohedamycin B and C* (**5** and **6**, respectively). The NMR spectra of **5** and **6** (see *Tables 2* and *3*) suggested that the two compounds were diastereoisomers. The ^{1}H-NMR spectrum of **6** (see *Table 2*) showed that the main chromophore as well as ring F remained the same as in **1**, and that ring E had the enol structure found in **3**. However, the side chain at C(2) was altered as could be seen from the absence of the singlet resonance corresponding to the $H_3C(15)$ group and the appearance of a new CH_3 resonance at 1.52 ppm as

a doublet. Extensive decoupling experiments showed that this part of the molecule contained a CH_3–CH–CH– fragment, which had to be part of a CH_3-substituted oxirane as indicated by the coupling constants observed (5 and 2 Hz):



One proton of this oxirane was further coupled to a methine proton, whose chemical shift of 4.08 ppm pointed to an attached OH group. The signal of this latter proton was obscured by the resonance of H–C(2″). Recording the spectrum in a mixture of $CDCl_3$ and deuterobenzene, however, yielded separated signals. The resonance of the methine proton at 4.08 ppm was further split by coupling with another methine proton and this coupled to a CH_3 group (1.52 ppm). The structures thus derived for **5** and **6** were corroborated by the ^{13}C-NMR spectra; all the resonances could be assigned in a straightforward manner. It was not possible, however, to establish the relative configurations at the side-chain C-atoms. We assume, that the configuration of the terminal epoxide remains the same as in the starting material **1**, but we cannot tell, whether **5** and **6** differ in the configuration at C(14) or at C(16).

2.3. *Photohedamycin D* (**7**). The 400-MHz ^{1}H-NMR spectrum of **7** (see *Table 2*) clearly showed that the main chromophore and ring F were the same as in **1**, and the side chain at C(2) corresponded to that of **5** and **6**; the chemical shifts rather pointed to the configurations of **5**. However, it was noted that the signal of the Me_2N group of ring E was absent and that the resonances that should belong to this part of the molecule were distinctly shifted and altered. The ^{1}H-NMR spectrum further revealed the resonances of two olefinic protons around 6.4–6.7 ppm with a common coupling constant of 16 Hz, indicating the (*E*)-configuration for the corresponding double bond. One of these protons was coupled with a resonance at 4.32 ppm (*t*), which further coupled with a methine resonance at 3.92 ppm. The latter was split in addition by a CH_3 group. The two protons with resonances around 4 ppm pointed to O functions, presumably OH groups. The (*E*)-configuration at the double bond precluded a cyclic structure similar to the original ring E. The ^{13}C-NMR spectrum (see *Table 3*) revealed a ketone carbonyl resonance at 196.1 ppm. All these findings lead to the following structure for the substituent at C(8):

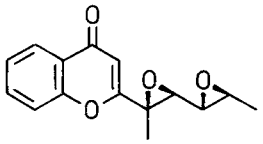


The ^{13}C-resonances were easily assigned (see *Table 3*); the resonances found for the new substituent at C(8) corroborated the structure proposed for **7**. The relative configurations of the C-atoms in the substituent at C(8) could not be determined. We assume, however, that the configurations of C(4') and C(5') are the same as those of the corresponding C-atoms (C(3') and C(2'), respectively) in **1**.

2.4. *Photokidamycin A* (**4**). The structure of **4** was evident after a comparison of its ^1H-NMR spectrum (see *Table 2*) with that of **3**, bearing in mind the spectral differences between **1** and **2** [3] [8].

3. Discussion. – Hedamycin (**1**) was irradiated in a variety of solvents for different periods of time. These results are summarized in *Table 1*. The experiments carried out in the presence of oxygen may not be directly compared with those carried out in the absence of oxygen since different apparatus were used. It is noteworthy that in the presence of oxygen – the conditions best imitating the laboratory conditions under which the photodeactivation of **1** was first observed by *White* and *White* [4] – only one photoproduct could be isolated. No effort was undertaken to elucidate the reaction mechanisms leading to the different photoproducts. But one might assume that the enol-ether function in ring E of **3** is formed in the following way: the first step is probably the reduction of the anthraquinone part of **1** to the corresponding hydroquinone in analogy to the well-known photoreduction of anthraquinone itself [9] [10]. The sterically well positioned ring E may serve as an intramolecular H-donor and thus lose two H-atoms. The hydroquinone system is then reoxidized to the quinone. This occurs at the latest during workup in the presence of air. A similar, although somewhat different example for such a reaction sequence was published recently by *Lynch* and *Meth-Cohn,* involving intramolecular H-abstraction from a piperidine substituent attached to an anthraquinone derivative [11]. The fact that the irradiation of kidamycin (**2**) yielded the analogous photoproduct, photokidamycin A (**4**), showed that the observed reaction is typical for all antibiotics of the pluramycin type.

In the two diastereoisomeric photoproducts **5** and **6**, ring E was the same as in **3**, but the side chain at C(2) was transformed with respect to **1**. Two pathways might lead to **5** and **6**. Either, irradiation of **1** first changes the structure of the side chain, which would give two products that were not isolated, and which, with a second photon, would react under H-abstraction at ring E to give **5** and **6**. The second possibility would be that **5** and **6** are secondary products of the photolabile **3**. Since **3** was found in large quantities, we conclude that this second pathway is probably the more important one. The photoreaction at the side chain of **3** involves formally a reductive opening of the oxirane ring at the C(14)-O bond. A similar reaction was not observed during our studies with a 4*H*-chromen-4-one (4-chromone) model compound (**8**) with the hedamycin side chain [5]. This is evident, since the formation of a side chain as in **5** and **6** is dependent on a H-donor, which was absent in the model studies mentioned.



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Accordingly, *Keller et al.* observed an analogous opening of the oxirane rings after the irradiation of epoxy ketones in the presence of Bu_3SnH [12]. We, therefore, may conclude that in the formation of **5** and **6**, a derivative of **1** in the anthrahydroquinone form acts as the H-donor. To ascertain a sufficient level of such hydroquinone derivatives, the solution should be as free of oxygen as possible. Consequently, **5** and **6** should not be formed in the presence of oxygen. Indeed, we could not detect them under such conditions. In principle, the opening of the oxirane ring could involve a change of configuration at C(14) and/or at C(16). Thus, four diastereoisomeric products would be expected. Since only two of them were found[3]), we assume that only one of the two C-atoms mentioned was epimerized. Attempts to investigate the relative configurations at C(14) and C(16) with the aid of model compounds in analogy to our earlier work [13] failed so far.

Photohedamycin D (**7**) has an unchanged hedamycin chromophore and ring F. The side chain at C(2) is the same as in **5**. However, instead of ring E, **7** features an open-chain substituent at C(8). The way of formation of **7** is not clear. Formally, it is formed in two steps. Hydrolysis of the enol ether in a compound like **5** or **6** leads to the 1'-carbonyl group and the 5'-hydroxy group. Further, Me_2NH must be eliminated. A similar thermal elimination of Me_2NH was described by *Furukawa et al.* using the triacetate of **2** and a *Cope*-type elimination with perbenzoic acid [2]. Compound **7** was shown to have the same configurations as **5** at the C-atoms of the C(2)-side chain. One would expect a second isomer with the configurations of **6**; this compound could, however, not be detected.

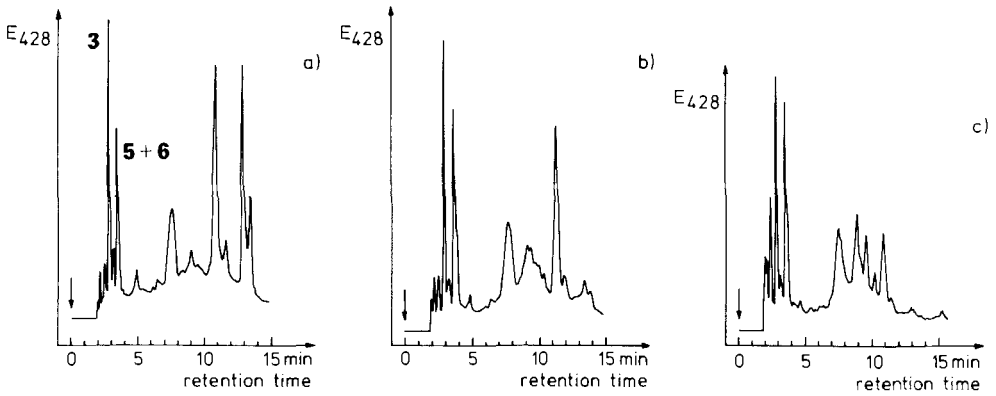

Fig. 2. *HPLC traces of the product mixture obtained from a 40-min photolysis of* **1** *in toluene in the absence of oxygen.* Analytical silica gel column *(Nucleosil 50-5)*, CH_2Cl_2/EtOH/Et_3N, 4/5 H_2O-sat., 94.5:5:0.5→84:15:1, 1.5 ml/min. *a*) Injection immediately after workup of the mixture, *b*) injection *ca.* 45 min after workup, *c*) injection *ca.* 90 min after workup.

Hedamycin (**1**) was not only irradiated in organic solvents but also in aqueous buffer solutions in the absence of oxygen. The photoproducts were then extracted with CH_2Cl_2; a blank test with **1** showed this extraction to be quite reproducible. However, even after extraction with CH_2Cl_2, the aqueous phases of the photolyses were still orange or red, which pointed to rather polar degradation products. In general, it was quite difficult to

[3]) Additional stereoisomers would have been recognized in the HPLC of the photoreaction in MeOH, even if their amount would have been one tenth of that of **6**; a tenfold stereoselectivity of this reaction in MeOH is thus certain.

isolate distinct products from the photolysis mixtures. The products were mostly very unstable. This is best illustrated by *Fig. 2*. Samples of the same product mixture were purified by chromatography in intervals of about 45 min. It can clearly be seen that the product composition rapidly changed with time.

We assume that such polar compounds are formed under all photolysis conditions. This is in accord with our earlier observation, that some of the photodecomposition products of **1** were readily water-soluble. Our model studies with the 4-chromone derivative **8** showed that a complex mixture of polar products was obtained, which was difficult to separate. Therefore, we did not further investigate the polar photoproducts of **1**. In analogy to our results with the photolysis of **8** [5], we assume that also **1** and probably some of its photoproducts dimerize and polymerize, especially in the absence of oxygen.

The irradiation of **1** in the presence of oxygen gave only one isolable photoproduct, **3**, besides a vast number of intractable minor products, whereas **8** under the same conditions yielded some well-defined products. These differences in behavior might be explained by the formation of singlet oxygen in the quenching reaction with the excited triplet state of the hedamycin chromophore. This singlet oxygen may then react with **1**, and the photoproducts formed, yielding the many minor products observed.

We thank Dr. *W. T. Bradner*, Syracuse, for a generous gift of hedamycin. Financial support by the *Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung* is gratefully acknowledged.

Experimental Part

1. *General.* The apparatus for the photolyses consisted of a 75-W Hg high-pressure lamp *TQ 81* (*Quarzlampen GmbH*, Hanau) fitted with a silica water cooling jacket. Photolyses in the presence of oxygen were carried out in an open *Pyrex* test tube, 32 mm ∅, which was placed next to the cooling jacket of the lamp. The soln. was presaturated with technical grade oxygen and stirred with a magnetic stirrer. Reactions in the absence of oxygen were carried out in a special vessel consisting of a 200-ml round bottomed flask with a ground glass joint that could be sealed off with a *Rotaflo* stopcock. To this flask was fused a cyclindrical side arm, ∅ 26 mm, 40 ml. The soln. to be irradiated was placed in the main part of the apparatus and degassed at 10^{-5} bar by three freeze-pump-thaw cycles. Then the soln. was transferred to the cylindrical part, which was placed next to the Hg lamp. The solution was stirred magnetically during the reaction time.

HPLC: *Spectra-Physics* pump and gradient mixer *SP 8700*, variable wavelength detector *SP 8400*, *Hewlett-Packard* integrator *3380 A*. Columns: semiprep.: silica gel 8 × 250 mm, *LiChrosorb Si 60*, 7 μm (*Knauer AG*, Oberursel, FRG); anal.: silica gel 4.6 × 250 mm, *Nucleosil 50-5* (*Macherey-Nagel*, Düren, FRG)[4]).

Solvents were removed *in vacuo* at 30–35° using a rotary evaporator.

UV/VIS spectra were measured in 96% EtOH (*Fluka*, 'for UV spectroscopy') on a *Varian Cary 219* spectrometer. NMR spectra were recorded on the following instruments: *Bruker WH 90* (spectral laboratory of our Institute, *K. Aegerter*), *Bruker WP 200 SY* (Pharmazeutisches Institut der Universität Basel), *Bruker WH 360* and *Bruker WM 400* (*Ciba-Geigy AG*, Basel, *P. Hug*). Xe FAB-MS were measured on an *AEI MS 902* mass spectrometer equipped with a *Kratos FAB* source and a *VG DS 20-50* data system (*F. Hoffmann – La Roche & Co. AG*, Basel, Dr. *W. Vetter, P. Meyer*). A small amount of a soln. of the sample in MeOH was diluted with thioglycerol and applied to the FAB target.

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[4]) Column chromatography had heardly been used so far with hedamycin (**1**). Even on TLC **1** was not easy to handle. It moved satisfactorily on silica-gel plates only in a mixture of $CHCl_3$ and Et_3N. When the base was omitted from the eluent, hedamycin stayed at the start. The use of silica-gel columns with solvents containing Et_3N proved to be tricky inasmuch as no reproducible retention times could be achieved. The columns tended to be deactivated by the base content of the eluent, leading to increasingly shorter retention times for **1**. Besides Et_3N, NH_3 was also investigated as the basic component of the eluent. However, the results with this base were even less reproducible than with Et_3N due to the higher volatility of NH_3.

Table 2. *^{1}H-NMR Data of* **1** *and* **3**–**7**[a])

	1[b])	**3**	**4**	**5**	**6**	**7**
H–C(3)	6.46 (*s*)	6.46 (*s*)	6.38 (*s*)	6.33 (*s*)	6.33 (*s*)	6.33 (*s*)
H–C(6)	8.00 (*d*, *J* = 0.6)	7.96 (*d*, *J* = 1)	7.93 (br. *s*)	7.95 (*s*)	7.96 (*s*)	7.93 (*s*)
H–C(9)	8.33 (*s*)	7.72 (*d*, *J* = 1)	7.72 (br. *s*)	7.72 (*s*)	7.72 (*s*)	7.68 (*s*)
H_3C(13)	2.99 (br. *s*)	3.00 (*s*)	3.01 (*s*)	3.02 (*s*)	3.01 (*s*)	2.98 (*s*)
H–C(14)				3.03 (*qd*, *J* = 7 and 5)	3.11 (*qd*, *J* = 7 and 5)	3.03 (*quint.*)
H_3C(15)	1.96 (*s*)	1.96 (*s*)	2.01 (*s*)	1.56 (*d*, *J* = 7)	1.52 (*d*, *J* = 7)	1.55 (*d*, *J* = 7)
H–C(16)	3.32 (*d*, *J* = 6)	3.33 (*d*, *J* = 5)	7.47 (*q*, *J* = 6)	4.08 (*m*)	4.08 (*m*)	4.08 (*m*)
H–C(17)	2.89 (*dd*, *J* = 5 and 2)	2.89 (*dd*, *J* = 5 and 2)		2.91 (*t*, *J* = 2)	2.90 (*t*, *J* = 2)	2.90 (*t*, *J* = 2)
H_3C(17)			2.04 (*d*, *J* = 8)			
H–C(18)	3.11 (*qd*, *J* = 5 and 2)	3.11 (*qd*, *J* = 5 and 2)		3.15 (*qd*, *J* = 5 and 2)	*ca.* 3.00 (*m*)	3.15 (*m*)
H_3C(19)	1.44 (*d*, *J* = 5)	1.44 (*d*)*		1.27 (*d*, *J* = 5)	1.23 (*d*, *J* = 5)	1.32 (*d*, *J* = 5)
H–C(2′)	3.55 (*m*)	4.33 (*qd*, *J* = 6 and 9)	4.33 (*qd*, *J* = 6 and 9)	4.33 (*qd*, *J* = 6 and 9)	4.32 (*qd*, *J* = 6 and 9)	6.65 (*d*, *J* = 16)
H–C(3′)	3.19 (*t*, *J* = 9)	3.68 (*t*, *J* = 9)	3.67 (*t*, *J* = 9)	3.72 (*t*, *J* = 9)	3.68 (*t*, *J* = 9)	6.47 (*dd*, *J* = 16 and 5)
H–C(4′)	2.93 (br.)	3.48 (*dd*, *J* = 9 and 2)	3.48 (*dd*, *J* = 9 and 2)	3.62 (*dd*, *J* = 9 and 2)	3.52 (*dd*, *J* = 9 and 2)	4.32 (*t*, *J* = 5)
H–C(5′)	*ca.* 1.2*	4.98 (*d*, *J* = 2)	4.99 (*d*, *J* = 2)	4.96 (*d*, *J* = 2)	4.98 (*d*, *J* = 2)	3.92 (*m*)
	ca. 2.5*					
H–C(6′)	5.45 (*m*)					
H_3C(6′)						1.12 (*d*, *J* = 6)
H_3C(7′)	1.43 (*d*, *J* = 6)	1.43 (*d*)*	1.42 (*d*, *J* = 5)	1.43 (*d*, *J* = 6)	1.42 (*d*, *J* = 6)	
$(CH_3)_2$N–C(4′)	2.32 (*s*)	2.42 (*s*)	2.43 (*s*)	2.54 (*s*)	2.46 (*s*)	
H–C(2″)	4.04 (br. *q*)	4.09 (*qd*, *J* = 6 and 2)	4.08 (*qd*, *J* = 6 and 2)	4.08 (*m*)	4.08 (*m*)	4.04 (*m*)
H–C(3″)	3.35 (br. *s*)	3.44 (*d*, *J* = 2)	3.43 (*d*, *J* = 2)	3.44 (*d*, *J* = 1)	3.40 (*d*, *J* = 1)	3.43 (*d*, *J* = 1)
H–C(5″)	*ca.* 2.1 (*m*)*	2.17 (*dd*, *J* = 14 and 6)	*ca.* 2.1 (*m*)	2.20 (*dd*, *J* = 14 and 6)	*ca.* 2.15 (*m*)	*ca.* 2.02 (*m*)
	ca. 2.7 (*m*)*	2.32 (*dd*, *J* = 14 and 6)	*ca.* 2.3 (*m*)	2.33 (*dd*, *J* = 14 and 6)	*ca.* 2.33 (*m*)	*ca.* 2.3 (*m*)
H–C(6″)	5.45 (*m*)	5.43 (*t*, *J* = 6)	5.43 (*t*, *J* = 6)	5.41 (*t*, *J* = 6)	5.39 (*t*, *J* = 6)	5.44 (*t*, *J* = 6)
H_3C(7″)	1.51 (*d*, *J* = 6)	1.45 (*d*)	1.45 (*d*, *J* = 5)	1.45 (*d*, *J* = 6)	1.44 (*d*, *J* = 6)	1.42 (*d*, *J* = 6)
H_3C(8″)	0.71 (*s*)	0.87 (*s*)	0.84 (*s*)	0.87 (*s*)	0.82 (*s*)	0.85 (*s*)
$(CH_3)_2$N–C(4″)	2.22 (*s*)	2.26 (*s*)	2.25 (*s*)	2.29 (*s*)	2.25 (*s*)	2.26 (*s*)

[a]) Chemical shifts given in ppm downfield from internal TMS (= 0 ppm). Solvent: $CDCl_3$. Assignments with asterisks may be interchanged.

[b]) Data from [3].

Table 3. *^{13}C-NMR Data of* **1**, **3**, *and* **5–7**[a])

C-Atoms	1[b])	3	5	6	7	C-Atoms	1[b])	3	5	6	7
C(1a)	156.1	156.0	156.3	156.4	156.5	C(1′)					196.1
C(2)	166.3	166.1	169.6	169.6	169.8	C(2′)	77.3	76.7	76.8	76.7	131.4**
C(3)	110.0	110.1	112.7	112.5	112.8	C(3′)	71.9	68.2*	68.3*	68.2*	144.8
C(4)	178.7	178.8	178.8	178.7	178.6	C(4′)	67.4	66.1*	66.6*	66.7*	74.8
C(4a)	126.2*	126.1	126.1	126.1	125.9	C(5′)	28.3	95.2	94.4	95.1	69.9
						C(6′)	75.2	156.7	157.4	157.2	17.7
C(5)	149.7	150.1	150.5	150.3	150.8	C(7′)	18.9	17.6	17.6*	17.6*	
C(6)	125.9	125.9	125.6	125.6	126.7	$(CH_3)_2N$–C(4′)	40.4	40.8	40.6	40.8	
C(6a)	137.3	137.1	136.9	137.2	135.6						
C(7)	183.1	181.2	181.2	181.2	181.3	C(2″)	67.3	67.7	67.6	68.0	67.8
C(7a)	125.8*	128.8*	128.7*	129.2*	128.5*	C(3″)	70.9	71.1	71.1	71.4	71.2
						C(4″)	57.3	57.4	57.5	57.9	57.6
C(8)	140.2	129.0*	129.1*	129.2*	133.8*	C(5″)	33.7	34.8	34.6	35.5	35.3
C(9)	133.1	136.2	136.1	136.2	131.9**	C(6″)	69.6	69.0	68.7	69.1	68.1
C(10)	138.6	139.5	139.3	139.7	141.2	C(7″)	17.6	17.6	17.6*	17.5*	17.5*
C(11)	159.8	160.2	160.3	160.3	160.2	C(8″)	12.3	13.3	13.4	13.6	13.7
C(11a)	116.1	116.6	116.6	116.8	115.7	$(CH_3)_2N$–C(4″)	36.8	37.2	37.2	37.4	37.4
C(12)	188.0	188.0	188.4	188.4	188.0						
C(12a)	119.2	119.0	118.8	119.0	119.1						
C(13)	24.1	24.3	24.4	24.1	24.3						
C(14)	57.7	57.6	43.7	43.6	43.8						
C(15)	14.5	14.4	15.5	13.6	15.4						
C(16)	63.9	63.9	71.2	72.2	71.3						
C(17)	55.4	55.4	60.7	60.3	60.6						
C(18)	51.8	51.9	51.7	52.0	51.9						
C(19)	17.2	17.3	17.0	17.1	17.0						

[a]) Chemical shifts given in ppm downfield from internal TMS (= 0 ppm). Solvent: $CDCl_3$. Assignments with asterisks may be interchanged.
[b]) Data from [8].

2. *Photohedamycin A (8-[4-(Dimethylamino)-3-hydroxy-2-methyl-3,4-dihydro-2*H*-pyran-6-yl]-2-(3,3'-dimethyl[2,2'-bioxiran]-3-yl)-11-hydroxy-5-methyl-10-[2,3,6-trideoxy-3-(dimethylamino)-3-*C*-methyl-α-L-lyxo-hexopyranosyl]-4*H*-anthra[1,2-b]pyran-4,7,12-trione; **3**). Hedamycin (2-(3,3'-Dimethyl[2,2'-bioxiran]-3-yl)-11-hydroxy-5-methyl-8-[2,3,6-trideoxy-3-(dimethylamino)-β-D-arabino-hexopyranosyl]-10-[2,3,6-trideoxy-3-(dimethylamino)-3-C-methyl-α-L-lyxo-hexopyranosyl]-4*H*-anthra[1,2-b]pyran-4,7,12-trione,* **1**, 10 mg 13 μmol) was dissolved in 51 ml of toluene. The soln. was saturated with oxygen and irradiated for 15 min in the apparatus described above. After removal of the solvent, a light brown resinous lac was obtained, which contained **3** (48%) and **1** (20%) according to HPLC. The products of several similar photolyses were pooled to give 90 mg containing *ca.* 25 mg of **3** (HPLC). The mixture was dissolved in 1 ml of CH_2Cl_2 and separated by HPLC on the semiprep. silica gel column (17 injections; CH_2Cl_2/i-PrOH/25% aq. NH_3 93:7:0.25, H_2O-sat.; 4 ml/min) yielding 4.2 mg of **1** ($t_R \approx 3.5$ min, orange lac) and 16 mg of **3** ($t_R \approx 9$ min, orange lac). The photoproduct **3**, which was 98% pure (anal. HPLC), was rechromatographed as described above (10 injections) and 13.4 mg of pure **3** were obtained as an orange lac. UV/VIS (EtOH): 211 (39,000), 243 (48,000), 263 (sh, 35,000), 385 (8,200), 422 (8,900). ^{1}H-NMR (360 MHz, 0.015M in $CDCl_3$): see *Fig. 1* and *Table 2*[2]). ^{13}C-NMR (90.5 MHz, 0.015M in $CDCl_3$): see *Table 3*[2]). FAB-MS: (*M* + thiglycerol + H) 853, (*M* + 3H) 747, (*M* + H) 745; mass number for $C_{41}H_{48}N_2O_{11}$: 744.

3. *Photokidamycin A (8-[4-(Dimethylamino)-3-hydroxy-2-methyl-3,4-dihydro-2*H*-pyran-6-yl]-11-hydroxy-5-methyl-2-[(E)-1-methyl-1-propenyl]-10-[2,3,6-trideoxy-3-(dimethylamino)-3-C-methyl-α-L-lyxo-hexopyranosyl]-4*H*-anthra[1,2,-b]pyran-4,7,12-trione* **4**). *Kidamycin (11-Hydroxy-5-methyl-2-[(E)-1-methyl-1-propenyl]-8-[2,3,6-trideoxy-3-(dimethylamino)-β-D-arabino-hexopyranosyl]-10-[2,3,6-trideoxy-3-(dimethylamino)-3-C-methyl-α-L-lyxo-hexopyranosyl]-4*H*-anthra[1,2-b]pyran-4,7,12-trione,* **2**, 7.3 mg, 11 μmol) was irradiated in the presence of oxygen for 7 min in the same way as described above for **1**. After removal of the solvent, the products were dissolved in 250 μl of CH_2Cl_2 and separated by HPLC under the same conditions as stated above for **3** (8 injections) to give 1.7 mg of **2** and 2.6 mg of **4**, both as orange lacs. ^{1}H-NMR (200 MHz, *ca.* 0.008M in $CDCl_3$): see *Table 2*[2]).

4. *Photohedamycins B and C (8-[4-(Dimethylamino)-3-hydroxy-2-methyl-3,4-dihydro-2*H*-pyran-6-yl]-11-hydroxy-2-[2-hydroxy-1-methyl-2-(3-methyloxiran-2-yl)ethyl]-5-methyl-10-[2,3,6-trideoxy-3-(dimethylamino)-3-C-methyl-α-L-lyxo-hexopyranosyl]-4*H*-anthra[1,2-b]pyran-4,7,12-trione; **5** and **6**, resp.*). Hedamycin (**1**, 10.5 mg, 14 μmol) was dissolved in 40 ml of abs. MeOH. The soln. was degassed and then irradiated for 40 min in the apparatus described above. Then the solvent was removed. The product mixtures obtained in this way from several photolyses were pooled (total 48 mg) and dissolved in a mixture of 90 μl of i-PrOH and 360 μl of CH_2Cl_2. HPLC separation was achieved on the semiprep. silica gel column (22 injections; CH_2Cl_2/i-PrOH/25% aq. NH_3 91:9:0.08, sat. with H_2O; 4 ml/min). Three fraction were obtained. Fraction 1 ($t_R \approx 3.7$ min): 4.0 mg of **3**; fraction 2 ($t_R \approx 5.4$ min): 3.0 mg of **5** (94% pure, anal. HPLC); fraction 3 ($t_R \approx 6$ min): 8.2 mg of **6** (95% pure).

Data of **5**. Orange lac. IR ($CHCl_3$): 3500 br., 3000, 2950, 2860, 2800, 1650, 1590, 1480, 1450, 1430, 1380, 1310, 1160, 1130, 1100, 1080, 1050, 1000, 950, 920, 860, 840. ^{1}H-NMR (400 MHz, 0.02M in $CDCl_3$): see *Table 2*[2]). ^{1}H-NMR (400 MHz, 0.02M in $CDCl_3/C_6D_6$ 1:1[2]): 7.82 (*s*, H–C(6)); 7.81 (*s*, H–C(9)); 6.16 (*s*, H–C(3)); 5.48 (*t*, *J* = 6, H–C(6″)); 4.91 (*d*, *J* = 2, H–C(5′)); 4.43 (*qd*, *J* = 6 and 10, H–C(2′)); 3.98 (*qd*, *J* = 6 and 2, H–C(2″)); 3.84 (*dd*, *J* = 6 and 3, H–C(16)); 3.71 (*t*, *J* = 9, H–C(3′)); 3.50 (*dd*, *J* = 8 and 2, H–C(4′)); 3.31 (*d*, *J* = 2, H–C(3″)); 2.99 (*qd*, *J* = 5 and 2, H–C(18)); 2.87 (*s*, $H_3C(13)$); 2.61 (*m*, H–C(14) and H–C(17)); 2.31 (*s*, $(CH_3)_2N$–C(4′)); 2.06 (*s*, $(CH_3)_2N$–C(4″)); 1.48 (*t*, $H_3C(7')$ and $H_3C(7'')$); 1.35 (*d*, *J* = 7, $H_3C(15)$); 1.12 (*d*, *J* = 5, $H_3C(19)$); 0.71 (*s*, $H_3C(8'')$). ^{13}C-NMR (100.6 MHz, 0.02M in $CDCl_3$): see *Table 3*[2]). FAB-MS: (*M* + 3H) 749, (*M* + H) 747; mass number for $C_{41}H_{50}N_2O_{11}$: 746.

Data of **6**. Orange lac. IR ($CHCl_3$): 3500 br., 2980, 2970, 2840, 2790, 1650, 1590, 1460, 1440, 1420, 1380, 1310, 1160, 1130, 1090, 1070, 1040, 1010, 990, 950, 910, 850, 840. ^{1}H-NMR (200 MHz, *ca.* 0.02M in $CDCl_3$): see *Table 2*[2]). ^{13}C-NMR (22.63 MHz, *ca.* 0.2M in $CDCl_3$) see *Table 3*[2]). FAB-MS: (*M* + 3H) 749, (*M* + H) 747; mass number for $C_{41}H_{50}N_2O_{11}$: 746.

5. *Photohedamycin D (8-[(E)-4,5-Dihydroxy-1-oxo-2-hexenyl]-11-hydroxy-2-[2-hydroxy-1-methyl-2-(3-methyloxiran-2-yl)ethyl]-5-methyl-10-[2,3,6-trideoxy-3-(dimethylamino)-3-C-methyl-α-L-lyxo-hexopyranosyl]-4*H*-anthra[1,2-b]pyran-4,7,12-trione,* **7**). Hedamycin (**1**, 53 mg, 71 μmol) was dissolved in 53 ml of toluene. A 10-ml aliquot of this soln. was diluted with toluene to a final volume of 40 ml, was degassed and then irradiated for 50 min in the apparatus described above. Then, the solvent was removed and the product mixture was stored at –20° for *ca.* 30 d before further workup. The product mixtures obtained in this way from several photolyses were pooled (total amount 110 mg), dissolved in a mixture of 0.2 ml of i-PrOH and 0.8 ml of CH_2Cl_2, and chromatographed on the semiprep. silica gel column (22 injections; CH_2Cl_2/i-PrOH/25% aq. NH_3 92.5:7.5:0.08, H_2O-sat.; 4 ml/min) to give 10 mg of **7** as a reddish-brown lac. Rechromatography on the same column (8 injections;

CH_2Cl_2/i-PrOH/25% aq. NH_3 94:6:0.4, H_2O-sat.; 4 ml/min) gave 6 mg of **7** as a reddish-brown lac, which according to ^{1}H-NMR proved to be uniform. IR ($CHCl_3$): 3500 br., 2960, 2920, 2780, 1650, 1580, 1460, 1440, 1410, 1370, 1310, 1150, 1120, 1090, 1040, 990, 950, 900, 850. ^{1}H-NMR (400 MHz, 0.02M in $CDCl_3$): see *Table 2*[2]). ^{13}C-NMR (100.6 MHz, 0.02M in $CDCl_3$): see *Table 3*[2]). FAB-MS: (M + thiglycerol + H) 828, (M + H) 720; mass number for $C_{39}H_{45}N_2O_{12}$: 719.

6. *Quantitative Determination of the Product Ratios.* – 6.1. *Photolyses in the Presence of Oxygen.* Hedamycin (**1**, 10 mg) was dissolved in 50 ml of the appropriate solvent (see *Table 1*) and irradiated as described above. Aliquots of 2 ml each were drawn from the mixture before the irradiation and then at certain times. The solvent of these samples (except CH_2Cl_2) was blown off with N_2 and the residue taken up in 2 ml of CH_2Cl_2. These solns. were then used to fill the 20 µl sample loop of the HPLC apparatus, and the samples were then chromatographed on the analytical silica gel column (CH_2Cl_2/EtOH/Et_3N 98.2:1.5:0.3, 3/4 H_2O-sat., 1.5 ml/min, UV monitoring at 428 nm). The yields were obtained by dividing the product peak areas by the area of the peak of **1** from the reference sample withdrawn before the irradiation[5]).

6.2. *Photolyses in the Absence of Oxygen.* Solns. of **1** for the photolyses in different solvents and reference samples for HPLC were prepared as follows, depending on the different solubilities of **1** in the solvents in question:

Solvent	Stock soln.	Reference sample	Soln. for photolysis
Toluene	11.8 mg/12 ml	1 ml of stock soln.	10 ml of stock soln., diluted to 41 ml
CH_2Cl_2	50.5 mg/53 ml	1 ml of stock soln., diluted to 5 ml	10 ml of stock soln., diluted to 41 ml
CH_3CN	11.9 mg/44 ml	2 ml of stock soln.	40 ml of stock soln.
Acetone	24.9 mg/50 ml	2 ml of stock soln.	20 ml of stock soln., diluted to 40 ml
CH_3OH	11.8 mg/23 ml	2 ml of stock soln.	20 ml of stock soln., diluted to 41 ml

Aq. buffer solns.: a stock soln. was prepared by dissolving **1** (25.3 mg) and KHphthalate (1.02 g) in 100 ml of H_2O (giving 0.05M phthalate buffer pH 4). *Reference samples:* two 2-ml aliquots of the stock soln. were treated as follows: each sample was taken up in 10 ml of 10% aq. $KHCO_3$ soln. and then extracted with CH_2Cl_2 (4 × 20 ml). The org. phases were washed once with sat. NaCl soln., pooled, dried (Na_2SO_4) and the solvent removed *in vacuo*. The residue was taken up in 5.0 ml of CH_2Cl_2. The resulting soln. was used for HPLC.

The solns. for the photolyses were degassed as detailed above and then irradiated. Then, the solvents were removed; the products were obtained as orange lacs. Aq. solns. were worked up after the irradiation by concentrating them to about 10 ml by lyophilization. Then, *ca.* 2 ml of 2N Na_2CO_3 soln. were added for neutralization. The mixture was then taken up in 15 ml of 10% aq. $KHCO_3$ solution and then extracted with CH_2Cl_2 (5 × 20 ml). The red coloration of the aq. phases persisted even after extraction, indicating polar products still left in the aq. phase. The org. phases were washed once with sat. NaCl soln., pooled, dried (Na_2SO_4) and the solvent removed *in vacuo*. The products were obtained as orange lacs.

The product mixtures thus obtained were each dissolved in 5.0 ml of CH_2Cl_2, injected into the 20 µl sample loop of the HPLC apparatus and chromatographed on the analytical silica gel column: CH_2Cl_2/EtOH/Et_2N, 4/5 H_2O-sat., gradient 98:1.5:0.5→94.5:5:0.5→84:15:1, 1.5 ml/min, *ca.* 55 bar, monitoring at 270 and 428 nm.

The yields were calculated dividing the product peak areas by the area of the hedamycin reference peak, taking into account the different degrees of dilution[5]).

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[5]) The product ratios were calculated using the same absorption coefficient at 428 nm (the long-wavelength absorption maximum of **1**) for all the photoproducts, assuming that different substituents at C(2) would not greatly affect the absorption coefficient (*cf.* [5]); unknown, however, was the influence of conjugated substituents at C(8). Yet, **1** with the saturated ring and **3** with the unsaturated ring at C(8) had practically the same UV spectrum (see [3] and above). So we believe that the product ratios given in *Table 1* are correct.

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