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THE STRUCTURE OF THE ANTIBIOTIC HEDAMYCIN-II†

COMPARISON OF HEDAMYCIN AND KIDAMYCIN

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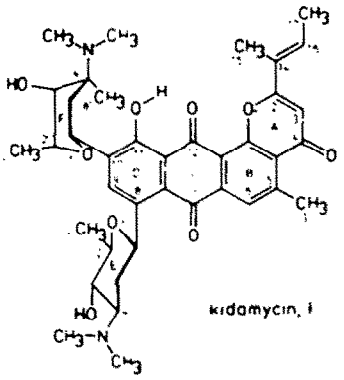
Abstract—A detailed spectral comparison between kidamycin 1 and hedamycin 13 shows that the two antibiotics have almost identical structures. However, the olefinic side chain at C-2 of kidamycin is replaced in hedamycin by a 2,3,4,5-diepoxy-2-hexyl group.

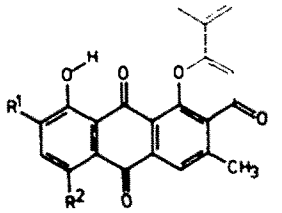
The antitumor antibiotic hedamycin was classified as being of the "pluramycin type".[2] From this group of antibiotics, kidamycin 1 has a ^{1}H-NMR spectrum which strikingly resembles that of hedamycin.[3] Furthermore, kidamycin is the only compound of the pluramycin group the structure of which has been fully elucidated; X-ray analyses of two different derivatives were made.[4,5] As kidamycin shows also many similarities with the partial structures derived so far for hedamycin,[1] a closer comparison of the two antibiotics was necessary.



kidamycin, 1

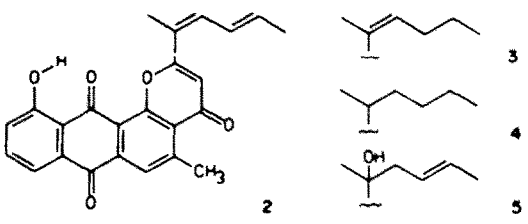
Kidamycin is a metabolite of *Streptomyces phaeoverticillatus*.[5] It has a molecular formula $C_{39}H_{46}N_2O_9$ and thus contains $C_2H_2O_2$ (and one equivalent of unsaturation) less than hedamycin. Kidamycin shows similar colour reactions to those of hedamycin (purple with alkali, purple with Mg^{2+}, blue with Ni^{2+}, yellowish brown with Fe^{3+}).

Both compounds have almost identical UV spectra (see Table 1). This suggests that both compounds contain the same main chromophore (drawn with heavy lines in the Scheme below), perhaps even with the same substitution pattern. Hedamycin, of course, cannot contain an olefinic side chain at the pyrone ring. However, the conjugated extension in kidamycin from the pyrone ring into the olefinic side chain probably does not contribute very much to the overall spectrum.



The effect of different side chains on this chromophore was investigated by Fricke[6] in connection with a structural study of the indomycins. Four indomycinone derivatives[6,7] (isolated or obtained by partial synthesis) were compared (spectra in methanol/dioxane 1:1): α-indomycinone 2, dihydro-α-indomycinone 3, tetrahydro-α-indomycinone 4 and β-indomycinone 5 (see Table 2). They have similar UV spectra, the main differences being in the band around 270 nm, which is shifted according to the number of double bonds in the side chain conjugated to the pyrone. The extension of the chromophore by one double bond, as in dihydro-α-indomycinone 3, shifts the absorption by 5 nm, from 267 to 272. A similar difference is seen between hedamycin, at 264, and kidamycin, at 270 nm, confirming the idea that hedamycin possesses the same chromophore as kidamycin but with a saturated substituent at the pyrone ring.

Comparison of kidamycin 1 with the aliphatic fragments derived from the ^{1}H-NMR double resonance experiments[1] shows that hedamycin most probably contains very similar, if not the same tetrahydropyran units as kidamycin. This, together with the information



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†Presented in part at the fall meeting of the Swiss Chemical Society in Aarau, 4 October, 1975. Previous paper of this series.[1]

Table 1. UV spectra of hedamycin and kidamycin in methanol (values for methanol/dioxane 1:1 in parentheses)

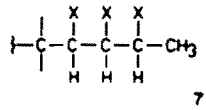
Hedamycin	λ:	213 (213)	244 (244)	264s(264)	434 (228) nm
	ε:	34000	46200	29600	8900
Kidamycin	λ:	216s(216)	243 (243)	270s(269)	434 (229) nm
	ε:	37600	47000	33000	8500

s = shoulder

Table 2. UV spectra of indomycinone derivatives in methanol/dioxane 1:1[a]

α-Indomycinone (2)	λ:	242	282	418 nm
	ε:	34600	24000	9800
Dihydro-α-indomycinone (3)	λ:	240	272	416 nm
	ε:	39400	28100	8800
Tetrahydro-α-indomycinone (4)	λ:	241	267	416 nm
	ε:	48000	25200	8800
β-Indomycinone (5)	λ:	239	267	417 nm
	ε:	37400	22600	8500

gained from the analysis of the UV spectra, leads to the proposition of structure **6** for hedamycin. The substituent *R* in formula **6** must be consistent with fragment *A* from the decoupling experiments[1] and thus has to contain the partial structure **7** as well as all the structural elements that have not yet been accounted for in formula **6**, i.e. a C-methyl group, two ether oxygen atoms and two rings. This is summarized in **8**, where *X* is oxygen. Structures **9**–**11** seem possible for *R*. The most appealing one is the diepoxide **9**, as this type of side chain resembles (and in terms of biosynthesis is equivalent to) the ones found in naturally-occurring indomycines and indomycinones (**2** and **5**).[6,7]



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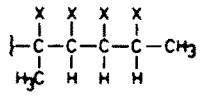

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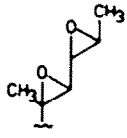

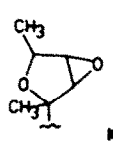

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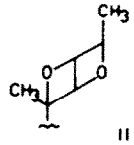
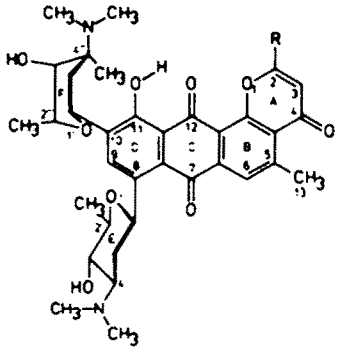


11

Whether the substitution pattern of the anthraquinone/pyrone in hedamycin is really the same as in kidamycin still remains to be proved.

The IR spectra too, share many common features, some of them even in the fingerprint region (see Fig. 1). The most prominent difference is seen in the carbonyl bands. Whereas hedamycin shows two clearly resolved peaks, kidamycin has only one, which is very strong and rather broad. The additional double bond conjugated to the pyrone carbonyl is expected to lower the frequency of this latter group. And the olefinic double bond will also absorb in the region of 1600–1650 cm^{-1}. The sum of these two absorptions and those of the quinone carbonyls, apparently, gives the broad band observed.



The ^{1}H-NMR spectra of hedamycin and kidamycin are very much alike (see Fig. 2 and Table 3). Common to both spectra are the OH signal at ~14 ppm, the three aromatic singlets, the broad doublet at 5.5 ppm, the singlet methyl at 3.0, the two dimethylamino groups and the singlet methyl at ~0.7 ppm. The major differences are as follows: A vinyl proton can be seen in the spectrum of kidamycin as a quartet at 7.5 ppm. It is coupled to the adjacent methyl group and further, by allylic coupling, to the second methyl group of the side chain. These two methyl groups have resonances at 2.04 (doublet) and 2.00 ppm (singlet). This latter signal obviously does not correspond to the methyl singlet at 1.96 ppm in hedamycin, which therefore must belong to the saturated substituent at the pyrone.

The hedamycin spectrum features a cluster of three methyl doublets around 1.5 ppm. The decoupling experiments[1] have shown that the two signals at 1.51 (J = 5.9 Hz) and 1.43 ppm (J = 5.9 Hz) belong to the

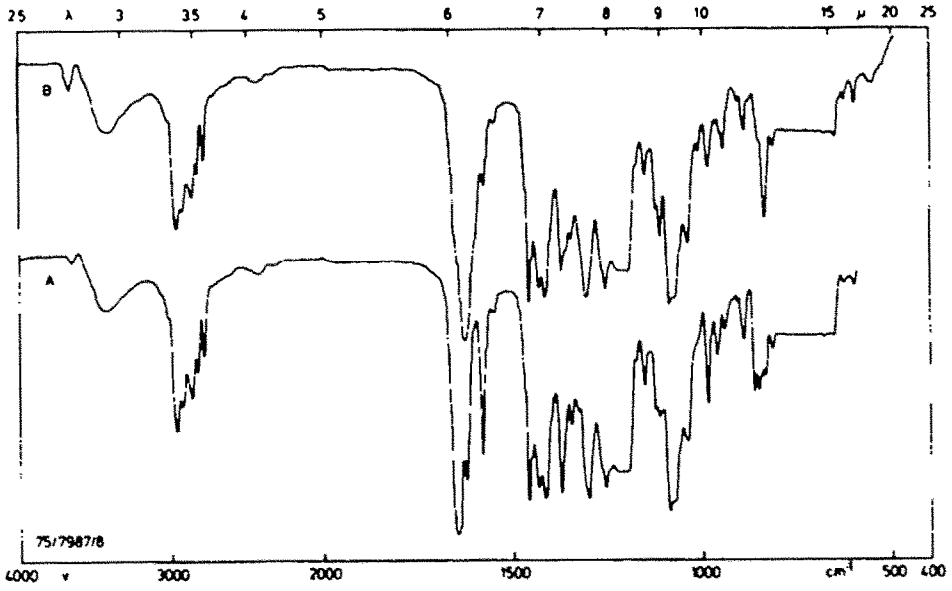


Fig. 1. IR spectra of hedamycin (A) and kidamycin (B) in $CHCl_3$.

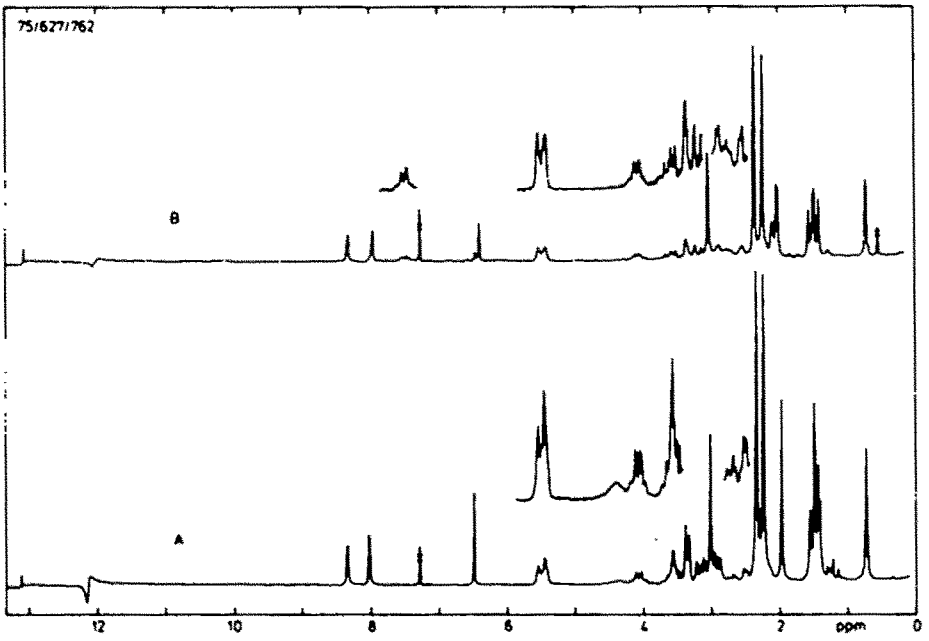


Fig. 2. 90 MHz FT NMR spectra of hedamycin (A) and kidamycin (B) in $CDCl_3$.

tetrahydropyran fragments, whereas the methyl with the smaller coupling constant (1.44 ppm, $J = 5.3$ Hz) belongs to the epoxide fragment.† In kidamycin the same cluster consists of only two doublets at 1.50 ppm ($J = 6.2$ Hz) and 1.42 ppm ($J = 5.9$ Hz), which is in reasonable agreement with the hedamycin values.† The doublet corresponding to the methyl group of the epoxide fragment in hedamycin, is in the kidamycin spectrum, of course, absent.

Further differences between the two spectra can be seen in the region around 3 ppm. This is where the absorptions of the methine protons of the epoxide fragment are in the hedamycin spectrum.

The NMR spectra seem to be suitable for a further elucidation of the substitution pattern of the anthraquinone/pyrone system. It can be most probably excluded that the two tetrahydropyran rings are in an ortho relationship in hedamycin, as in such a case strong steric interaction between the tetrahydropyran rings would lead to chemical shift differences, both in ^1H- and ^{13}C-NMR (see below).

One or more of the following changes in the substitution pattern still have to be considered: (a) the two tetrahydropyran rings have changed places; (b) the aromatic methyl group is in the other free position of

†Due to overlap of the methyl doublets, the coupling constants cannot be determined very accurately, see Fig. 2.

Table 3. ^{1}H-NMR spectra of hedamycin and kidamycin

Protons	Hedamycin at 270 MHz in C_5D_5N	Hedamycin at 90 MHz in $CDCl_3$	Kidamycin at 90 MHz in $CDCl_3$
H-3	6.60 s	6.46 s	6.38 s
H-6	8.02 s	8.00 d (0.6)	7.96 d (0.6)
H-9	8.81 s	8.33 s	8.32 s
11-OH		14.1 br	14.1 br
3 H-13	2.93 s	2.99 s br	3.01 s br
3 H-15	1.97 s	1.96 s	2.00 s
H-16	3.57 d (6)	3.32 d (6)	7.49 q (7)
H-17	3.05 dxd (6/<3)	2.89 dxd(4.7/2.1)	-
3 H-17	-	-	2.04 d (7)
H-18	3.12 dxq (<3/<6)	3.11 dxd(5.0/2.2)	-
3 H-19	1.14 d (<6)	1.44 d (5.3)	-
H-2'	3.73 dxq (6/9)	3.55 m	3.56 m
H-3'	3.37 t (9)	3.19 t (9)	3.20 t (9)
H-4'	3.07 br	2.93 br	2.97 m[5]
2 H-5'	1.37 q (12) 2.37 d br (12)	~1.2* ~2.5*	~1.2* ~2.6*
H-6'	5.65 d (10)	5.45 m	5.48 m
3 H-7'	1.54 d (6)	1.43 d (5.9)	1.42 d (5.9)
H-2"	4.31 dxq (3/6)	4.04 q br (6)	4.05 q br (6)
H-3"	3.65 d (4)	3.35 s br	3.37 d (3.5)[5]
2 H-5"	1.83 m 2.45 dxd (5/15)	~2.1 m* ~2.7 m*	~2.1* ~2.6*
H-6"	5.78 m	5.45 m	5.48 m
3 H-7"	1.68 d (6)	1.51 d (5.9)	1.50 d (6.2)
3 H-8"	0.81 s	0.71 s	0.70 s
4'-$N(CH_3)_2$	2.27 s	2.32 s	2.33 s
4"-$N(CH_3)_2$	2.26 s	2.22 s	2.21 s

The chemical shifts are given as δ-values in ppm from internal TMS. Assignments denoted with * may be interchanged. Coupling constants in Hz are given in parentheses after the multiplicities. The spectral width in 90 MHz FT measurements was 1200 Hz with 8k/4k data points, corresponding to 0.29 Hz per point or 0.0033 ppm per point.

ring *B*; (c) one of the tetrahydropyran rings and the aromatic methyl group have changed places. Of these possibilities (b) and (c) can be ruled out considering additional information from the ^{1}H-NMR spectra. The three aromatic protons have the following chemical shifts (in $CDCl_3$): kidamycin: 6.38, 7.96 (d, $J \sim 0.6$ Hz), 8.32; and hedamycin: 6.46, 8.00 (d, $J \sim 0.6$ Hz), 8.33.

The line showing the largest shift difference is that for the pyrone proton, the shift difference coming from the additional conjugated double bond in kidamycin. The δ-value of about 6.4 ppm falls well in the range of 5.9–6.6 ppm exhibited by similar pyrone protons.[8,9] This value also proves that the side chain cannot be in position 3 of the pyrone ring, as the proton, which then would be at C-2, would have a chemical shift of about 7.7 ppm.[8]

The line at about 8 ppm, which on close examination shows long range coupling, corresponds to the proton ortho to the aromatic methyl group, the signal of which is somewhat broadened and in some spectra even split with a coupling constant of *ca.* 0.6 Hz (confirmed by a ^{1}H,^{1}H decoupling experiment). Since this resonance at 8 ppm and the remaining one at 8.3 ppm have identical chemical shifts in both spectra, the substitution patterns must be the same in both antibiotics. The only difference still possible is the interchange of the two tetrahydropyrans.

The two ^{13}C-NMR spectra again are strikingly alike (see Fig. 3 and Table 4). In the downfield region, the ten lines between 188 and 137 ppm as well as the three signals between 119 and 110 ppm in the hedamycin spectrum seem to have their counterparts in the kidamycin spectrum showing the same multiplicities upon off-resonance decoupling and similar chemical shifts. Six of these 13 lines have identical δ_C-values in both spectra (±0.2 ppm), the other seven show differences in the range of 0.2 to 2.6 ppm. In the region between 135 and 125 ppm a one-to-one relationship between the signals in the spectra of the two antibiotics could not be established. However, whereas hedamycin has two doublets and two singlets (off-resonance decoupling multiplicities), kidamycin shows three doublets and three singlets in this region. These additional two lines correspond, of course, to the two olefinic carbon atoms in the side chain.

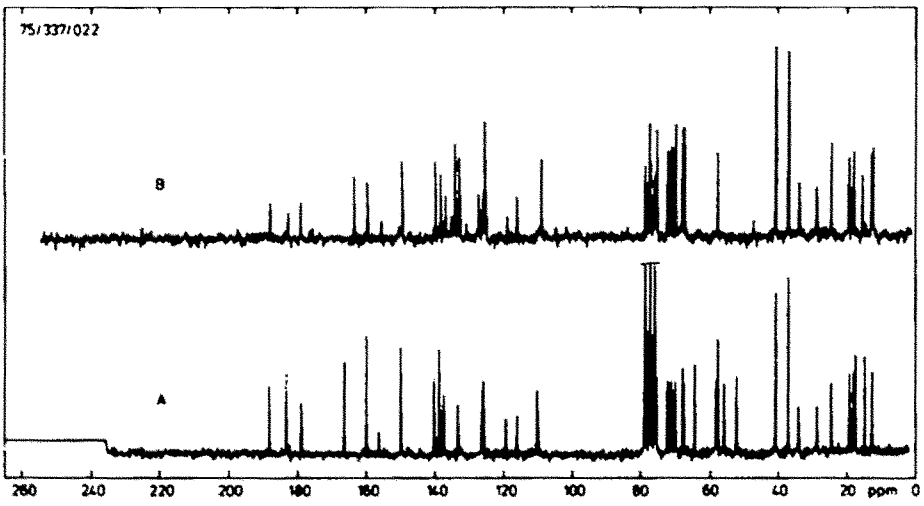


Fig. 3. 22.63 MHz ^{13}C-NMR spectra of hedamycin (A) and kidamycin (B) in $CDCl_3$.

The most spectacular resemblence, however, is in the upfield region (0 to 80 ppm), where 16 resonances, representing 18 carbon atoms, have identical chemical shifts in both spectra (±0.1 ppm) and the same multiplicities upon off-resonance decoupling. In addition, in the spectrum of kidamycin, six lines which were observed in the hedamycin spectrum are absent, i.e. 63.9 (d), 57.7 (s), 55.4 (d), 51.8 (d), 17.2 (q) and 14.5 ppm (q). On the other hand, two new signals at 14.9 (q) and 12.1 ppm (q) were observed. This is exactly what is

Table 4. Comparison of the ^{13}C-NMR spectra of hedamycin and kidamycin

Hedamycin	Kidamycin	Hedamycin	Kidamycin
188.0 s	188.2 s	67.4 d	67.4 d
183.1 s	183.0 s	67.3 d	67.2 d
178.7 s	179.3 s	63.9 d	-
166.3 s	163.7 s	57.7 s	-
159.8 s	159.7 s	57.3 s	57.4 s
156.1 s	155.7 s	55.4 d	-
149.8 s	149.6 s	51.8 d	-
140.2 s	140.0 s	40.4 q	40.4 q
138.6 s	138.4 s	36.8 q	36.8 q
137.3 s	137.0 s	33.7 t	33.6 t
	134.2 d	28.3 t	28.3 t
133.2 d	133.0 d	24.1 q	24.0 q
126.2 s	127.2 s	18.9 q	18.9 q
125.9 d	125.8 s	17.6 q	17.6 q
125.8 s	125.4 d	17.2 q	-
	125.0 s		
		-	14.9 q
119.2 s	118.9 s	14.5 q	-
116.1 s	116.1 s	12.4 q	12.3 q
110.0 d	108.7 d		12.1 q
77.3 d	77.3 d		
75.2 d	75.2 d		
71.9 d	71.9 d		
70.9 d	70.8 d		
69.6 d	69.6 d		

The chemical shifts are δ_C-values in ppm from internal TMS in $CDCl_3$. Spectral width 6000 Hz, 8k/4k data points, corresponding to 1.5 Hz per point or 0.065 ppm per point.

expected from the differences between hedamycin and kidamycin which have so far been recognized; the two methyl groups in the side chain have different chemical shifts as they belong either to a saturated system (hedamycin) or an olefinic residue (kidamycin). The four resonances between 64 and 51 ppm missing in the kidamycin spectrum correspond to the four carbon atoms bonded to oxygen in the epoxide side chain of hedamycin; three of them are CH's, one is quaternary.

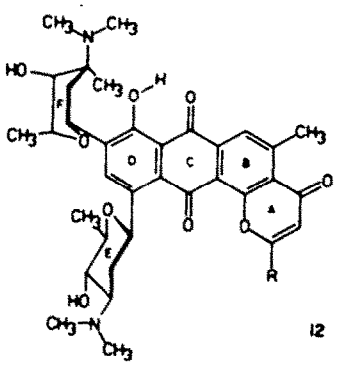
The chemical shifts of the six carbons belonging to the C-2 side chain in hedamycin do not permit a decision about the structure of this side chain, since hardly any suitable model compounds for the structural fragments 9–11 are available. It is noteworthy, however, that the two methyl shifts observed correspond very well to those reported for similar methyl groups in epoxides like asperlin,[10] aspyrone,[11] and ovalicin,[12] and thus support the diepoxide 9. Further evidence for structure 9, however, can be obtained from C–H coupling data. The three resonances belonging to the CH groups of the hedamycin side chain show direct C–H coupling constants of 173–177 Hz, whereas the couplings for the remaining CH groups bonded to oxygen are in the range of 136–146 Hz. The large values measured for the side chain resonances are a clear indication that the corresponding carbon atoms must be part of oxirane rings, since the coupling in oxirane itself is 176 Hz.[13] Thus, structure 9 is indeed the correct representation of the C-2 substituent of hedamycin; yet the stereochemistry of this substituent sill remains to be established.

From the fact that the 18 carbon atoms having identical chemical shifts in both spectra, comprise all the carbon atoms of the two tetrahydropyran rings, including the methylene groups, methyl and dimethylamino substituents, the following conclusion must be drawn: The two tetrahydropyran rings in hedamycin are, in fact, the same as those found in kidamycin, with respect to constitution, relative configuration and even conformation. Any change in conformation would change the steric relationship of the carbon atoms and thus alter the carbon chemical shifts.

Field desorption (FD) mass spectra of hedamycin and kidamycin, and electron impact (EI) spectra† of their tris(trimethylsilyl) derivatives unambiguously confirmed the molecular weights of the two antibiotics as 746 and 688, respectively. Both compounds show similar fragmentation patterns in the FD and EI mass spectra, which further corroborates the proposed close relationship between hedamycin and kidamycin.

From all the data described above the conclusion must be drawn that hedamycin is extremely closely related to kidamycin. Chromophore, substitution pattern (the two tetrahydropyrans might be interchanged) and two of the major substituents seem to be identical. The third substituent in hedamycin has been shown to be the 2,3,4,5-diepoxy-2-hexyl group.

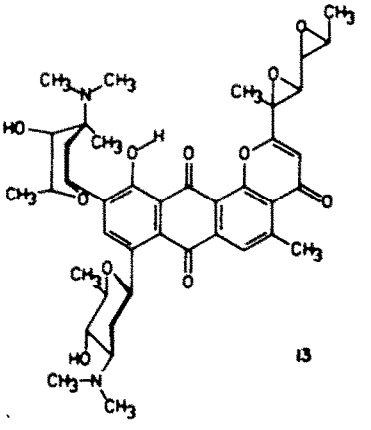
Another structural possibility for hedamycin 12, which has not been discussed so far and which features a different annelation of the pyrone ring, can be ruled out considering C–H coupling data. The hydrogen at C-6 is expected to split the resonance of a vicinal carbonyl with a coupling constant of 3–5 Hz.[14] In kidamycin the two anthraquinone carbonyl resonances are easily assigned from their chemical shifts (188.2 for the hydrogen



12

bonded C-12, 183.0 for C-7). In a fully proton coupled ^{13}C-NMR spectrum of kidamycin the line at 183.0 ppm is indeed split into a doublet with a coupling constant of 3 Hz, whereas the line at 188.2 ppm is a sharp singlet. Exactly the same behaviour is shown by the two corresponding carbonyl resonances in hedamycin, thus precluding structure 12 where the hydrogen bonded carbonyl should show vicinal C–H coupling.

It is the aim of further studies to elucidate the stereochemistry of the substituent at the pyrone ring and to give unambiguous confirmation of the arrangement of the tetrahydropyran rings. So far, the most probable structure for hedamycin is represented by formula 13.



13

EXPERIMENTAL

Mass spectra were run on a Varian MAT CH 5 DF spectrometer with a combination FD/FI/EI ion source. Silylations were carried out with an excess of N,O-bis-(trimethylsilyl)-acetamide at 100°C for 15 min. UV spectra were recorded on Beckman spectrophotometers models DK2 and 25; a Perkin-Elmer IR grating spectrometer model 125 was used for IR spectra. NMR spectra were measured at 90 MHz (^{1}H) and 22.63 MHz (^{13}C) on a Bruker WH 90 spectrometer.

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†Mass spectral analyses were carried out by Dr. W. J. Richter, Ciba-Geigy AG, Basel; details will be reported elsewhere.

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