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Structures and Biological Properties of Kinamycin A, B, C, and D[1]

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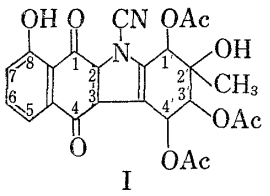
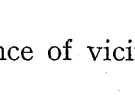
A series of new antibiotics, Kinamycin A, B, C, and D which are mainly effective against gram-positive bacteria, were extracted with chloroform from the broth filtrate of *Streptomyces murayamaensis* sp. *nov.* Hata et Ohtani. Kinamycin C (I), $C_{24}H_{20}O_{10}N_2$, *m/e* 496 (M^+), was determined to have an 8-hydroxynaphthoquinone skeleton, nitrile, acetoxyl, and tertiary methyl groups from its ultraviolet (UV), infrared (IR), and nuclear magnetic resonance (NMR) spectra, and was further found to have a unique structure of N–C–N from some chemical reactions and X-ray diffraction. Structure relationship among I and other components A, B, and D was assumed to be due to the difference in the number and position of the acetoxyl group from analyses of IR, NMR, and mass spectra of their acetylated compounds.

The antimicrobial activity of the four kinamycins increases with the decreasing number of acetoxy group, in the order of kinamycin C, A, D, and B. In addition, some derivatives obtained during structural studies on I were found to have nearly equal or increased antimicrobial activity compared with I and kinamycin D (XI).



I

The molecular formula ($C_{24}H_{20}O_{10}N_2$) and molecular weight (496) of kinamycin C[3,4] (I) were deduced from mass spectra and elementary analyses of the derivatives obtained during structural studies on I, and p*Ka'* values were determined as 3.1 and 9.45 in aqueous 85% ethanol solution. The infrared (IR) spectrum of I showed absorptions for hydroxyl at 3500 cm^{-1}, for nitrile or isonitrile group at 2155 cm^{-1}, acetoxyl-CO at 1740 cm^{-1}, hydrogen-bonded quinone-CO at 1625 cm^{-1}, and a free quinone-CO at 1660 cm^{-1} (Fig. 1). The ultraviolet (UV) and visible spectra of I showed an absorption maxima at 246, 275, 370, 388, and 448 nm, which are characteristic absorptions for naphthoquinone-type chromophores.[5] A red shift of their absorption in 0.1N sodium hydroxide-methanol solution suggests the presence of a phenolic hydroxyl.

The NMR spectrum (Fig. 2) of I indicated the presence of a tertiary methyl at 1.3 δ (s), three alcoholic acetoxyl groups at 2.0—2.3 δ (s), a hydroxyl at 2.57 δ (s) (which disappeared in D_2O), an isolated proton on a carbon carrying the alcoholic acetoxyl group at 5.4 δ (s), and vicinal protons on carbons carrying two alcoholic acetoxyl groups at 5.6 and 6.2 δ (d, J=7.2 Hz), which suggests presence of vicinal acetoxyl groups in  as a structural moiety.

1) S. Ōmura, A. Nakagawa, H. Yamada, T. Hata, A. Furusaki, and T. Watanabe, *Chem. Pharm. Bull.* (Tokyo), **19**, 2428 (1971).
2) *a,b) Shirokane, Minato-ku, Tokyo; c) Nishinomiya-shi, Hyogo.*
3) S. Itō, T. Matsuya, S. Ōmura, M. Otani, A. Nakagawa, H. Takeshima, Y. Iwai, M. Ohtani, and T. Hata, *J. Antibiotics*, **23**, 315 (1970).
4) T. Hata, S. Ōmura, Y. Iwai, A. Nakagawa, M. Otani, S. Itō, and T. Matsuya, *J. Antibiotics*, **24**, 353 (1971).
5) I. Singh, R.T. Ogata, R.E. Moore, C.W. Chang, and P.J. Scheuer, *Tetrahedron*, **24**, 6053 (1968).

Three aromatic protons signaled at 7.13 (1H, dd, J=8.0 Hz, J=2.0 Hz), 7.5 (1H, t, J=8.0 Hz), and 7.6 δ (1H, dd, J=8.0, J=2.0 Hz), which seemed to be based on a typical absorption of ABX splitting pattern.

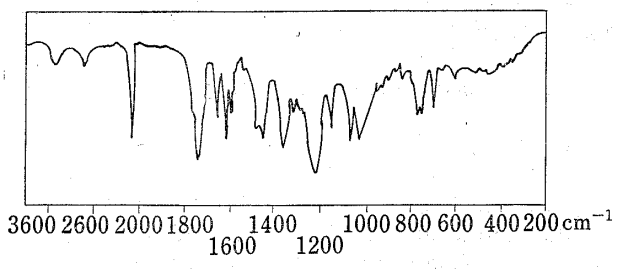


Fig. 1. IR Spectrum of I (KBr)

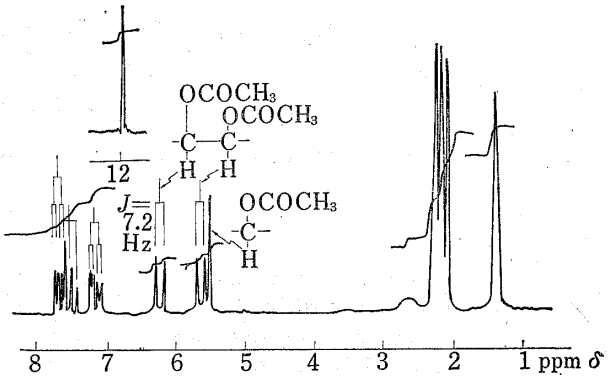


Fig. 2. NMR Spectrum of I (100 MHz: $CDCl_3$)

A hydrogen-bonded phenolic hydroxyl signaled at 12.0 δ (1H, s) (disappearing in D_2O) as a singlet peak. The IR spectra of the diacetate (II), obtained by acetylation of I with anhydrous sodium acetate-acetic anhydride and methyl-kinamycin C (III), obtained by methylation with diazomethane, indicated free quinone-CO at 1650 cm^{-1}, and the hydrogen-bonded quinone-CO observed at 1625 cm^{-1} in I disappeared in II and III, as shown in Fig. 3b and 3c. Consequently, the presence of a phenolic hydroxyl on peri-position was determined for I, and further three aromatic protons in II and III were also determined by typical absorption of ABX splitting pattern in their nuclear magnetic resonance (NMR) spectra.

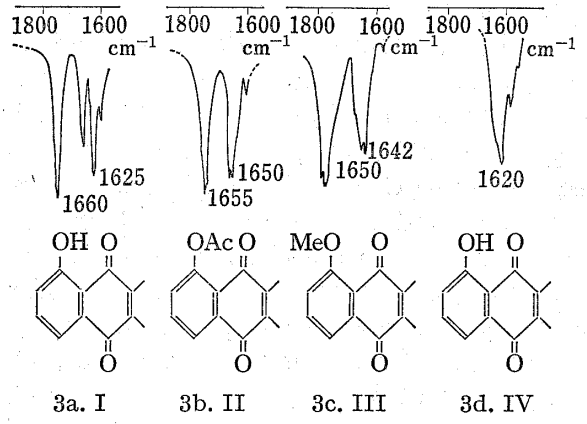


Fig. 3. IR Spectra of I, II, III and IV (in KBr)

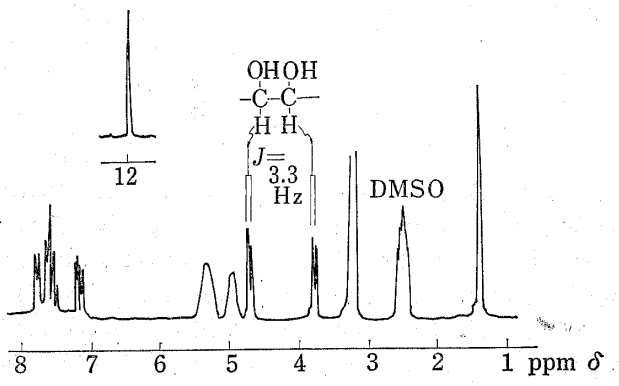


Fig. 4. NMR Spectrum of IV (100 MHz; DMSO-d_6)

Hydrolysis of I with 0.2N sodium hydroxide afforded a deacetyl-kinamycin C (IV), which has lost three acetoxyl groups. The IR spectrum of IV showed an absorption for hydrogen-bonded quinone-CO at 1620 cm^{-1} (Fig. 3d), but not the free quinone-CO observed at 1663 cm^{-1} in I. In the NMR spectrum of IV (Fig. 4), vicinal protons at 5.6 and 6.20 δ, and a singlet proton at 5.4 δ in I shifted to a higher magnetic field to 3.8 (d, 1H, J=3.3 Hz), 4.63 (d, 1H, J=3.3 Hz), and 4.65 δ (s, 1H), respectively. From these results, presence of a 8-hydroxy-naphthoquinone, vicinal protons on carbon atoms each carrying the alcoholic acetoxyl group, an isolated proton on a carbon carrying the alcoholic acetoxyl group, a tertiary-methyl, and a nitrile or an isonitrile group was confirmed as a partial structure of I. IV gave an Isopropylidenekinamycin C (V), $C_{21}H_{18}O_7N_2$, when refluxed with anhydrous acetone and a trace

of *p*-toluenesulfonic acid as a catalyst.[6] The NMR spectrum of V (Fig. 5a) showed the presence of three methyl groups at 1.4—1.55 δ (s) due to $-O\diagdown\!\!\diagup CH_3$ and tertiary methyl, vicinal protons at 3.96 and 4.49 δ (d, J=9.0 Hz), and a proton isolated on a carbon carrying the secondary hydroxyl at 4.83 δ (s). Since a proton at 4.83 δ in V has the same chemical shift value as that of IV, this secondary hydroxyl is not disturb by the formation of an isopropylidene grouping, and is situated on an isolated position at C-1′ on the cyclohexene ring.

The IR spectrum of V showed an absorption for the two hydrogen-bonded quinone–CO groups at 1620 cm^{-1}, and that of diacetate (VI), obtained by acetylation of V with acetic anhydride–pyridine, showed two free quinone–CO groups at 1650 cm^{-1}, these facts suggest that, of the two quinone–CO groups, the one at C-1 is in hydrogen–bonding with the phenolic hydroxyl at C-8 and that in C-4 with the alcoholic hydroxyl at C-4′ in the cyclohexene ring, both remaining undisturbed by the formation of an isopropylidene grouping, and both hydroxyl groups replaced the acetoxyl groups but not that at C-1′ in VI.

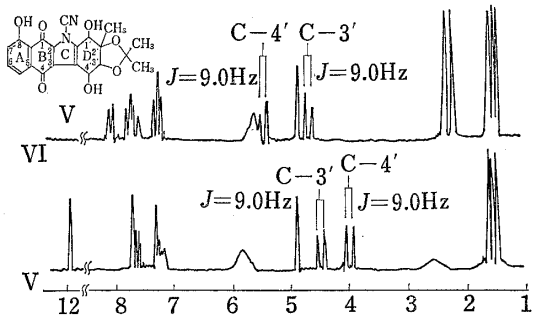


Fig. 5. NMR Spectra of V and VI (100 MHz; $CDCl_3$)

This was further borne out by the NMR spectrum of VI (Fig. 5b), which showed signals for two new acetoxyl groups at 2.22 and 2.44 δ, and the latter is an aromatic acetoxyl group formed by acetylation of the phenolic hydroxyl at 12.0 δ. The fact that a proton at 3.96 δ in V shifted to a lower field to 5.47 δ in VI suggests that a hydroxyl at C-3′ of vicinal secondary hydroxyl groups at C-3′ and C-4′ in IV takes parts in the formation of the isopropylidene group, but the hydroxyl at C-4′ does not. Therefore, the hydroxyl at C-4′ in V was replaced by an acetoxyl group at 2.22 δ in VI. Since a proton isolated on a carbon carrying the secondary hydroxyl at 4.83 δ in V has the same chemical shift as that of VI, the secondary hydroxyl at C-1′ is not acetylated, and the isopropylidene group in V must be formed between the secondary hydroxyl at C-3′ and the tertiary hydroxyl at C-2′ on the carbon carrying the tertiary methyl which appears as a broad signal at 2.57 δ in I.

Furthermore, since V is not oxidized with sodium periodate in dioxane, the isopropylidene group is not formed between C-1′ and C-2′, or C-3′ and C-4′.

In order to establish the binding position of the hydroxyl groups in the cylohexene ring in IV, IV was oxidized with sodium periodate in dioxane and it gave an oxide (VII). The NMR spectrum of VII (Fig. 6) showed absorptions for an aldehyde proton attached to an unsaturated bond at 10.52 δ (s), a methyl adjacent to the carbonyl produced by oxidation at 2.4 δ (s), and a secondary hydroxyl and a proton on the same carbon at 6.28 and 4.28 δ respectively. From these observations, it was found that a tertiary methyl and a tertiary hydroxyl are present on the same carbon (C-2′) in I. The IR spectrum of VII showed, an absorption for a free quinone-CO at 1660 cm^{-1} and hydrogen-bonded quinone-CO at 1620 cm^{-1}, and an aldehyde produced by sodium periodate oxidation indicated presents of vicinal secondary hydroxyl groups at C-3′ and C-4′ in IV. From the IR and NMR spectra of the derivatives obtained during structural studies on I, partial structure for I was determined as shown in Fig. 7.

Position of the remaining group CN_2 was investigated by X-ray diffraction[7] on *p*-bromobenzoate derivative (VIII) which was obtained by treatment of I with *p*-bromobenzoyl chloride

6) M. Miyamoto, K. Morita, Y. Kawamatsu, S. Noguchi, R. Marumoto, M. Sasai, A. Nohara, Y. Nakadaira, Y.Y. Lin, and K. Nakanishi, *Tetrahedron*, **22**, 2761 (1966).
7) A. Furusaki, M. Matsui, T. Watanabe, S. Ōmura, A. Nakagawa, and T. Hata, *Israel J. Chem.*, **10**, 173 (1972).

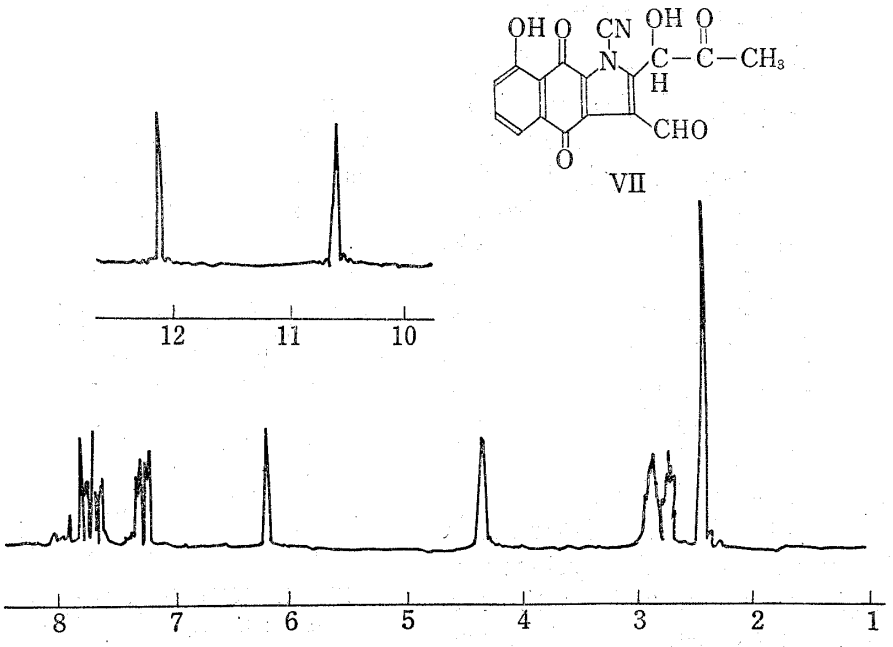


Fig. 6. NMR Spectrum of VII (100 MHz; DMF-d_7)

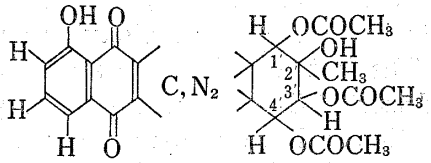
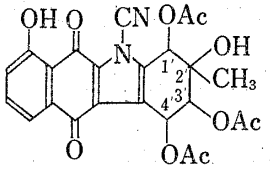


Fig. 7. Partial Structure of I

in chloroform-pyridine and crystallized from benzene. The structure with a nitrile or isonitrile group attached to a five-membered ring was presumed for the unknown partial structure. The three possible structures **1**, **2**, and **3**, were considered for the binding form of carbon and nitrogen atoms undefined by X-ray diffraction.



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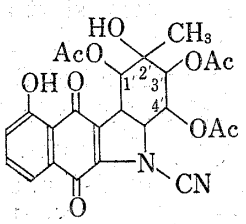

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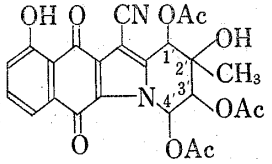


3

Hydrolysis[8] of IV by refluxing with 30% potassium hydroxide for 1 hr gave a residue whose distillate was assayed for formic acid by its reduction to formaldehyde, but formic acid was not determined with chromotropic acid. A stream of purified N_2 was passed through the refluxing solution of IV with 10% hydrochloric acid-methanol for 1 hr, and the resulting liberated ammonia was determined by Nessler's reagent. Consequently, kinamycins were confirmed to have a nitrile group.

From the fact that the absorption of only a hydrogen-bonded quinone-CO appeared at 1620 cm^{-1} in IV, and a proton on a carbon carrying an aldehyde group was not observed and an aldehyde proton at 10.52 δ attached to unsaturated bond appeared in VII, the possibility of structures **2** and **3**[9] can be denied. In view of these observations it was confirmed that I has a structure in which a nitrile group is attached to the nitrogen atom in the pyrrole ring

8) D.B. Borders, K.J. Saz, J.E. Lancaster, W.K. Hausmann, L.A. Mitscher, E.R. Wetzel, and E.L. Patterson, *Tetrahedron*, **26**, 3123 (1970).

9) The NMR spectrum of Tyrosin signaled an N-formyl proton at 7.9 δ: R.B. Morin, M. Gorman, R.L. Hamill, and P.V. Demarco, *Tetrahedron Letters*, **54**, 4737 (1970).

From the three dimensional X-ray diffraction study of VIII, a naphthoquinone and a pyrrole ring were assumed to be in a plane and less twisted, and the cyclohexene ring takes a half chair conformation.

On the basis of the structure of I, the structural relationship among other components, A (IX), $C_{24}H_{20}O_{10}N_2$ (M^+=496), B (X), $C_{20}H_{16}O_{18}N_2$ (M^+=412), and D (XI), $C_{22}H_{18}O_9N_2$

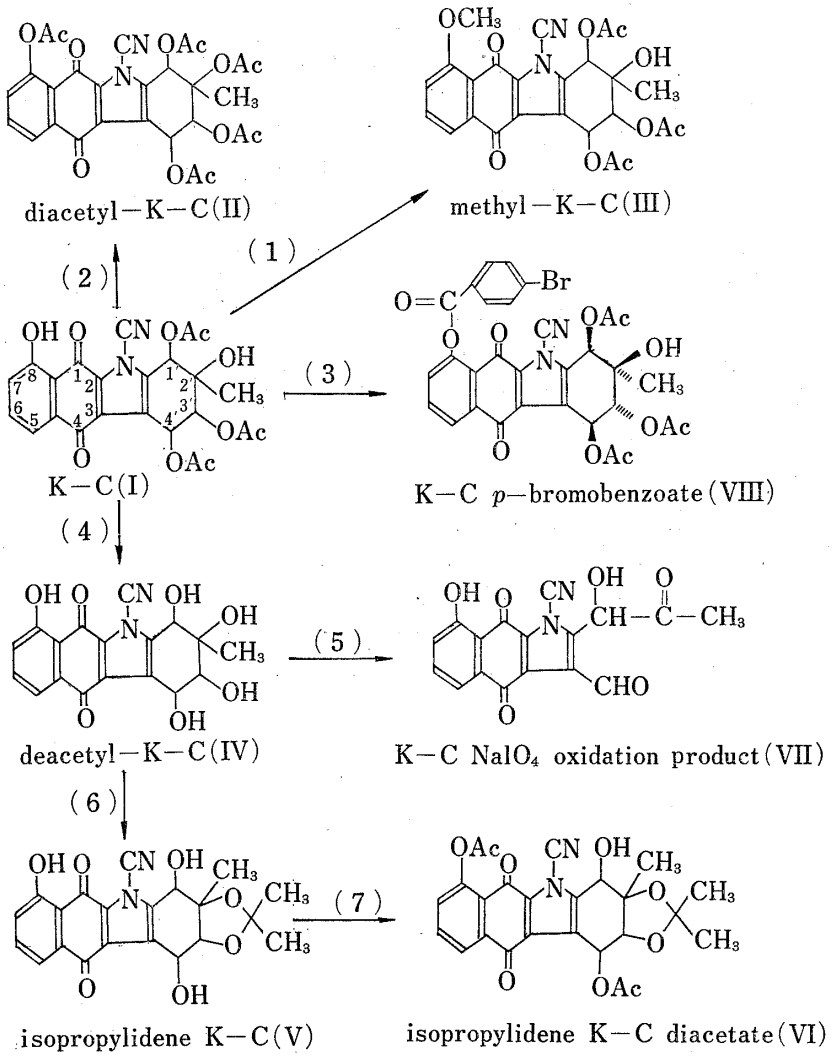
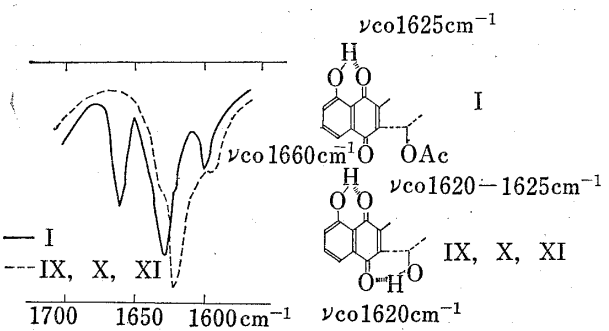


Chart 1

* K-C: kinamycin C
reagents
(1) CH_2N_2 in ether, (2) Ac_2O, AcONa in pyridine, (3) *p*-bromobenzoyl chloride in $CHCl_3$, (4) 0.2N NaOH, (5) $NaIO_4$ in H_2O and dioxane, (6) *p*-toluenesulfonic acid in absolute acetone, (7)Ac_2O in pyridine



Fg. 8. IR Spectrum of I, IX, X, and XI

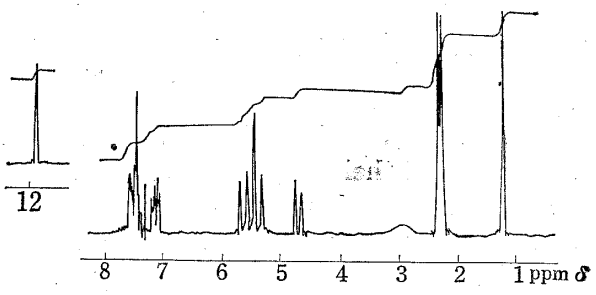
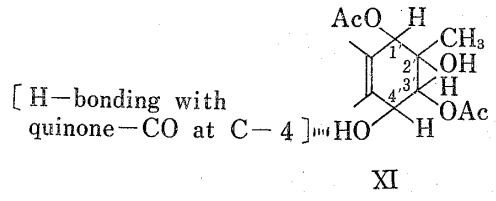


Fig. 9. NMR Spectrum of XI (100 MHz, $CDCl_3$)

(M^+=452), could be clarified. UV, IR, NMR and mass spectra of the four compounds suggest that the structural difference is based on the number and position of the acetoxyl groups. The IR spectra of components IX, X, and XI show the absorptions for hydroxyl (3500 cm^{-1}), nitrile (2260 cm^{-1}), and acetoxyl-CO (1740 cm^{-1}) as functional groups common to I, excluding that of quinone-CO (1660—1620 cm^{-1}). The IR absorption of quinone-CO region in the four compounds is shown in Fig. 8.

The absorptions due to the free and hydrogen-bonded quinone-CO respectively, observed at 1660 cm^{-1} and 1625 cm^{-1} in I, were observed as hydrogen-bonded quinone-CO at *ca.* 1620 cm^{-1} in IX, X, and XI. This fact suggests that an acetoxyl group at C-4′ position of the cyclohexene ring in I is replaced by a hydroxyl group in IX, X, and XI. As shown in Fig. 9, the NMR spectrum of XI indicated the presence of a tertiary methyl at 1.3 δ (s, 3H), two acetoxyl groups at 2.22 and 2.67 δ (three acetoxyl groups in I), two hydroxyl groups at 2.90 and 5.29 δ (disappeared in D_2O), vicinal protons on carbon atoms each carrying the secondary hydroxyl and acetoxyl at 4.68 and 5.60 δ (d, J=8.0 Hz), respectively, a proton isolated on a carbon carrying the acetoxyl at 5.42 δ, three aromatic protons of ABX splitting pattern at 7.02—7.70 δ, and a phenolic hydroxyl at 11.88 δ (s, 1H).

From the comparison of NMR and IR spectra of I, XI was considered to have a secondary hydroxyl at C-4′ position in the cyclohexene ring. This was further confirmed from the spectroscopic data of triacetyl-kinamycin D (XII) obtained by acetylation of XI with acetic anhydride-pyridine. The absorption for hydrogen-bonded quinone-CO observed at 1620 cm^{-1} in XI shifted to a higher wave-number side and was observed as a free quinone-CO at 1640 and 1655 cm^{-1} in XII. In the NMR spectrum of XII, three acetoxyl signals appeared, indicating the acetylation of phenolic hydroxyl (11.98 δ) and two hydroxyl groups (2.90 and 5.29 δ) in XI shifts to a lower magnetic field by *ca.* 1 ppm on acetylation, the proton was found to be situated on the carbon carrying the hydroxyl at C-4′ in the cyclohexene ring in XI.



XI

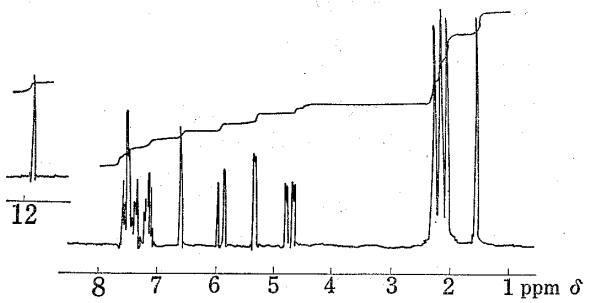


Fig. 10. NMR Spectrum of IX (100 MHz, $CDCl_3$)

Comparative examination of II and XII from their IR, NMR, and mass (M^+, *m/e* 580) spectra and silica gel thin-layer chromatography (TLC) (solvent system: ethyl acetate: chloroform=3: 2) confirmed them to be the same substance. Therefore, the only difference between I and XI was that the acetoxyl group at C-4′ in the cyclohexene ring in I was replaced by hydroxyl in XI.

Components IX and X have the same chromophore, 8-hydroxynaphthoquinone, as I and XI from their IR, NMR, and UV spectral data. IR spectra of IX and X showed the absorption for a hydrogen-bonded quinone-CO at 1625 cm^{-1}, suggesting the presence of a secondary hydroxyl group at C-4′ on a cyclohexene ring. The NMR spectrum of IX (Fig. 10) exhibited the presence of a tertiary methyl at 1.58 δ, three acetoxyl groups at 2.1—2.2 δ, a proton on the carbon carrying the hydroxyl at 4.66 δ (dd, J=1.8 and 8.5 Hz), hydroxyl at 5.25 δ (d, J=1.8 Hz) (disappearing in D_2O), and two protons on carbon atoms each carrying

the acetoxyl group at 5.84 δ (d, J=8.5 Hz) and 6.53 δ (s). In X, only one acetoxyl at 2.3 δ and a tertiary methyl at 1.54 δ was observed.

From the comparison of chemical shift values for the tertiary methyl group in the four compounds, I, IX, X, and XI, and their acetylate (II), monoacetyl-kinamycin A (XIII), tetraacetyl-kinamycin B (XIV), and (XII), the signal for the tertiary methyl proton in IX, X, II, XII, XIII, and XIV has shifted to a lower magnetic field by *ca.* 0.3 ppm than that in I and XI, in which the tertiary hydroxyl on the same carbon at C-2′ has not been acetyalted (Table I).

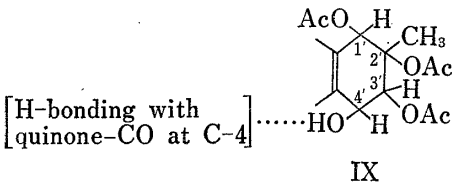
Table I. Chemical Shift of Tertiary-CH_3 at C-2′ on Cyclohexene Ring in Each Compound and Their Acetylates

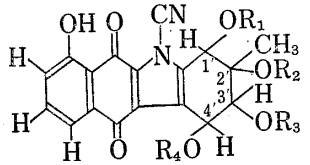
Compound	I	XI	IX	X	II	XII	XIII	XIV
tert-CH_3 (in $CDCl_3$)	1.31	1.26	1.58	1.54	1.57	1.59	1.62	1.60[a]

a) DMSO-d_6

In view of the above facts, the most reasonable conclusion for the structures of IX and X is that the secondary hydroxyl at C-1′, tertiary hydroxyl at C-2′, and secondary hydroxyl at C-3′ in each are replaced by three acetoxyl groups in IX, and secondary hydroxyl at C-4′ is free, and the tertiary hydroxyl at C-2′ is replaced by acetoxyl, and secondary hydroxyl at C-1′, C-3′, and C-4′ are all free in X.

Acetylation of IX with acetic anhydride-pyridine using a trace of *p*-toluenesulfonic acid as a catalyst afforded XIII, whose NMR spectrum showed acetylation of the hydroxyl at C-4′ but not the phenolic hydroxyl, and this fact was also confirmed by the absorptions of a free quinone-CO at 1655 and a hydrogen-bonded quinone-CO at 1625 cm^{-1} in its IR spectrum. Mass spectrum of XIII indicated the peaks at *m/e* 538 (M^+) and 496 (M^+—42). Tetraacetyl-kinamycin B (XIV), obtained by acetylation in a similar manner as IX, exhibited the peaks at *m/e* 580 (M^+), 538 (M^+—42), and 496 (M^+—84), and was confirmed to be identical with II and XII obtained by acetylation of I and XI, respectively, from the comparison of their IR, NMR and mass spectra, and silica gel thinlayer chromatography. The structural relationships among these four compounds was determined as shown in Chart 2.





Compound	R_1	R_2	R_3	R_4
A (IX)	$COCH_3$	$COCH_3$	$COCH_3$	H
B (X)	H	$COCH_3$	H	H
C (I)	$COCH_3$	H	$COCH_3$	$COCH_3$
D (XI)	$COCH_3$	H	$COCH_3$	H

Chart 2. Structures of Kinamycin A, B, C, and D

The *in vitro* antimicrobial activities of kinamycins by the agar dilution method are listed in Table II. As can be seen from Table II, they are all strongly active against gram-positive bacterial but less active against gram-negative bacteria. Among these four, B (X) and D (XI) are more active than A (IX) and C (I). In relationship between antimicrobial activities and structures of each component the antimicrobial activity increases with decreasing number of AcO group in the order of kinamycin C, A, D, B. The minimum inhibitory concentration of X are 0.012 μ/ml against *Bacillus subtilis* PCI-219, *Bacillus anthracis*, *Staphylococcus*

aureus, and *Staphylococcus albus*, and is 0.09—0.19 μ/ml against *Vibrio comma*. The derivatives obtained during structural studies on I as described above were submitted to antibacterial testing with the results shown in Table III. Derivatives of deacetyl-kinamycin C (IV) showed a marked activity against *Mycobacterium* ATCC 607 and gram-negative organisms like *Escherichia coli* NIHJ, *Klebsiella pneumoniae*, and *Shigella sonnei*. On the other hand, derivative of diacetyl-kinamycin C (II) had a decreased activity against gram-postive bacteria, and showed no activity against gram-negative bacteria by acetylation. Derivatives of kinamycin $NaIO_4$ oxide (VII) showed a markedly strong activity against gram-positive and -negative bacteria.

Table II. Antimicrobial Spectra of Kinamycin A, B, C, and D

Test organism	Minimum inhibitory concentration (μg/ml)			
	A	B	C	D
Bacillus subtilis PCI-219	0.024	0.012	0.19	0.012
Bacillus anthracis	0.19	0.012	0.19	0.024
Staphylococcus aureus FDA 209P	0.78	0.012	0.78	0.024
Staphylococcus albus	0.024	0.012	0.39	0.024
Mycobacterium ATCC 607	25	6.25	6.25	6.25
Escherichia coli NIHJ	>100	3.12	>100	12.5
Vibrio comma	>100	0.19	25	12.5
Vibrio comma Inaba 904	50	0.09		
Klebsiella pneumoniae	>100	12.5	>100	25
Pseudomonas aeruginosa P-2	>100	>100	>100	>100
Salmonella typhosa 901W	>100	6.25	>100	12.5
Shigella dysenteriae	>100	25	>100	25

Table III. Antimicrobial Spectra of Kinamycin Derivatives

Test organism	Minimum inhibitory concentration (μg/ml)						
	1	2	3	4	5	6	7
Bacillus subtilis PCI 219	0.024	0.078	1.25	1.25	25	0.31	0.078
Staphylococcus aureus 209P	0.024	0.024	1.56	0.024	5.0	0.012	0.012
Sarcina lutea	0.78	0.39	1.56	0.012	2.5	0.012	0.024
Escherichia coli NIHJ	>100	0.78	>100	6.25	>100	25	25
Mycobacterium ATCC 607	>100	1.56	>100	0.78	>100	12.5	12.5
Klebsiella pheumoniae	>100	12.5	>100	0.39	>100	50	50
Shigella sonnei	>100	1.56	>100	0.78	50	25	25
Salmonella typhimurium	6.25	6.25	6.25	100	10	2.5	2.5
Pseudomonas aeruginosa P-2	>100	>100	>100	>100	>100	>100	>100

1: kinamycin C
2: deacetyl-kinamycin C
3: diacetyl-kinamycin C
4: kinamycin C $NaIO_4$ oxide
5: isopropylidene-kinamycin diacetate
6: isopropylidene-kinamycin C
7: kinamycin D

Experimental

Melting points were taken on the MRK Micro-melting point Determinator. The p*Ka'* values were determined with the Metrohm E336A Potentiometer. The IR spectra were taken with the JASCO DS-403G Spectrometer, the NMR spectra with the JNM-4H-100 and the Hitachi H-60 Spectrometer (tetramethylsilane as the internal standard), the Ultraviolet spectra with the Hitachi EPS-3T recording Spectrophotometer, and the mass spectra with the Hitachi RMU-7L Spectrometer.

Diacetyl-kinamycin C (II)——Kinamycin C (100 mg) was refluxed for 2 hr with Ac_2O (3 ml) containing AcONa (80 mg). The reaction mixture was poured into ice H_2O, neutralized with $NaHCO_3$, and extracted with EtOAc. The extract was dried over Na_2SO_4 and evaporated *in vacuo*, the residue was chromato-

graphed over silica gel (2 g) eluted with $CHCl_3$–EtOAc (6: 1) to give 72 mg of II which was crystallized from EtOAc, mp 268—271° (decomp.). *Anal.* Calcd. for $C_{28}H_{24}O_{12}N_2$: C, 57.93; H, 4.13; N, 4.83. Found: C, 57.79; H, 4.18; N, 4.94. Mol. wt. 580 (mass spectrum). IR (CCl_4) cm^{-1}: 2150 (–C≡N), 1755 (acetoxy C=O), 1655 and 1650 (free quinone C=O). UV λ_{max}^{EtOH}: 260 nm (ε 8.7×10^3). NMR $\delta_{ppm}^{CDCl_3}$: 1.57 (3H, s, CH_3), 1.96 (3H, s, CH_3 of acetate), 2.1 (3H, s, CH_3 of alcoholic acetate), 2.4 (3H, s, CH_3 of phenolic acetate), 5.78 (1H, d, J=6 Hz), 6.13 (1H, d, J=6 Hz), 6.48 (1H, s), 7.11 (1H, dd, J=2, J=8 Hz aromatic proton), 7.49 (1H, t, J=8 Hz), 7.61 (1H, dd, J=2, J=8 Hz).

Methyl-kinamycin C (III)——Ethereal CH_2N_2 (2 ml) was added to a suspension of I (80 mg) in EtOH to give a clear solution, which was allowed to stand for 1 hr at room temperature. The reaction mixture was evaporated to dryness *in vacuo* and the residue was chromatographed over silica gel (2.4 g) eluted with $CHCl_3$–EtOAc (6: 1) to give 53 mg of III, mp 140—144° (decomp.).

Deacetyl-kinamycin C (IV)——I (1 g) was dissolved in 150 ml of 0.2N NaOH solution and the solution was stirred for 50 min at room temperature. After adjusting to pH 5 with 0.5N HCl, the precipitate produced was collected by filtration and dried *in vacuo* to give 760 mg of IV, mp 133—136° (decomp.). IR (KBr) cm^{-1}: 3300 (OH), 2120 (–C≡N), 1620 (hydrogen-bonded quinone C=O), 1320 (alcoholic OH). NMR $\delta_{ppm}^{DMSO\text{-}d_6}$: 1.3 (3H, s, CH_3), 3.75 (1H, d, J=3.4 Hz), 4.55 (1H, d, J=3.4 Hz), 6.95—7.65 (3H, aromatic proton).

Isopropylidene-kinamycin C (V)——IV (200 mg) was dissolved in anhyd. acetone (16 ml) containing a small amount of *p*-toluenesulfonic acid as a catalyst, the reaction mixture was refluxed for 12 hr, and the resulting precipitate was extracted with ether. The ether extract was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness. The residue was chromatographed over silica gel (6 g) and eluted with $CHCl_3$–EtOAc to afford a solid (120 mg), mp 185—188° (decomp.). UV λ_{max}^{EtOH}: 264.5 nm (ε 2.46×10^2). IR (KBr) cm^{-1}: 3300 (OH), 2160 (–C≡N), 1620 (hydrogen-bonded quinone C=O), 1376 (CH_3), 1330 (alcoholic OH), 1150, 1090 (–C–O–C–). NMR $\delta_{ppm}^{CDCl_3}$: 1.43 (3H, s, CH_3), 1.51 (3H, s, CH_3), 1.56 (3H, s, CH_3), 2.55 (1H, s, OH), 3.96 (1H, d, J=9.0 Hz), 4.49 (1H, d, J=9.0 Hz), 4.83 (1H, s), 5.88 (1H, s, OH), 7.12—7.65 (3H, aromatic proton).

Isopropylidene-kinamycin C Diacetate (VI)——V (60 mg) was dissolved in pyridine (2 ml) and Ac_2O (0.5 ml). The reaction mixture was allowed to stand for overnight, then poured into ice H_2O, and neutralized with $NaHCO_3$, and extracted with EtOAc. The extract was dried over Na_2SO_4 and evaporated *in vacuo*. The residue (55 mg) was chromatographed over silica gel (2 g) and eluted with $CHCl_3$–EtOAc, giving 40 mg of VI. Recrystallization from EtOAc gave yellow crystals, mp 195—197° (decomp.). UV λ_{max}^{EtOH}: 261.5 nm (ε 2.96×10^3). IR (KBr) cm^{-1}: 3300 (OH), 2920 (CH_3), 2150 (–C≡N), 1765, 1745 (acetoxy C=O), 1640, 1635 (free quinone C=O), 1380 (CH_3), 1315, 1305 (alcoholic OH), 1250, 1190 (–C–O–C–). NMR $\delta_{ppm}^{CDCl_3}$: 1.40 (3H, s, CH_3), 1.48 (3H, s, isopropyl CH_3), 1.55 (3H, s, isopropyl CH_3), 2.22 (3H, s, CH_3 of alcoholic AcO), 2.44 (3H, s, CH_3 of phenolic AcO), 4.65 (1H, d, J=9.0 Hz), 5.47 (1H, d, J=9.0 Hz), 4.80 (1H, s), 5.57 (1H, s, OH), 7.27—8.02 (3H, aromatic proton).

Periodate Oxidation Product (VII)——An aqueous solution of 0.0015M $NaIO_4$ (321 mg) in H_2O was added to a suspension of IV (600 mg) in dioxane (15 ml). The mixture was stirred for 1 hr at room temperature, and then, after adding H_2O, the resulting precipitate was extracted with EtOAc. The extract was dried over Na_2SO_4 and evaporated to dryness to give a crude powder (500 mg), which was chromatographed over silica gel (20 g) eluted with $CHCl_3$–EtOAc (3: 1) to afford 110 mg of VII as orange powder, mp 117—119° (decomp.). IR (KBr) cm^{-1}: 2140 (–C≡N), 1720 (C=O), 1700 (–CHO), 1650 (free quinone C=O), 1620 (hydrogen-bonded quinone C=O). UV λ_{max}^{EtOH}: 252.5 nm (ε 1.06×10^4). NMR $\delta_{ppm}^{DMF\text{-}d_7}$: 2.4 (3H, s, CH_3), 4.28 (1H, d, J=0.6 Hz), 6.28 (1H, s, OH, J=0.6 Hz), 7—8 (3H, aromatic proton), 10.52 (1H, s, aldehyde proton), 12.03 (1H, s, phenolic OH).

Kinamycin C *p*-Bromobenzoate (VIII)——A solution of I (300 mg) in 4 ml $CHCl_3$ dissolved in a solution of *p*-bromobenzoyl chloride in 2 ml pyridine was heated for 90 min at 70° in an oil bath. After removal of pyridine-HCl produced, the filtrate was washed with 0.5N H_2SO_4 (5 ml×2) and extracted with $CHCl_3$ (5 ml). The organic layer was washed with H_2O (10 ml×2), dried over Na_2SO_4, and evaporated to dryness to give 215 mg of a crude benzoate, which was chromatographed over silica gel (8.5 g) eluted with $CHCl_3$–EtOAc (5: 1). Recrystallization from benzene gave yellow plates, mp 203—205° (decomp.). *Anal.* Calcd. for $C_{31}H_{23}O_{11}N_2Br$: C, 58.81; N, 3.65; H, 3.79; Br, 9.97. Found: C, 54.71; H, 3.56; N, 4.11; Br, 11.76. Mol. wt. 679 (mass spectrum). $[\alpha]_D^{25}$ −19.4° (c=0.5, $CHCl_3$). IR (KBr) cm^{-1}: 2175 (–C≡N), 1745 (acetoxy C=O), 1662 (free quinone C=O), 1172, 1134, 1070 (–C–O–C–). UV λ_{max}^{EtOH}: 249 nm (ε 1.09×10^4). NMR $\delta_{ppm}^{CDCl_3}$: 1.22 (3H, s, CH_3), 2.06 (3H, s, CH_3 of acetate), 2.11 (3H, s, CH_3 of acetate), 2.11 (3H, s, CH_3 of acetate), 5.45 (1H, s), 5.6 (1H, d, J=7.8 Hz), 6.25 (1H, d, J=7.8 Hz), 7—9 (3H, aromatic proton).

Quantitative Determination of Formic Acid——Compound IV (100 mg) was suspended in 30% KOH (10 ml) and heated under reflux for 1 hr. The hydrolysate was adjusted to pH 3 and subjected to distillation. An aliquot of the distillate was assayed for formic acid by its reduction to formaldehyde, but formic acid was not determined with chromotropic acid.[10]

10) W. Morton Grant, *Anal. Chemistry*, **20**, 267 (1948).

Qualitative Determination of Ammonia——Compound IV (300 mg) was suspended in 10% HCl–MeOH (6 ml) and heated under reflux for 1 hr. A stream of purified N_2 was passed through the refluxing solution and resulting liberated gas was tested to give yellow precipitate by Nessler's reagent. From above results, this gas was determined as ammonia.

Triacetyl-kinamycin D (XII)——Compound XI (200 mg) was dissolved in a mixture of pyridine (3 ml) and Ac_2O (1.5 ml), and the mixture was allowed to stand at room temperature for overnight. The reaction mixture was treated with H_2O, extracted with ether, and the extract was washed with H_2O, dried over Na_2SO_4, and evaporated to dryness (210 mg). The residue was chromatographed over slica gel (8 g) and eluted with $CHCl_3$–EtOAc (10: 1) to give 120 mg of XII which was crystallized from EtOAc, mp 247—252° (decomp.). *Anal.* Calcd. for $C_{28}H_{24}O_{12}N_2$: C, 57.93; H, 4.13; N, 4.83. Found: C, 57.89; H, 4.16; N, 5.03. Mol. wt. 580 (mass spectrum).

Monoacetyl-kinamycin A (XIII)——Compound IX (100 mg) was dissolved in a mixture of pyridine (1.3 mg), Ac_2O (0.7 ml) and a trace of *p*-toluenesulfonic acid as a catalyst, and the mixture was allowed to stand at room temperature for overnight. By a similar procedure as for XII, a powder (100 mg) was obtained and chromatographed over silica gel (4 g) eluted with $CHCl_3$–EtOAc (8: 1) to give 70 mg of XIII, mp 161—163° (decomp.). $C_{26}H_{22}O_{11}N_2$, mol. wt. 538 (mass spectrum). NMR $\delta_{ppm}^{CDCl_3}$: 1.63 (3H, s, CH_3), 2.0 (3H, s, CH_3 of acetate), 2.1 (3H, s, CH_3 of acetate), 2.12 (6H, s, two CH_3 of acetate), 2.14 (3H, s, CH_3 of acetate), 5.83 (1H, d, J=7.5 Hz), 6.16 (1H, d, J=7.5 Hz), 6.53 (1H, s), 7.2—7.6 (3H, aromatic proton).

Tetraacetyl-kinamycin B (XIV)——Compound X (100 mg) was acetylated with Ac_2O (0.8 ml) and a trace of *p*-toluenesulfonic acid as a catalyst in pyridine (1.3 ml) by a similar procedure as for XIII, and the residue was purified by chromatography over silica gel (4 g) eluted with $CHCl_3$–EtOAc (10: 1) to give 75 mg of XIV, mp 201—204° (decomp.). $C_{28}H_{24}O_{12}N_2$, mol. wt. 580 (mass spectrum).

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