NEW PRODUCTS RELATED TO KINAMYCIN FROM *STREPTOMYCES MURAYAMAENSIS*

II. STRUCTURES OF PRE-KINAMYCIN, KETO-ANHYDROKINAMYCIN, AND KINAMYCINS E AND F

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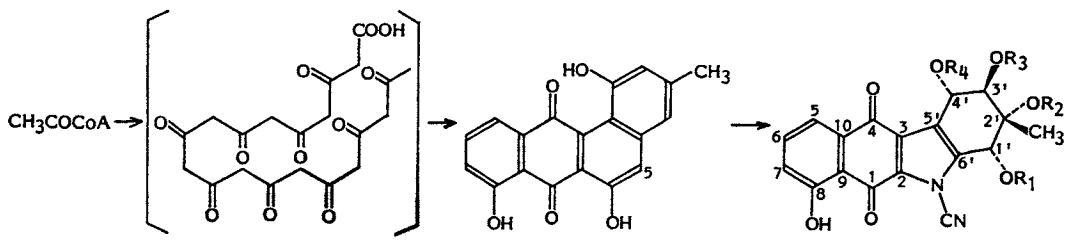
Four new colored components of *Streptomyces murayamaensis* sp. nov. Hata et Ohtani, the producer of the kinamycins, have been isolated and their structures determined by a combination of mass spectral, high field NMR and biosynthetic techniques. The first compound with the benz[*b*]carbazole skeleton in the biosynthetic pathway of kinamycin D has been named "pre-kinamycin" (**7**). A keto-epoxide kinamycin intermediate has been labeled "keto-anhydrokinamycin" (**9**), and the 1′-monoacetate of kinamycin has been called kinamycin E (**12**). Natural production of deacetylkinamycin (**13**, now labeled kinamycin F) by *S. murayamaensis* has been confirmed by an isotope trapping experiment. The role of these new intermediates in kinamycin biosynthesis is discussed.

The kinamycin antibiotics, **1**~**4**, were isolated and characterized by ŌMURA *et al.*,[1~5] and are active against Gram-positive and, to a lesser extent, Gram-negative organisms. Weak antitumor activity was exhibited by kinamycin C against Ehrlich ascites carcinoma and against sarcoma-180.[2] We have been investigating the biosynthesis of these unusual benz[*b*]carbazole structures,[6~8] and have established the pathway shown in Scheme 1. The kinamycins are derived from a decaketide **5** that cyclizes to the benzanthraquinone **6** which undergoes a remarkable process of oxidation/nitrogen-insertion/ring-contraction.[8] TLC analysis of ethyl acetate extracts of broths from the fermentation of *Streptomyces murayamaensis* revealed numerous colored spots in addition to those due to the kinamycins, **6**, and murayaquinone.[9] By manipulation of the composition of the fermentation medium we have been able to improve production of a number of these other compounds,[10] and have determined the structures of four of these that are relevant to the pathway of kinamycin biosynthesis.

Scheme 1.



5 **6**

1 $R_1=R_2=R_3=Ac$ $R_4=H$
2 $R_2=Ac$ $R_1=R_3=R_4=H$
3 $R_1=R_3=R_4=Ac$ $R_2=H$
4 $R_1=R_3=Ac$ $R_2=R_4=H$

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Results

Fermentation in a medium composed of oatmeal and trace metals was most effective for the production of useful quantities of a number of colored metabolites in addition to the kinamycins. Chromatography on Silicar CC-4 packed in methylene chloride led to separation into nonpolar and polar fractions, as well as purification of a few of the individual compounds. Initial elution with methylene chloride first afforded pure dehydrorabelomycin (**6**) and then yielded a mixture of five compounds, one of which was murayaquinone. Further elution with 30% ether in methylene chloride gave a mixture of kinamycins A and B and an unknown substance, followed by pure kinamycin D, and finally yielded a mixture of three unknowns. Further flash chromatography on silica gel provided, in succession, a purple compound (P1), a second purple compound (P2), and a yellow compound (Y1) from the nonpolar fraction; murayaquinone was destroyed by the silica gel. An aliphatic contaminant of Y1 was next removed by rechromatography, eluting with 20% ethyl acetate in hexane. Flash chromatography of the mixture of polar unknowns yielded pure PY1, PY2 and PY3, all of them orange crystalline materials.

We have determined the structures of P1, Y1, PY1 and PY3. All four have a cyanamide functionality as evidenced by their IR spectra. The ^{1}H NMR spectral data are presented in Table 1. The same data for kinamycin D are included for comparison.

P1 (7)

Elemental analysis of P1 gave a formula of $C_{18}H_{10}N_2O_4$. In addition to the cyanamide at 2162 cm^{-1}, the IR spectrum also indicated the presence of a quinone and of an hydroxyl group. Examination of the proton spectrum of P1 revealed the pattern typical of the three adjacent hydrogens found in the A-ring of the kinamycins (5-, 6-, and 7-H). In addition, there were two resonances that could be assigned to *meta*-protons on a tetra-substituted aromatic ring (δ 6.60 and 6.69, d, J=1.5 Hz, clearly observable only when the methyl resonance at δ 2.39 was irradiated), and a three proton singlet at δ 2.39 for an aromatic methyl group. The remaining resonances belonged to two exchangeable hydrogens which, from their chemical shifts (δ 11.60 and 12.32), were clearly hydrogen-bonded.

The hydrogen-bonded resonances were no longer present in the spectrum of the diacetate, prepared by treatment with acetic anhydride and a catalytic amount of concentrated sulfuric acid. P1 was extremely insoluble in all solvents, but the diacetate **8** was sufficiently soluble to obtain a ^{13}C NMR spectrum when run in a 10-mm sample tube. In this case twenty-two resonances were observed, including quinone carbonyls at δ 174.14 and 192.48, two ester carbonyls at δ 170.28 and 170.64, and a cyanamide resonance at δ 83.71. Much of this spectrum was highly reminiscent of those of the kinamycins.

The data suggested a benz[*b*]carbazole such as had been predicted as an intermediate in kinamycin biosynthesis,[7] in this case already containing the cyanamide. Structure **7** was confirmed by the enrichments observed in the ^{13}C NMR spectrum of a sample obtained biosynthetically by feeding sodium [2-^{13}C]acetate (feeding conditions were the same as those described in ref 9). This placed the phenolic groups at unenriched sites. Of the two structures that allow hydrogen bonding of both hydroxyls to a quinone carbonyl, only **7** is consistent with the biosynthetic results.

Y1 (9)

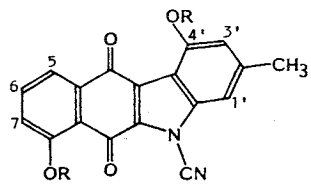
The ^{1}H NMR spectrum of the third compound from the nonpolar fraction was very similar to that of the kinamycins, but lacked any acetate and also lacked one of the D-ring hydrogens. Two exchange-

Table 1. ^{1}H NMR spectral data of *Streptomyces murayamaensis* metabolites.

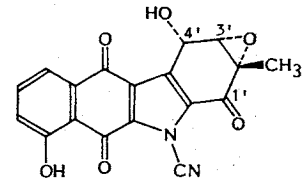
	P1 (7)[a]	P1A$_2$ (8)[a]	Y1 (9)[b]	PY1 (11)[b]	PY3 (12)[b]	Kinamycin D (4)[b]
8 (OR)	12.32[d] (1H, s)	2.48 (3H, s)	12.06[d](1H, s)	12.03[d](1H, s)	12.17[d](1H, s)	12.4[d](1H, s)
7	7.04 (1H, dd, J=0.9, 7.2 Hz)	7.14 (1H, dd, J=1.1, 7.4 Hz)	7.27 (1H, d)	7.06 (1H, dd, J=1.5, 7.8 Hz)	7.22 (1H, dd, J=1.1, 7.7 Hz)	7.22 (1H, dd, J=1.1, 7.9 Hz)
6	7.17 (1H, dd, J=7.0, 7.2 Hz)	7.34 (1H, dd, J=7.4, 7.0 Hz)	7.63 (1H, t, J=7.7 Hz)	7.46 (1H, t, J=7.9 Hz)	9.58 (1H, t, J=7.9 Hz)	7.58 (1H, t, J=7.8 Hz)
5	7.24 (1H, dd, J=0.9, 7.0 Hz)	7.52 (1H, dd, J=1.1, 7.2 Hz)	7.76 (1H, d, J=7.6 Hz)	7.50 (1H, dd, J=1.5, 7.8 Hz)	7.71 (1H, dd, J=1.1, 7.1 Hz)	7.71 (1H, dd, J=1.1, 7.3 Hz)
4′			5.34 (1H, dd, J=1.7, 2.9 Hz)	6.49 (1H, d, J=5.5 Hz)	4.61 (1H, dd, J=1.3, 7.9 Hz)	4.79 (1H, d, J=8.1 Hz)
4′ (OR)	11.60[d] (1H, s)	2.49 (3H, s)	5.94[d](1H, d, J=1.6 Hz)	5.05[d](1H, br s)	5.71[d](1H, d, J=1.3 Hz)	5.50[d](1H, br s)
3′	6.60 (1H, d, J=9.1 Hz)[c]	6.95 (1H, br s)	3.89 (1H, d, J=2.7 Hz)	5.39 (1H, d, J=5.5 Hz)	4.24 (1H, d, J=7.9 Hz)	5.59 (1H, d, J=8.1 Hz)
3′ (OR)				2.15 (3H, s)	3.0[d](1H, br)	2.27 (3H, s)
2′ (OR)				3.2~4.2[d](1H, br s)	2.8[d](1H, br)	2.95[d](1H, br)
2′ (CH$_3$)	2.39 (3H, s)	2.46 (3H, s)	1.64 (3H, s)	1.28 (3H, s)	1.48 (3H, s)	1.22 (3H, s)
1′	6.69 (1H, d, J=9.1 Hz)[c]	6.84 (1H, d, J=1.2 Hz)		4.55 (1H, s)	5.53 (1H, s)	5.43 (1H, s)
1′ (OR)				3.2~4.2[d](1H, br s)	2.62 (3H, s)	2.19 (3H, s)

[a] CD_2Cl_2. [b] $CDCl_3$. [c] Decoupled from δ 2.39. [d] Exchanges with D_2O.

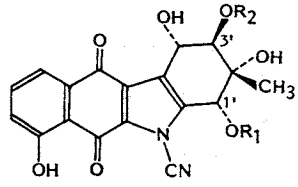
Fig. 1. New compounds obtained from cultures of *Streptomyces murayamaensis*.



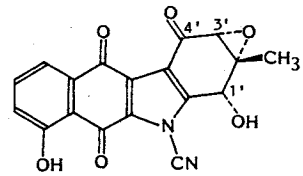
7 R=H
8 R=Ac



9



11 R_1=H R_2=Ac
12 R_1=Ac R_2=H
13 R_1=R_2=H



10

able hydrogens were identified at δ 5.94 and 12.06, the latter being phenolic and hydrogen-bonded. The IR spectrum was consistent with a quinone and, in addition, contained a band at 1671 cm^{-1} for an additional unsaturated carbonyl. This was confirmed by the ^{13}C NMR spectrum, which contained resonances at δ 183.67 and 180.85 for the quinone and at δ 188.62 for a ketone. These data and the empirical formula of $C_{18}H_{10}N_2O_6$ obtained by high resolution (HR)-MS suggested structure **9** or **10**.

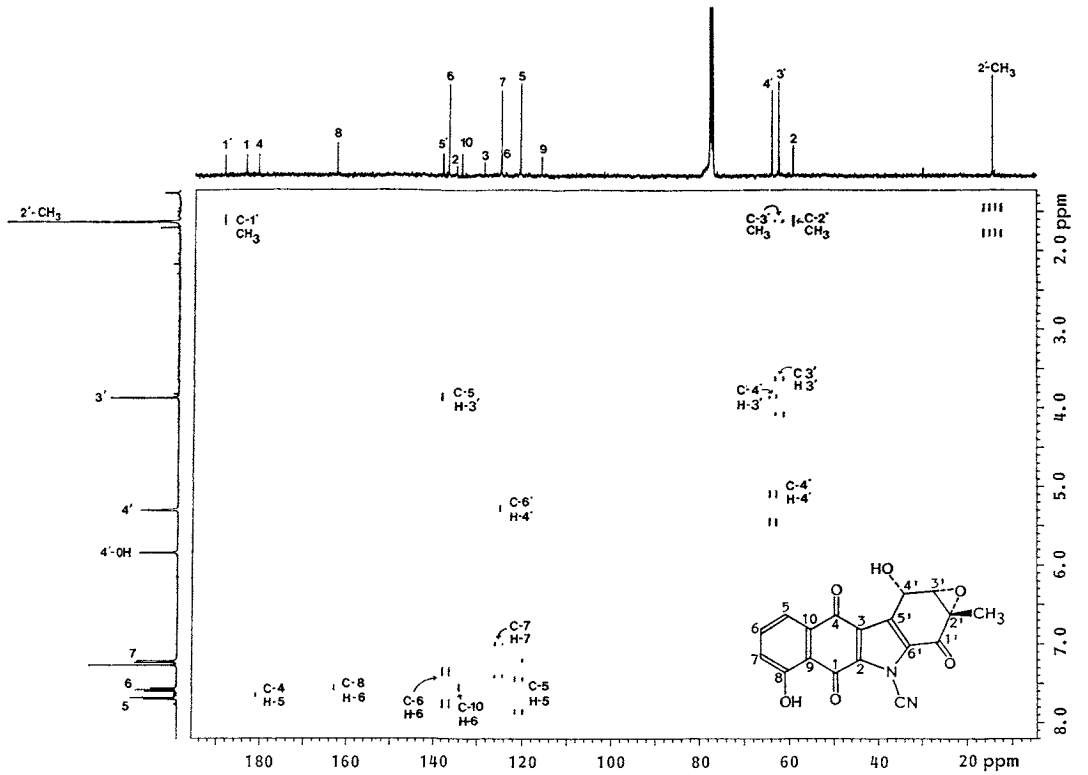
The correct regiochemistry was recognizable from the coupling (J=2.7 Hz) between the resonances at δ 3.89 and 5.34, attributable to 3′-H and 4′-H, respectively. The resonance at δ 5.34 also showed coupling to the hydroxyl at δ 5.94. A LR HETCOSY[9,11] spectrum, shown in Fig. 2, confirms nearly all the NMR assignments and—most importantly—shows long range coupling ($^3J_{CH}$) between the methyl hydrogens (δ 1.53) and the ketone carbonyl (δ 188.62), confirming that the ketone must be at C-1′. A difference nuclear Overhauser effect (NOE) spectrum obtained by irradiating the methyl resonance at δ 1.64 showed positive enhancements of the resonances at δ 3.89 and 5.34. Thus, all these hydrogens must be on the same side of the ring and the structure must be **9**.

PY1 (**11**) and PY3 (**12**)

Two of the polar compounds have also been characterized. Their ^1H NMR spectra clearly indicated that they were mono-acetates of the essential kinamycin skeleton (deacetylkinamycin[3]). HR-MS gave the same exact mass consistent with this conclusion and the ^{13}C NMR spectra were as would be predicted. The ^1H NMR spectrum of PY3 had one resonance shifted to δ 4.24 relative to kinamycin D (δ 5.59), which could be assigned to C-3′, and the spectrum of PY1 had the resonance assignable to C-1′ shifted to δ 4.55 relative to kinamycin D (δ 5.43), establishing the acetylation pattern of each compound as **11** and **12** for PY1 and PY3, respectively.

It was possible to prepare PY1 and PY3 by careful hydrolysis of kinamycin D. Thus, treatment of kinamycin D with potassium carbonate in methanol at 0°C afforded, in addition to some starting material and some deacetylkinamycin, predominantly PY1 and a small amount of PY3. Furthermore, stirring kinamycin D with silica gel overnight in 10% methanol - methylene chloride yielded a small amount of

Fig. 2. LR HETCOSY spectrum of **9**.



PY1 in addition to mostly recovered starting material.

Deacetylkinamycin (13)

Although deacetylkinamycin (**13**) was prepared by ŌMURA *et al.*[8] by hydrolysis of kinamycins A~D, the Japanese group did not detect this compound in the fermentation broth of *S. murayamaensis*. We noticed a spot with the Rf and color of **13** on the TLC of extracts obtained when the oatmeal - trace metals medium was used for fermentation. To confirm that **13** was being produced, an isotope trapping experiment was carried out in which sodium [2-^{14}C]acetate was fed to *S. murayamaensis*. The fermentation was terminated 10 hours later and the cells were disrupted by sonication. *De novo*-produced **13** was trapped by addition of authentic material. Re-isolation of **13** yielded radioactive material and recrystallization to constant specific activity afforded a 0.33% incorporation of the radioactive acetate that had been fed.

Discussion

Careful examination of "minor" metabolites – those produced in relatively small quantity by an organism – can provide critical links in a biosynthetic pathway. In the present case, after manipulation of the fermentation medium to increase the accumulation of a number of such compounds to levels sufficient for isolation and characterization, this approach has been very useful.

Four new structures in the kinamycin pathway have been elucidated. P1 (**7**), the first compound with the benz[*b*]carbazole skeleton we have named "pre-kinamycin". The cyanamide is derived from C-5 of **6**, so is always present in the benz[*b*]carbazoles.[12] While PY1 appears to be an artifact of the

Scheme 2.

6 ⟶ 7 ⟶ 9 ⟶ 13 ⟶ 12 ⟶ 4

work-up, PY3 has been confirmed as a natural product. Thus, when [^{14}C]deacetylkinamycin was fed to *S. murayamaensis*, work-up of the fermentation yielded strong incorporation into kinamycin D and into PY3 (feeding conditions and isolation of metabolites will be reported in a subsequent paper). Therefore, we have named PY3 "kinamycin E" and, since deacetylkinamycin has now also been shown to be produced by this organism, we have re-named it kinamycin F. The fourth new compound, **9**, is fully consistent with our previous feeding results[7] and has been named keto-anhydrokinamycin.

On the basis of the structures now known from *S. murayamaensis* it seems likely that the biosynthetic pathway to kinamycin D is as shown in Scheme 2. Our examination of the unusual metabolism of this organism is continuing.

Experimental

General

MP's were determined in a Hoover capillary mp apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were taken on a Bruker AM 400 spectrometer at 400 and 100.6 MHz, respectively, using 5-mm or 10-mm multinuclear probes. All ^{13}C spectra were taken under Waltz proton-decoupling conditions. NMR spectra are reported as ppm downfield from Me_4Si (δ 0.0). IR spectra were obtained with a Nicolet FT-IR Model 5DXB spectrometer, and UV spectra were obtained on an IBM 9420 instrument. Microsamples were weighed on a Cahn Model 29 electrobalance. All radioactive measurements were carried out in a Beckman LS 7800 liquid scintillation counter using the Beckman HP scintillation cocktail. All measurements were done in duplicate to a $\pm 4\%$ standard deviation. Counting efficiencies were determined automatically by using the Beckman DPM program and sealed ^{14}C-quenched standards purchased from Beckman, and the standard curves were then tested for accuracy with standards prepared from kinamycin D and *n*-[^{14}C]hexadecane. TLC were done on Merck precoated Silica gel 60 PF-254 plates, 0.25 mm thick. Column chromatographies were done on Silicar CC-4 brand of silicic acid (Mallinkrodt 7086) or on flash grade silica (EM Kieselgel 60). Fermentations were carried out in a Lab-Line Model-3595 gyrotory incubator shaker. Broths were centrifuged using either a Sharples T-1 continuous centrifuge or an IEC B-20A centrifuge. Cells were disrupted by sonication with an Ultrasonics Model W-225R sonicator. Evaporation *in vacuo* refers to solvent removal on a rotary evaporator at aspirator pressure and 25~35°C.

Sodium [2-^{14}C]acetate was purchased from ICN Pharmaceuticals. D_2O was purchased from Cambridge Isotope Laboratories. Strains of *S. murayamaensis* were gifts from Professors ŌMURA and HORNEMANN; all incorporation experiments were done with the former strain. Reference samples of kinamycin D were provided by Professors ŌMURA and HORNEMANN. All chemicals were of reagent grade, and all solvents were distilled prior to use.

Culture Maintenance and Fermentation Conditions

Standard culture conditions have been described previously,[7] and fermentation conditions are described in the previous paper.[10]

Isolation

Broth from a 1-liter fermentation of *S. murayamaensis* in the oatmeal - trace elements medium (5×200 ml in 1-liter Erlenmeyer flasks) was acidified with 6 N HCl to pH 3.0. The mycelia were removed by centrifugation (10,000 rpm, 10 minutes, IEC Model B-20A Centrifuge), resuspended in 50 ml distilled H_2O, sonicated (4°C, 5 minutes, continuous, maximum power) and recombined with the broth. The entire volume was stirred with toluene (400 ml) for 1 hour and the resulting emulsion was filtered through a Celite pad, which was then rinsed with acetone (100 ml). After further extraction of the aqueous layer with EtOAc (2×200 ml), the combined organics were dried (Na_2SO_4) and concentrated *in vacuo* to give a brown gum (0.94 g).

The residue was dissolved in $CHCl_3$ (2 ml) and applied to a Silicar CC-4 column (3×20 cm) packed

in $CHCl_3$. Elution with $CHCl_3$ first gave a dark green fraction containing **6** plus nonpolar oils (0.425 g after concentration *in vacuo*). Trituration of the oily residue with hexane gave **6** as dark green needles (5.5 mg). Continued elution with 0.5% EtOAc in $CHCl_3$ gave a fraction (0.242 g) containing the two purple components (P1 and P2) and the nonpolar yellow component (Y1), along with contaminating oils. Further elution of the column with 10% EtOAc in $CHCl_3$ yielded a mixture (0.118 g) of kinamycins A and B (**1** and **2**) and an unknown colorless metabolite (UK). Kinamycin D was eluted with 20% EtOAc in $CHCl_3$, and was directly crystallized from EtOAc - hexane as an orange powder (55 mg). Finally, washing the column with EtOAc gave a mixture of the polar metabolites, PY1, PY2 and PY3 (0.123 g).

The nonpolar metabolites (P1, P2 and Y1) were further purified by column chromatography with flash grade SiO_2 (2×10 cm, 0.5% Et_2O in CH_2Cl_2). The two purple components were cleanly separated and each was directly crystallized from CH_2Cl_2 - hexane. P1 (**7**) crystallized as dark purple needles (15 mg) and P2 crystallized as dark purple brown needles (6 mg). Y1 (**9**) was contaminated with oils and required further chromatographic purification through a flash SiO_2 column (2×10 cm, 10% EtOAc - hexane). Fractions containing pure Y1 were combined, concentrated and **7** (12 mg) was crystallized from $CHCl_3$ - hexane.

The polar components (PY1, PY2 and PY3) were separated by column chromatography through flash grade SiO_2 (2×10 cm, 5% MeOH in CH_2Cl_2). Traces of PY1 (**11**) eluted first, followed by PY3 (**12**) and finally PY2. **12** crystallized from EtOAc - hexane as an orange powder (14.5 mg) and PY2 crystallized from $CHCl_3$ - hexane as yellow orange crystals (9.4 mg).

Physico-chemical Properties

Pre-kinamycin (**7**): MP 300°C (dec); UV $\lambda_{max}^{CHCl_3}$ nm (ε) 254 (5.50×10^3), 288.4 (2.17×10^4), 342 (sh, 5.77×10^3), 574 (3.77×10^4); IR ν_{max}^{KBr} cm^{-1} 3300, 2900, 2162, 1733, 1729, 1718, 1616, 1458, 1239, 1228, 1067; ^1H NMR (see Table 1).

Anal Calcd for $C_{18}H_{10}N_2O_4$: C 67.92, H 3.17, N 8.80.
Found: C 67.57, H 3.00, N 8.61.

Pre-kinamycin Diacetate (**8**): Pre-kinamycin (**7**) (15 mg) was suspended in Ac_2O (10 ml). Upon addition of a catalytic amount of H_2SO_4, the dark purple suspension immediately turned into a clear, deep red solution. After 15 minutes, the reaction mixture was poured into a mixture of ice, $NaHCO_3$ and CH_2Cl_2 and stirred vigorously for 30 minutes. The layers were separated and the aqueous layer was washed with CH_2Cl_2 (25 ml). The combined organic layers were dried (Na_2SO_4) and concentrated. Chromatography of the residue (SiO_2 flash, 5% Et_2O in CH_2Cl_2) and crystallization from CH_2Cl_2 - hexane gave 15 mg (79%) of **8** as dark red needles: MP >300°C (dec); UV $\lambda_{max}^{CHCl_3}$ nm 257, 493; IR ν_{max}^{film} cm^{-1} 2914, 2119, 1763, 1761, 1719, 1613, 1366, 1200; ^1H NMR (see Table 1); ^{13}C NMR (CD_2Cl_2, 10 mm probe, 27°C) δ 21.58, 21.74, 22.89, 83.71, 113.49, 118.01, 122.15, 131.01, 131.33, 131.38, 133.24, 133.66, 138.33, 142.76, 143.36, 145.50, 149.21, 170.28, 170.64, 174.14, 192.48; electron impact (EI)-MS *m/z* (abundance) 402 (M^+, 4.5%), 374 (19%), 332 (30%), 290 (100%), 206 (10%), 176 (13%).

Keto-anhydrokinamycin (**9**): MP >300°C (dec); UV $\lambda_{max}^{CHCl_3}$ nm (ε) 235.8 (4.33×10^4), 255.0 (2.81×10^4), 305.6 (1.86×10^4), 410.0 (9.01×10^3), 452.8 (9.79×10^3); IR ν_{max}^{film} cm^{-1} 3340, 2900, 2164, 1671, 1622, 1477, 1469, 1444, 1233, 1087; ^1H NMR (see Table 1); ^{13}C NMR ($CDCl_3$) δ 14.28, 58.77, 61.98, 63.47, 63.57, 115.77, 120.63, 124.95, 125.03, 128.81, 133.90, 134.98, 136.78, 138.25, 162.76, 180.85, 183.65, 188.62; fast atom bombardment (FAB)-MS *m/z* (abundance) 351 ($M+H^+$, 4%), 309 (16%), 155 (67%), 152 (32%), 135 (47%), 119 (100%), 103 (53%), 85 (88%), 59 (43%); EIHR-MS *m/z* 350.0552 (M^+ calcd for $C_{18}H_{10}N_2O_6$: 350.0539).

Kinamycin E (**12**): MP >200°C (dec); UV λ_{max}^{EtOH} nm (ε) 255.6 (2.17×10^4), 277.0 (1.41×10^4), 295.0 (9.22×10^3), 408.8 (8.85×10^3), 446.4 (8.76×10^3); IR ν_{max}^{film} cm^{-1} 3550, 2920, 2162, 1733, 1729, 1718, 1616, 1459, 1328, 1228, 1066; ^1H NMR (see Table 1); ^{13}C NMR ($CDCl_3$) δ 17.64, 20.04, 68.87, 69.74, 72.64, 74.03, 114.90, 119.49, 123.98, 127.40, 128.55, 131.97, 132.09, 133.18, 135.40, 161.68, 170.34, 180.07, 182.85; FAB-MS *m/z* (abundance) 413 ($M+H^+$, 1%), 309 (5%), 251 (9%), 155 (46%), 135 (42%), 119 (100%), 103 (48%), 85 (87%), 73 (69%); EIHR-MS *m/z* 412.0959 (M^+ calcd for $C_{20}H_{16}N_2O_8$: 412.0906).

Hydrolysis of Kinamycin D to **11**, **12** and **13**: Solid potassium carbonate was added to a solution

of kinamycin D (**4**, 105 mg, 0.23 mmol) in methanol (100 ml) at 0°C. After 15 minutes, TLC showed that almost all of the kinamycin D had been consumed and the reaction was terminated by acidification of the reaction mixture with 1 N HCl. After concentration, the residue was chromatographed (flash grade SiO_2, 2×10 cm column, 5% MeOH - CH_2Cl_2). Fractions containing pure kinamycin D (**4**), PY1 (**11**), kinamycin E (**12**) or kinamycin F (**13**) were pooled, concentrated and weighed. **11** (68.3 mg, 72%) crystallized from $CHCl_3$ - hexane as a bright orange powder: MP >200°C (dec); UV λ_{max}^{EtOH} nm (ε) 247.2 (2.29×10^4), 276.0 (1.29×10^4), 295.0 (8.04×10^3), 402.4 (7.34×10^3), 464.0 (6.73×10^3); IR ν_{max}^{film} cm^{-1} 3402, 2980, 2147, 1738, 1734, 1620, 1487, 1457, 1374, 1335, 1230, 1076, 1039; ^1H NMR (see Table 1); ^{13}C NMR ($CDCl_3$) δ 20.59, 20.98, 66.16, 69.21, 72.62, 75.52, 76.17 (CN ?), 115.44, 119.89, 124.67, 128.11, 129.43, 131.77, 133.67, 135.85, 136.04, 162.15, 170.44, 181.00, 183.12; FAB-MS *m/z* (abundance) 413 ($M+H^+$, 14%), 395 (15%), 335 (7%), 307 (12%), 155 (49%), 152 (30%), 119 (100%), 103 (51%), 85 (91%); EIHR-MS (*m/z*) 412.0890 (M^+ calcd for $C_{20}H_{16}N_2O_8$: 412.0906).

Kinamycin E (**12**, 4.5 mg, 4.2%) crystallized from $CHCl_3$ - hexane as a yellow-orange powder. The mp and ^1H NMR for this product were identical to those of **12** isolated from fermentation broths.

Isotope Dilution Experiment for Kinamycin F (**13**): A cultured broth (200 ml in a 1-liter Erlenmeyer flask; glycerol 3%, asparagine 0.1%, K_2HPO_4 0.1%, $MgSO_4$ 0.04%, $FeSO_4$ 0.01%) was inoculated with 10 ml of the seed culture and incubated at 26°C, 285 rpm. At 14 hours, [2-^{14}C]acetate (3.36×10^7 dpm) in 5 ml doubly distilled H_2O was added through a sterile micropore filter. At 24 hours, **13** (15 mg in 500 μl dimethyl sulfoxide) was added and the fermentation was immediately terminated with the addition of 1 N HCl (5 ml). The broth was then sonicated (5 minutes, continuous, full power, 4°C) and the resulting suspension was extracted with EtOAc (4×100 ml). The combined extracts were dried (Na_2SO_4) and concentrated. The residue was chromatographed (Silicar CC-4, 5% MeOH - CH_2Cl_2) and fractions containing pure **13** (by TLC analysis) were pooled and concentrated (10 mg). **13** was crystallized five times in a Craig tube using EtOAc - hexane. After each crystallization, samples were removed and dried for radioactivity counting. Recrystallizations 3, 4 and 5 had specific activities of 2.684×10^6, 2.770×10^6 and 2.759×10^6 dpm/mmol, respectively. Based on the average of 2.738×10^6 dpm/mmol, a 0.33% incorporation of [2-^{14}C]acetate into **13** had been obtained.

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