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STRUCTURE AND CHEMISTRY OF KIDAMYCIN

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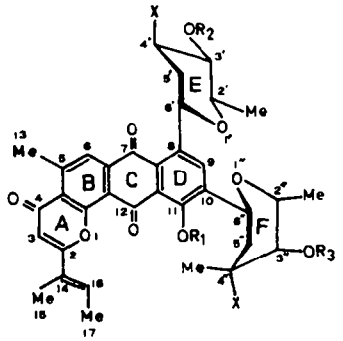
Abstract—By means of chemical studies as well as an X-ray analysis of kidamycin (**1a**), an antitumor antibiotic produced by a *Streptomyces* species, we have elucidated its structure and stereochemistry. Kidamycin represents a new type of polycyclic C-glycosyl microbial metabolites.

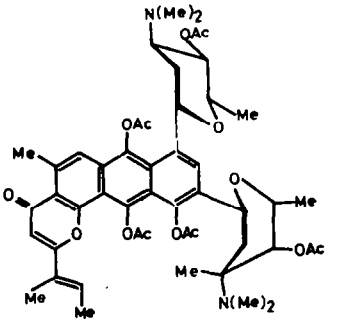
From the metabolites of *Streptomyces phaeoverticillatus*, Hata *et al.* obtained a series of compounds named iyomycin.[1] A variant strain was later found to produce, in the presence of anthraquinone-2,7-disulfonic acid, similar metabolites from which kidamycin, an antitumor antibiotic, was isolated.[2,3] The earlier investigations involved a preliminary chemical characterization of the compound, showing that it forms a triacetate. Subsequent studies by chemical and crystallographic methods have established structure **1a** for kidamycin. Brief communications on the X-ray experiments have already been described.[4,5] The present paper reports the results of the chemical studies.



1a	X=NMe$_2$	R_1=R_2=R_3=H
1b	X=NMe$_2$	R_1=R_2=R_3=Ac
1c	X=NMe$_2$	R_1=H, R_2=R_3=Ac
1d	X=NMe$_2$	R_1=R_3=Ac, R_2=H
1e	X=NMe$_3$.I	R_1=R_2=R_3=Ac

The mass spectrum of kidamycin suggested molecular formula $C_{39}H_{48}N_2O_9$, which later proved correct by the X-ray analysis. The ^{1}H NMR spectrum indicated the presence of one tertiary C-Me, two secondary C-Me's, two vinyl Me's, four N-Me's, one aromatic Me, several methine protons, four vinyl or aromatic protons, one H-bonded OH, and two OH's (Table 1). The molecular formula showed the four N-Me's to be two NMe_2 groups. The IR spectrum contained bands due to OH, N-Me, aromatic and CO functions. From a strong CO absorption at 1640 cm^{-1}, the presence of a quinoid system was suspected. Kidamycin formed a metal-chelated complex.

Acetylation of kidamycin gave a triacetate (**1b**) whose NMR spectrum (Table 1) revealed signals due to two alkoxyacetyl and one phenoxyacetyl groups, and also for two, each independent, methine protons adjacent to the OAc groups, showing the presence of two secondary alcoholic and one phenolic OH groups in kidamycin. Partial acetylation afforded a diacetate (**1c**) which contained two alkoxyacetyl groups and one H-bonded OH. By brief treatment of **1b** with MeOH another diacetate (**1d**) was obtained, possessing an alkoxyacetyl and a phenoxyacetyl groups. In order to ascertain the presence of a quinoid group, reductive acetylation of **1b** with Ac_2O and Zn was undertaken, which introduced two additional phenoxyacetyl groups, giving dihydrokidamycin penta-acetate (**2**). These results suggested for kidamycin a quinoid structure with a phenolic OH group *peri* to one of the CO groups. The UV and IR data on the above compounds supported this view, and furthermore indicated the presence of an additional conjugated CO function as follows.



2

The UV spectrum of kidamycin showed absorptions at 244 (ϵ: 46600), 271 (ϵ: 32700), 343 (ϵ: 6400) and 427 nm (ϵ: 9100), which shifted in alkaline solution to 258 (ϵ: 43500), 323 (ϵ: 14200) and 543 nm (ϵ: 9500); **1b** absorbed at 238 (ϵ: 42900), 271 (ϵ: 42300) and 364 nm (ϵ: 10500); **2** exhibited a strong absorption at 260 nm (ϵ: 98000) and a weak absorption at 295 nm (ϵ: 23600), which were considered to reflect the parent aromatic system. The IR spectrum of **1b** contained bands at 1675 and 1640 cm^{-1}, whereas **2** showed in this region only a band at 1640 cm^{-1}. The 1675 cm^{-1} band was therefore assigned to the non-chelated quinoid CO's, and the 1640 cm^{-1} band to the other conjugated CO. The broad band centered at 1640 cm^{-1} of kidamycin is ascribed to overlapping the absorptions of the H-bonded and non-bonded quinone CO's and the third CO group.

Table 1. NMR data of kidamycin and its derivatives*

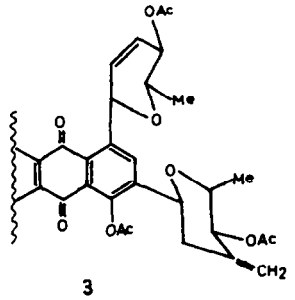
proton	(1a) in $CDCl_3$	(1b) in $CDCl_3$	(1b) in C_6D_6	(2a) in $CDCl_3$	(2b) in $CDCl_3$
4"-CH_3	0·77	0·99	0·84	1·21	1·19
2'-CH_3	1·46 d (6·0)	1·38 d (6·0)	1·38 d (6·5)	1·44 d (5·5)	1·29 d (6·3)
2"-CH_3	1·52 d (6·0)	1·44 d (6·0)	1·45 d (6·5)	1·50 d (6·3)	1·23 d (6·3)
C-15 CH_3	1·91	1·98	1·63	1·90	1·97
C-17 CH_3	1·94 d (7·0)	1·96 d (7·0)	1·68 d (7·0)	1·93 d (7·3)	1·98 d (7·0)
4'-,4"-$N(CH_3)_2$	2·25 2·41	2·29 2·33	2·19 2·28	2·22 2·36	2·18 2·34
C-13 CH_3	2·87	2·96	2·86	2·84	2·95
H-4'	2·97 m	3·10 m	3·21 m	2·84 m	3·11 m
H-3'	3·24 t (9·0)	4·90 t (10·0)	5·01 t (10·0)	3·30 t (9·0)	4·73 t (9·0)
H-3"	3·37 d (3·5)	5·22 d (3·5)	5·24 d (3·5)	3·30 d (2·5)	4·83 d (2·5)
H-2'	3·52 m	3·67 m	3·74 m	3·54 m	3·61 m
H-2"	4·07 m	4·32 m	4·28 m	3·83 m	3·92 m
H-6'	5·40 br.c	5·38 br.c	5·58 br.c	5·27 br.d	5·41 br.d
H-6"	5·40 br.c	5·38 br.c	5·58 br.c	4·83 br.d	4·88 br.c
H-3	6·14	6·32	6·16	6·14	6·28
H-16	7·26 q (7·0)	7·25 m	7·24 q (7·0)	7·22 q (6·8)	7·23 q (7·0)
H-6	7·75	7·81	7·58	7·72	7·77
H-9	8·29	8·33	8·86	8·32	8·32
11-OH	14·12			14·27	
11-OAc		2·51	2·34		2·49
3'-,3"-OH	4·15			3·47	
3'-,3"-OAc		2·15 2·19	1·84 1·87		2·14 2·26

* Measurement at 100MHz; chemical shifts in δ (ppm from $SiMe_4$); coupling constants (Hz) in parentheses: d=doublet, t=triplet, q=quartet, m=multiplet, c=complex, br=broad, all other resonances are singlet.

The last CO function, probably being due to an ester or a ketone, could not be characterized chemically. Alkaline hydrolysis of kidamycin, formation of its oxime or hydrazone, and reduction with LAH or $NaBH_4$, were attempted, but failed to give any informative products.

Of the nine oxygen function of kidamycin, the six were characterized as above and the others were assumed as ethereal oxygens. The presence of a glycosyl moiety as in many microbial metabolites seemed mostly likely. Attempted acid hydrolyses of kidamycin under various conditions, however, gave no homogeneous hydrolysis products. Since certain O-glycosidic linkages with an aminosugar resist hydrolysis or decompose completely under forced conditions, a desamino derivative of kidamycin was prepared by use of the Cope elimination for hydrolysis.

Oxidation of **1b** with perbenzoic acid in benzene gave a mixture of the corresponding di-N-oxide and its elimination products partiary or totally devoid of the N-oxide groups. Heating the mixture in benzene at a reflux temperature afforded a pure elimination product designated as bis[des(dimethylamino)] kidamycin triacetate (**3**), whose NMR spectrum contained signals for two *cis*-ethylenic protons (δ 5·80, 1H doublet J 10·0 Hz; 6·0, 1H doublet J 10·0 Hz) and two terminal methylene protons (δ 5·46, 1H doublet J 3·7 Hz; 6·43, 1H doublet J 3·7 Hz), but lacked peaks corresponding to the N-Me's or the tertiary C-Me. The results, being compatible with the *cis*-elimination process of the Cope elimination, indicated that the tertiary C-Me in kidamycin is adjacent to one of the NMe_2 groups. Attempts to hydrolyze **3** again failed, but the absence of an O-glycosidic linkage was suggested.

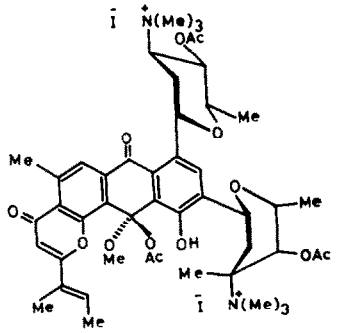


3

Kidamycin is sensitive to light, protic solvents, acid, and base, and its degradation reaction often resulted in a formation of resinous products. However, under weakly acidic conditions it was converted into a more stable

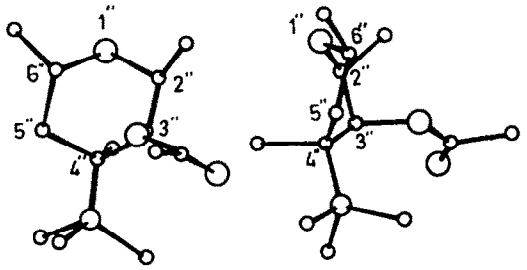
compound which proved to be an isomerized product, isokidamycin, from the following observations. The UV, IR and mass spectra of kidamycin were indistinguishable from those of isokidamycin, whereas the specific rotations of kidamycin and its triacetate differed, respectively, from those of the corresponding isocompounds. The difference between the two series was also observed in their NMR spectra but further details remained to be solved by an X-ray analysis.

A number of bromo and iodo derivatives of kidamycin were prepared in a search for crystallographically suitable compounds and firstly the methanolysis product (**1f**) of triacetylkidamycin bis(methylammonium iodide) (**1e**) described below was used for the analysis. Methylation of **1b** with MeI gave **1e**, crystallization of which from MeOH and EtOAc separated, after several months, large crystals of **1f** together with crystals of the unchanged compound. That methanolysis took place was evidenced by ready formation of the same product on treatment of **1e** with acidic MeOH and also by its NMR data which indicated the presence of one OMe, three Ac, and one phenolic OH groups. The product was triacetylmethoxykidamycin bis(methylammonium iodide) (**1f**) in contrast with **1d** obtained by methanolysis of **1b**.



1f

The X-ray analysis of **1f**,[4] coupled with the chemical characterization described above, disclosed its structure and absolute configuration as shown in structure **1f** which consists of a tetracyclic anthraceno[1,2-b]pyran ring system A, B, C, D and two tetrahydropyran rings E and F each linked through a C–C bond to ring D.

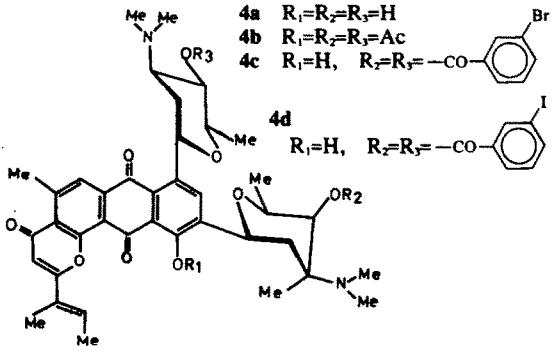


Projection of ring F in the crystal of **1f**.

†The nomenclature adopted for kidamycin (**1a**) in this paper is 2-(E-1-methyl-1-propenyl)-5-methyl-8-[(2'R, 3'S, 4'R, 6'R)-2'-methyl-3'-hydroxy-4'-(N,N-dimethylamino)-2'H-tetrahydropyran-6'-yl]-10-[(2"S, 3"S, 4"S, 6"R)-2",4"-dimethyl-3"-hydroxy-4"-(N,N-dimethylamino)-2"H-tetrahydropyran-6"-yl]-11-hydroxy-4H-anthraceno[1,2-b]pyran-4,7,12-trione. The numbering system is shown in structure **1a**.

The presence of two C-glycosidic linkages, instead of an O-glycosyl group, was shown. Ring E and F have the structures substituted similarly except for the tertiary Me at C-4" in ring F. The absolute configuration of ring E are 2'R, 3'S, 4'R, 6'R, while those of ring F are 2"S, 3"S, 4"S, 6"R; the conformation of ring E is a chair form while that of ring F is a somewhat twisted boat. The difference in the conformation between the two rings is associated with the difference in the configurations at C-2' and C-2", and also at C-4' and C-4". In both rings, the bulky $^{+}NMe_3$ groups attached to C-4' and C-4" are oriented in the equatorial direction. If the F ring would take a chair form, the 4"-$^{+}NMe_3$ group would suffer a 1,3-diaxial interaction with the 2"-Me, or the 4"-Me would do a similar interaction with the 6"-aryl group. As for the aromatic skeleton, the chemically uncharacterized CO function and one of the ethereal oxygens are located in the pyrone ring, A. The configuration with respect to the double bond of the 1-methyl-1-propenyl group at C-2 is E. The presence of the geminal OMe and OAc groups at C-12 with the *peri* OH group at C-11 indicates that the compound is formed by the methanolysis of **1e** with rearrangement.†

From structure **1f** the corresponding 7,12-diketo skeleton seemed probable for the aromatic portion of kidamycin. However, other structures, e.g. an *ana*-quinone structure with the 12-hydroxy-7,11-dicarbonyl grouping and structures of a dimeric anthraquinone-type, were not rigorously excluded. Furthermore, the available data could not provide conclusive evidence for the structure of isokidamycin. For these reasons isokidamycin bis-m-bromobenzoate (**4c**) and the corresponding apparently-isomorphous iodo derivative (**4d**) were subjected to an X-ray analysis.



The results showed the structure of isokidamycin to be represented as **4a**.[5] In contrast with structure **1f** of the methanolysis product, ring F of isokidamycin takes a chair conformation with the configurations of 2"S, 3"S, 4"S, 6"S. The chromophore was clearly proved to have the 7,12-diketo system. The crystal structure of **4c** was shown to have two molecules in an asymmetric unit, where the planes of the chromophores are stacked face to face.[5] The presence of two molecules in an asymmetric unit made it considerably difficult to complete the X-ray analysis of **4c** and **4d**, whilest the crystal of **1f** contained fortunately only one molecule in its asymmetric unit.[4] Apparently, the steric hindrance due to the OAc and OMe groups at C-12 of **1f** prevents such a stacking as in the crystal of **4c**.

The structural clarification of the above two compounds, **1f** and **4a** led to the assignment of structure **1a** for kidamycin which agreed well with the chemical and spectroscopic data. The presence of the same chromophore in kidamycin and isokidamycin was evi-

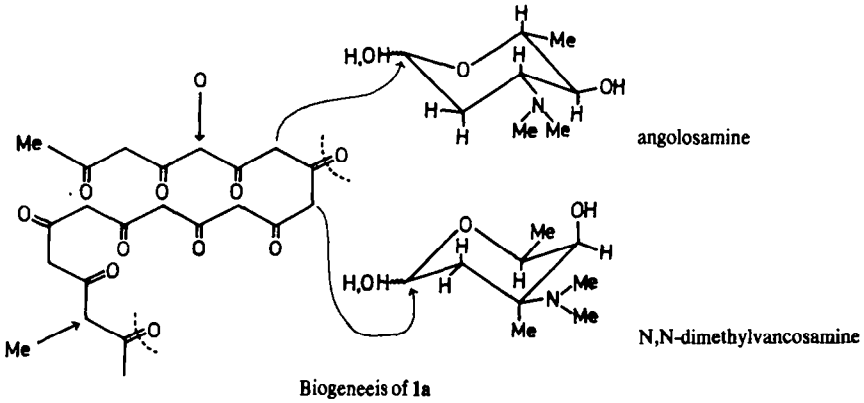
denced by the identical UV absorption of these two compounds and their triacetates and also by the essentially same chemical shifts of the aromatic protons of the two series. The formation of the methanolysis product, **1f** is explained by migration of the C-11 O-acetyl group of **1e**, accompanied by methoxylation, to the neighbouring carbonyl group.

Comparison of the configurations of ring F of kidamycin (2″*S*, 3″*S*, 4″*S*, 6″*R*) with those of isokidamycin (2″*S*, 3″*S*, 4″*S*, 6″*S*) reveals that the configurational difference between the two compounds is concerned only with the C-6″ position. It follows that the isomerization occurs at this position. The process can be explained in terms of protonation at the 1″ ethereal oxygen of kidamycin, followed by ring opening and recyclization with concomitant transformation of ring F from boat to chair. In connection with the acid isomerization, the sensitivity of kidamycin to base can be interpreted as being associated with the fission of ring F as shown in Chart 1.

The NMR signals of kidamycin, isokidamycin and their derivatives were compatible with structures **1a** and **4a**, and unambiguously assigned (Table 1). Comparison of the spectra of kidamycin and its acetate with those of the corresponding iso-compounds, showed that the resonances due to the ring E protons appear at almost the same positions and that the signals with different chemical shifts can be assigned for the ring F protons. Acetylation shifts observed for the H-3′ and H-3″ signals distinguished these from the H-2′ and H-2″ signals. The results of spin decoupling experiments (Table 2) supported the assignments and the nuclear Overhauser effect observed between the C-13 Me and the H-6 distinguished the latter signal from the H-9 signal. The 4″-Me signal of kidamycin appears at a field higher than that of isokidamycin does. The upfield shift is probably due to a shielding by the aromatic D ring: molecular models show that the 4″-Me of kidamycin has chances to lie in the diamagnetic region of ring D whereas that of isokidamycin has not, and that the rotation of the boat-form F ring around the C(10)–C(6″) bond is markedly restricted. The axial H-2″ of isokidamycin resonates at a field higher than the equatorial H-2″ of kidamycin does in agreement with the conformational difference in ring F between the two compounds.

On the basis of the NMR data, structure **1d** was assigned for the diacetate obtained by methanolysis of **1b**; the spectrum contained the signals for the H-3′ adjacent to the OH group and the H-3″ adjacent to the OAc group. Elimination of the C-3′ O-acetyl group of **1b** by methanolysis appears to be resulted from the participation of the neighbouring 4′-NMe_2 group. Although the methanolysis reaction of **1b** has not been examined in detail, formation of other products arising from methanolysis of the 3″- or 11-OAc group can not be excluded. Quarternarization of the NMe_2 groups as in **1e** prevents the amino-group participation and the 3′ and 3″-OAc of **1e** remain intact in the methanolysis.

Structure **3** was assigned for bis[des-(dimethylamino)]kidamycin triacetate on the basis of its NMR spectrum whose salient peaks have been described above. The F ring was assumed to take a chair conformation, since in this case the 4″-substituent suffers no 1,3-diaxial interaction. One of the NMR signals for the



Biogeneeis of **1a**

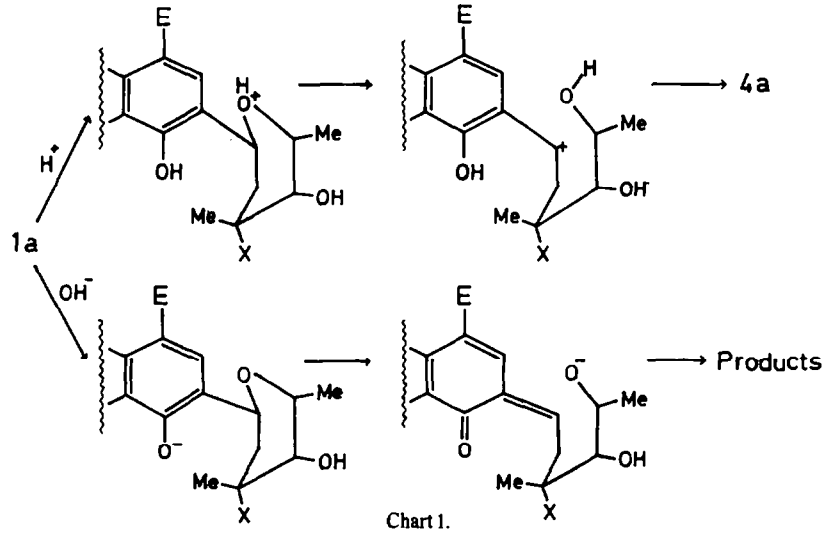


Chart 1.

Table 2. Results from the spin decoupling experiment*

1a in $CDCl_3$			2a in $CDCl_3$		
Signal irradiated (proton)	Signal observed (proton)	response	Signal irradiated (proton)	Signal observed (proton)	response
1·46 (2'-Me)	3·52 (H-2')	m → d	1·44 (2'-Me)	3·54 (H-2')	m → d
1·52 (2"-Me)	4·07 (H-2")	m → d	1·50 (2"-Me)	3·83 (H-2")	m → d
1·94 (C-17 Me)	7·26 (H-16)	q → br.s	1·93 (C-17 Me)	7·22 (H-16)	q → br.s
3·52 (H-2')	1·46 (2'-Me)	d → s	3·54 (H-2')	1·44 (2'-Me)	d → s
4·07 (H-2")	1·52 (2"-Me)	d → s	3·83 (H-2")	1·50 (2"-Me)	d → s
	3·37 (H-3")	d → s		3·30 (H-3")	d → s
7·26 (H-16)	1·94 (C-17 Me)	d → s	7·22 (H-16)	1·93 (C-17 Me)	d → s

1b in C_6D_6					
Signal irradiated (proton)	Signal observed (proton)	response	Signal irradiated (proton)	Signal observed (proton)	response
1·68 (C-17 Me)	7·24 (H-16)	q → br.s	5·01 (H-3')	3·21 (H-4')	m → dd
3·21 (H-4')	5·01 (H-3')	t → d		3·74 (H-2')	m → q
3·74 (H-2')	1·38 (H-3')	d → s	5·24 (H-3")	4·28 (H-2")	m → q
	5·01 (H-3')	t → d	7·24 (H-16)	1·68 (C-17 Me)	d → s
4·28 (H-2")	1·45 (2"-Me)	d → s	2·86 (C-13 Me)	7·58 (H-6)	s → s**
	5·24 (H-3")	d → br.s			

* Signals denoted in the same manner as in Table 1 except for s=singlet.

** NOE; 16% increase of integrated area of the H-6 signal.

4"-methylene protons appears at a lower field, probably due to a deshielding effect of the neighbouring equatorial 3"-OAc group.

Kidamycin constitutes a new class of polycyclic microbial metabolites, possessing a skeleton composed of an anthraquinone-fused pyrone ring as a chromophore and two C-glycosyl amino-sugar rings. The structure of the chromophore moiety conforms to the polyketide hypothesis of biogenesis and the similar chromophore has been found in indomycinone.[6] The E ring corresponds to angolosamine[7] and the F ring to N,N-dimethylvancosamine,[8] viz. 3-methylrhodosamine. Although C-glycoside formation in biogenesis is not uncommon,[cf9,10] the natural occurrence of a boat conformation as ring F has rarely been recorded[cf11] and biochemical formation of such a ring is of interest; conformational analysis suggests that substitution of the 1-OH group of N,N-dimethylvancosamine in its popular chair conformation with an axial bulky aromatic group results in formation of the boat-type F ring. The diminished antitumor and antibacterial activities exhibited by isokidamycin† indicate that the boat-form F ring of kidamycin also associates with the biological activities.

The structural similarities with kidamycin have been reported for iyomycin B_1, pluramycin, neopluramycin, haedamycin, rubiflavine[2,3] and indomycins.[4,6] The members of this class have also similar biological activities.

EXPERIMENTAL

M.ps are uncorrected. Unless otherwise stated, IR spectra were measured in KBr disc on a Hitachi-285 spectrophotometer, UV spectra in EtOH on a Hitachi-323 spectrophotometer, NMR spectra in $CDCl_3$ with Me_4Si as internal standard at 100 MHz on a JEOL 4H-100 instrument, and mass spectra on a JEOL JMS-01SG-2. Alumina refers to neutral Woelm grade III alumina. Microanalyses and NMR and Mass spectral measurements were performed under the direction of Dr. M. Sano at this Institute.

Kidamycin (**1a**). According to the reported procedure,[3a] crude kidamycin was chromatographed on alumina and crystallized

†Unpublished data obtained by Drs. N. Kanda and N. Ogawa of this Institute.

from $CHCl_3$-n-hexane to give a pure sample as orange prisms, m.p. 212–214° dec; $[\alpha]_D^{25}$ +476° (*c* 0·986, $CHCl_3$); UV λ_{nm} (ϵ): 244 (46600), 271 (32700), 343 (6400), 427 (9100); UV (ethanolic 0·01-N NaOH) $\lambda_{nm}(\epsilon)$: 210 (130000), 258 (43500), 323 (14200), 543 (9500); IR $\nu_{cm^{-1}}$: 3430 (OH), 2825, 2780 (N-Me), 1640 (C=O); NMR data in Table 1. (Found: mol wt 688·337 (mass spectrum). Calc. for $C_{39}H_{48}N_2O_9$: mol wt 688·334 Found: C, 67·37; H, 6·97; N, 4·06. $C_{39}H_{48}N_2O_9$. 1/2H_2O requires: C, 67·12; H, 6·93; N, 4·02%).

A methanolic solution of **1a** became purple on an addition of $Ni(OAc)_2$ or $Mg(OAc)_2$.

Kidamycin 11,3′,3″-*triacetate* (**1b**).[cf 3b] Acetylation of **1a** (5·2 g) in pyridine (60 ml) with Ac_2O (30 ml) at 25° for 24 hr and chromatography of the product in AcOEt over alumina (100 g) gave the acetate (6·2 g). Crystallization from AcOEt separated **1b** as yellow needles, m.p. 214–216° dec; $[\alpha]_D^{25}$ +256·2° (*c* 1·1, $CHCl_3$); UV $\lambda_{nm}(\epsilon)$: 238 (42900), 271 (42300), 364 (10500); IR $\nu_{cm^{-1}}$: 1775 (OAc), 1740 (OAc), 1675 (C=O), 1640 (C=O); NMR data in Table 1. (Found: C, 66·25; H, 6·57; N, 3·68, $C_{45}H_{54}N_2O_{12}$ requires: C, 66·32; H, 6·68; N, 3·44%).

Hydrolysis of **1b** (174 mg) in MeOH (60 ml) with 8% $KHCO_3$ aq (25 ml) at 40° for 2 hr, followed by chromatography of the product on alumina afforded **1a** (2·0 mg), m.p. 205–207° identified by IR and TLC.

Kidamycin 3′,3″-*diacetate* (**1c**). To a soln of **1a** (1·0 g) in Ac_2O (70 ml) was added AcONa (1·0 g). The mixture was stirred at room temp for 2 hr, poured into ice water, and neutralized with $NaHCO_3$. The product was extracted with $CHCl_3$ and the washed, dried $CHCl_3$ soln was concentrated. Addition of light petroleum gave a crystalline solid which after being dissolved in AcOEt, was purified by filtration through a column of Florisil (20 g). Concentration of the filtrate and crystallization of the residue (941 mg) from benzene–light petroleum gave **1c**, m.p. 194–196° dec; IR $\nu_{cm^{-1}}$: 1740 (OAc), 1640 (C=O); NMR δ: 2·17 ($OCOCH_3$), 2·27 ($OCOCH_3$), 13·9 (11-OH). (Found: C, 66·71; H, 6·77; N, 3·86, $C_{43}H_{52}N_2O_{11}$ requires: C, 66·82; H, 6·78; N, 3·63%).

Kidamycin 11,3″-*diacetate* (**1d**). A soln of **1b** (250 mg) in MeOH (75 ml) was refluxed for 30 min and concentrated. The residue was dissolved in $CHCl_3$ and light petroleum was added to precipitate a solid which was collected, dissolved in AcOEt and chromatographed over alumina (5·0 g). The product (66 mg) eluted with the first AcOEt-fraction (30 ml) was crystallized from AcOEt-n-hexane to give **1d**. m.p. 165–167°; IR $\nu_{cm^{-1}}$: 3425 (OH), 1765 (OAc), 1740 (OAc), 1665 (C=O), 1635 (C=O); NMR δ: 2·21 (3″-$OCOCH_3$), 2·55 (11-$OCOCH_3$). (Found: C, 67·07; H, 6·65; N, 3·62. $C_{43}H_{52}N_2O_{11}$ requires: C, 66·82; H, 6·78; N, 3·62%).

11,3′,3″-*Tri-O-acetylkidamycin bis*(*methylammonium iodide*) (**1e**). A soln of **1b** (100 mg) in MeI (10 ml) was allowed to stand at room temp. overnight. The separated crystals were crystallized from AcOEt-MeOH to give **1e** (61 mg), m.p. 194–195° dec; UV $\lambda_{nm}(\epsilon)$: 217·5 (59300), 241 (30300), 266 (28500), 275 (sh, 27200), 344 (8600); IR $\nu_{cm^{-1}}$: 1740 (OAc), 1675 (C=O), 1630 (C=O); NMR (CD_3SOCD_3) δ: 1·67 (4″-CH_3), 2·26 (3′-, or 3″-$OCOCH_3$), 2·34 (3′-, or 3″-$OCOCH_3$), 2·54 (11-$OCOCH_3$), 3·18 (N-$(CH_3)_3$), 3·19 (N-$(CH_3)_3$). (Found: C, 50·26; H, 5·54; N, 2·55; I, 22·78. $C_{47}H_{60}N_2O_{12}I_2$ requires: C, 50·18; H, 5·42; N, 2·51; I, 22·73%).

12,3′,3″-*Tri-O-acetyl*-12-*methoxykidamycin bis*(*methylammonium iodide*) (**1f**).† A soln of **1e** (240 mg) in MeOH (40 ml), AcOEt (20 ml) and AcOH (0·1 ml) was refluxed for 48 hr and concentrated. Addition of MeOH to the residue precipitated a solid which was crystallized from MeOH–AcOEt as **1f** (160 mg), m.p. 188–191° dec.; UV $\lambda_{nm}(\epsilon)$: 214 (69600), 264 (27200), 275 (sh, 26000), 295 (22600), 307 (sh, 18700), 343 (10800); IR $\nu_{cm^{-1}}$: 3400 (OH), 1735 (OAc), 1680 (C=O), 1620 (C=O); NMR (CD_3SOCD_3) δ: 1·62 (4″-CH_3), 2·24 (3′-, or 3″-$OCOCH_3$), 2·34 (3′-, or 3″-$OCOCH_3$), 2·97 (12-$OCOCH_3$), 2·97 (12-OCH_3), 3·18 (N-$(CH_3)_3$), 3·19 (N-$(CH_3)_3$), 9·05 (11-OH); (Found: C, 50·28; H, 5·98; N, 2·70; I, 21·86. $C_{48}H_{64}N_2O_{13}I_2 \cdot H_2O$ requires: C, 50·18; H, 5·78; N, 2·44; I, 22·09%). The same compound (**1f**) was obtained after slow crystallization of **1a** from MeOH–AcOEt at room temperature for 3 months.

†For the configuration at C-12, *cf* structure **1f**.

7,12-*Dihydrokidamycin* 7,11,12,3′,3″-*penta-acetate* (**2**). A mixture of **1b** (300 mg) in Ac_2O (14 ml) and Zn (600 mg) was heated at 50° for 2 hr, cooled and filtered. The filtrate was concentrated, water was added and the product was isolated by extraction with $CHCl_3$. Chromatography of the product over alumina (7·0 g) and elution with $CHCl_3$–AcOEt (3:1) afforded **2** (75 mg), which was crystallized from AcOEt-n-hexane, m.p. 237–239° dec; UV λ_{nm} (ϵ): 260 (98000), 295 (23600), 346 (10900); IR $\nu_{cm^{-1}}$: 1760 (OAc), 1735 (OAc), 1640 (C=O); NMR δ: 2·15 (3″-$OCOCH_3$), 2·21 (3′-$OCOCH_3$), 2·35 (11-$OCOCH_3$), 2·61 (7 or 12-$OCOCH_3$), 2·66 (7 or 12-$OCOCH_3$). (Found: C, 65·44; H, 6·88; N, 3·00. $C_{49}H_{60}N_2O_{14}$ requires: C, 65·32; H, 6·71; N, 3·11%).

Bis[*des*(*dimethylamino*)]*kidamycin* 11,3′,3″-*triacetate* (**3**). A mixture of **1b** (1·0 g; 1·24 mM) in benzene (100 ml) and perbenzoic acid (2·6 mM) was stirred at room temp. for 4 hr. The mixture was successively washed with 0·1-N $Na_2S_2O_3$ aq (100 ml), 5% $NaHCO_3$ aq (150 ml) and water (150 ml). The aqueous washings were extracted with $CHCl_3$ (200 ml) and the combined organic soln was dried and concentrated. Addition of light petroleum to the residue gave a ppt which was dissolved in $CHCl_3$ and chromatographed on alumina (30 g). All the fractions eluted with $CHCl_3$ and $CHCl_3$–MeOH (95:5) were shown to be a mixture by TLC. The combined eluate was dissolved in benzene (250 ml) and heated at reflux temp. for 5 hr. The solvent was evaporated and residue was chromatographed on silicagel (Merck, 30 g). The fraction eluted with benzene-AcOEt (2:1) was crystallized from benzene-$CHCl_3$ as **3** (234 mg), m.p. 238–240° dec; IR $\nu_{cm^{-1}}$: 1780 (OAc), 1740 (OAc), 1675 (C=O), 1640 (C=O); NMR δ: 5·46 (1H, 4″-methylene), 6·23 (1H, 4″-methylene), 5·8 (5′-H), 6·0 (4′-H). (Found: C, 67·66; H, 5·41. $C_{41}H_{40}O_{12}$ requires: C, 67·94; H, 5·56%).

Isokidamycin (**4a**). A mixture of **1a** (1·28 g) and anhydrous *p*-TsOH (1·7 g) in $CHCl_3$ (200 ml) was heated under reflux for 48 hr. The soln was washed with $NaHCO_3$ aq, and water, dried and concentrated. The residue was dissolved in CH_2Cl_2 and AcOEt was added to separate crystals (1·05 g) which were recrystallized from AcOEt to give **4a**, m.p. 200–201° dec; $[\alpha]_D^{25}$ +62·4° (*c* 1·1, $CHCl_3$); UV $\lambda_{nm}(\epsilon)$: 245 (44400), 272 (30800), 342 (sh, 6450), 426 (9400); IR $\nu_{cm^{-1}}$: 3540 (OH), 2825, 2780 (N-CH_3), 1640 (C=O); NMR data in Table 1. (Found: mol wt 688·337 (mass spectrum). Calc. for $C_{39}H_{48}N_2O_9$: mol wt 688·334. Found: C, 67·15; H, 6·95; N, 4·23, $C_{39}H_{48}N_2O_9 \cdot 1/2H_2O$ requires: C, 67·12; H, 6·93; N, 4·02%).

Isokidamycin 11,3′,3″-*triacetate* (**4b**). Acetylation of **4a** (360 mg) in pyridine with Ac_2O (5 ml) at room temp. overnight and purification of the product by chromatography over alumina, followed by crystallization from AcOEt-n-hexane gave **4b** (336 mg), m.p. 166° dec; UV $\lambda_{nm}(\epsilon)$: 238 (37400), 271 (37400), 365 (8800); IR $\nu_{cm^{-1}}$: 1775 (OAc), 1740 (OAc), 1675 (C=O), 1640 (C=O); NMR data in Table 1. (Found: C, 66·19; H, 6·58; N, 3·41. $C_{45}H_{54}N_2O_{12}$ requires: C, 66·32; H, 6·68; N, 3·44%).

Isokidamycin 3′,3″-*bis-m-bromobenzoate* (**4c**). To a stirred mixture of **4a** (193 mg) in benzene (40 ml) and K_2CO_3 (150 mg) was added a soln of m-bromobenzoyl chloride (150 mg) in benzene (10 ml) at room temp. and the mixture was stirred for 2 days. The solvent was evaporated and CH_2Cl_2 (50 ml) was added to the residue. The CH_2Cl_2 soln was washed with $NaHCO_3$ aq and water, dried and concentrated. The product was chromatographed on alumina (8·4 g) and eluted with benzene-$CHCl_3$ (1:1, 100 ml) and $CHCl_3$ (50 ml). The $CHCl_3$-fraction was rechromatographed similarly. The combined fraction (191 mg) eluted with benzene-$CHCl_3$ (1:1) was crystallized from benzene-acetone-n-hexane to give **4c**, m.p. 224–225° dec; IR $\nu_{cm^{-1}}$: 1725 (C=O), 1655 (C=O), (Found: C, 60·32; H, 5·23; N, 2·70; Br, 15·00. Calc. for $C_{53}H_{54}N_2O_{11}Br_2$: C, 60·35; H, 5·25; N, 2·59; Br, 15·15%).

Isokidamycin 3′,3″-*bis-m-iodobenzoate* (**4d**). This compound (552 mg) was prepared similarly as described above, from **4a** (580 mg) in benzene (180 ml), K_2CO_3 (3·0 g) and m-iodobenzoyl chloride (548 mg), and crystallized from benzene-acetone-n-hexane, m.p. 182–183° dec; IR $\nu_{cm^{-1}}$: 1725 (C=O), 1650 (C=O), 1640 (C=O). (Found: C, 55·62; H, 4·89; N, 2·41; I, 22·42. $C_{53}H_{54}N_2O_{11}I_2$ requires: C, 55·41; H, 4·74; N, 2·44; I, 22·00%).

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