

Table III.  $\Delta\delta_{\text{H}}$  Values for  $\text{H}_2$  and  $\text{H}_4$ 

H	$\delta_{\text{H}}(15)$	$\delta_{\text{H}}(9)$	stereo-chemistry <sup>a</sup>	$\Delta\delta_{\text{H}}$ (15 $\rightarrow$ 9)	H	$\delta_{\text{H}}(16)$	$\delta_{\text{H}}(17)$	stereo-chemistry <sup>a</sup>	$\Delta\delta_{\text{H}}$ (16 $\rightarrow$ 17)
$\text{H}_{2\text{eq}}$	1.76	2.69	cis	+0.93	$\text{H}_{2\text{eq}}$	2.05	3.02	cis	+0.97
$\text{H}_{2\text{ax}}$	1.38	1.54	trans	+0.16	$\text{H}_{2\text{ax}}$	1.59	1.56	trans	-0.03
$\text{H}_{4\text{eq}}$	3.45	4.44	cis	+0.99	$\text{H}_{4\text{ax}}$	4.64	4.60	trans	-0.04

<sup>a</sup> Stereochemistry of the protons at  $\text{C}_2$  and  $\text{C}_4$  relative to the heterosubstituent at  $\text{C}_3$ .

Table IV.  $\Delta\delta_{\text{C}}$  Values for  $\text{C}_2$  and  $\text{C}_4$ 

car- bon	$\delta_{\text{C}}$ (15) <sup>10</sup>	$\delta_{\text{C}}$ (9)	$\Delta\delta_{\text{C}}$ (15 $\rightarrow$ 9)	$\delta_{\text{C}}$ (18) <sup>12</sup>	$\delta_{\text{C}}$ (17) <sup>12</sup>	$\Delta\delta_{\text{C}}$ (18 $\rightarrow$ 17)
$\text{C}_2$	39.7	35.8	-3.9	43.3	39.4	-3.9
$\text{C}_4$	57.6	53.9	-3.7	77.8	77.4	-0.4

$[\theta]_{234} -326$ ,  $[\theta]_{280} +340$  ( $\text{CF}_3\text{CH}_2\text{OH}$ );  $\nu_{\text{max}}$  3430, 2980, 2950, 2940, 2900, 2840, 1735, 1550, 1508, 1230, 1123, 1055  $\text{cm}^{-1}$ ; and the  $\beta$  anomer (9), mp 180.5-181.5  $^{\circ}\text{C}$ ,  $[\alpha]_{\text{D}} +34.1^{\circ}$  ( $\text{CH}_3\text{OH}$ );  $\lambda_{\text{max}}$  ( $\text{CF}_3\text{CH}_2\text{OH}$ ) 199 nm ( $\epsilon$  4909);  $[\theta]_{234} -962$ ,  $[\theta]_{282} +2308$  ( $\text{CF}_3\text{CH}_2\text{OH}$ );  $\nu_{\text{max}}$  3440, 3000, 2955, 2900, 2860, 1730, 1550, 1515, 1315, 1235, 1065  $\text{cm}^{-1}$ . The  $^1\text{H}$  NMR (Table I) and  $^{13}\text{C}$  NMR data (Table II) were in agreement with the proposed 2,3,4,6-tetra-deoxy-4-(methoxycarbonylamino)-3-C-methyl-3-nitro-D-xylo-hexopyranosyl structures 8 and 9 ( $^4\text{C}_1$  conformation). The chemical shift of the 3-methyl ( $\delta_{\text{C}}$  26.4, 25.4) in 8 and 9 was in agreement with published values<sup>9</sup> for an equatorial C-methyl nitro sugar. Independent proof for the stereochemistry at  $\text{C}_3$  was obtained as follows. Methyl  $\beta$ -L-mycaroside (14) was converted by standard techniques into the 4-methoxycarbonylamino derivative (15). Comparison of the  $^1\text{H}$  NMR data for 15<sup>10</sup> with that of 9 (Table III) revealed marked deshielding of the vicinal cis protons  $\text{H}_{2\text{e}}$  and  $\text{H}_{4\text{e}}$  in going from the 3-axial hydroxy compound 15 to the 3-axial nitro compound 9, while little effect was observed on the vicinal trans proton  $\text{H}_{2\text{a}}$ . This was in excellent agreement with the results obtained in going from 16<sup>11</sup> to 17<sup>11</sup> (Table III). Comparison of the  $^{13}\text{C}$  NMR data for 15<sup>10</sup> with those of 9 (Table IV) revealed pronounced shielding of  $\text{C}_2$  and  $\text{C}_4$ , both of which bear vicinal cis protons to the axial nitro group.<sup>12</sup> These data were in excellent agreement with the observed shielding at  $\text{C}_2$ , which bears a vicinal cis proton, and the complete absence of any marked shielding at  $\text{C}_4$ , which does not bear a vicinal cis proton to the 3-O-acetate, in going from 18<sup>12</sup> to 17<sup>12</sup> (Table IV). Thus kijanose has the xylo configuration.

The EI mass spectra of 8 and 9 revealed no molecular ions, but ions at  $m/e$  184, 172, 156, 140, and 128 were diagnostic for structures 8 and 9. The CIMS gave  $\text{MH}^+$  ions for 8 and 9 at  $m/e$  263. Chemical proof for the presence of the nitro group was obtained by reduction of 8 and 9 by using Raney nickel to give 10 and 11 ( $\text{M}^+$ ,  $m/e$  232),<sup>10</sup> which on acetylation afforded 12 and 13.<sup>10</sup>

The application of Hudson's Rules of Isorotation to 8 and 9,  $[\text{M}]_{\text{D}} - [\text{M}]_{\text{D}} = 340.6^{\circ} - 89.3^{\circ} = 251.3^{\circ}$ , and 10 and 11,  $[\text{M}]_{\text{D}} - [\text{M}]_{\text{D}} = 281.7^{\circ} - (-11.4^{\circ}) = 292.1^{\circ}$ , indicated a D-configuration. Recently L-rubranitrose (19)<sup>13</sup> was claimed to have the L-configuration by comparison of the CD of 19 with that of L-evernitrose.<sup>14</sup> The published rotation of 19 did not agree with an L-sugar.<sup>13</sup> Comparison of the CD data for 9 with that reported for 1-O-acetyl- $\beta$ -L-rubranitrose (19) ( $\text{R} = \text{Ac}$ )<sup>13</sup> clearly indicates that both compounds have the same absolute stereochemistry. The published rotation for 19<sup>13</sup> is also in agreement with a D-config-

uration. We therefore propose that rubranitrose is a D-sugar and that the correct structure is 20. D-Kijanose represents the third naturally occurring nitro sugar to be discovered from an antibiotic.<sup>15</sup> The structure of the tetrasaccharide moiety and the location of the sugars on the aglycon in kijanimicin will be described in the following paper.

(15) Recently a nitro sugar having the same composition as D-kijanose has been isolated from the tetrocarcins,<sup>7</sup> but no structure has yet been published.

## Kijanimicin. 2.<sup>1</sup> Structure and Absolute Stereochemistry of Kijanimicin

Alan K. Mallams,\* Mohindar S. Puar, and  
Randall R. Rossman

Research Division, Schering-Plough Corporation  
Bloomfield, New Jersey 07006

Andrew T. McPhail\*

Paul M. Gross Chemical Laboratory, Duke University  
Durham, North Carolina 27706

Ronald D. Macfarlane

Department of Chemistry, Texas A&M University  
College Station, Texas 77843

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Kijanimicin<sup>1</sup> has been shown to have the novel tetrionic acid structure 1. Kijanimicin (1) was purified by preparative HPLC on silica gel using dichloromethane-methanol-triethylamine (98:1:1) as the eluant, affording a colorless amorphous solid, mp 174.5  $^{\circ}\text{C}$  dec;  $[\alpha]_{\text{D}} -124.2^{\circ}$  ( $\text{CH}_3\text{OH}$ );  $\text{pK}_{\text{a}}$  5.0,  $\lambda_{\text{max}}$  ( $\text{CF}_3\text{C}-\text{H}_2\text{OH}$ ) 200 nm ( $\epsilon$  42832), 241 (8946), 264 (sh) (9697), 274 (9446);  $\lambda_{\text{max}}$  ( $\text{CH}_3\text{OH} + 0.1 \text{ N HCl}$ ) 205 nm ( $\epsilon$  38313), 258 (9881);  $\lambda_{\text{max}}$  ( $\text{CH}_3\text{OH} + 0.1 \text{ N NaOH}$ ) 236 nm ( $\epsilon$  14677), 266 (sh) (12002), 276 nm (12002);  $\nu_{\text{max}}$  ( $\text{CHCl}_3$ ) 3625, 3550, 3480, 3440, 2980, 2940, 2910, 1755, 1730, 1605, 1545, 1510, 1230, 1130, and 1058  $\text{cm}^{-1}$ . The above data suggested the presence of a tetrionic acid moiety and hydroxyl, carbonyl, lactone, carbamate, nitro, and ether functions in the molecule. The  $^1\text{H}$  NMR spectrum of 1 revealed methyl groups at  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ ) (220 MHz) 0.65 (3 H, d,  $J = 5 \text{ Hz}$ , 6- $\text{CH}_3$ ), 1.07 (3 H, d,  $J = 7 \text{ Hz}$ , 8- $\text{CH}_3$ ), 1.18 (3 H, d,  $J = 6 \text{ Hz}$ , 6 $^{\text{E}}$ - $\text{CH}_3$ ), 1.20-1.40 (envelope of  $\text{CH}_3$  signals), 1.60 (3 H, s, 3 $^{\text{E}}$ - $\text{CH}_3$ ), 1.64 (3 H, s, 4- $\text{CH}_3$ ), 3.45 (3 H, s, 4 $^{\text{D}}$ - $\text{OCH}_3$ ) and 3.76 (3 H, s, 4 $^{\text{E}}$ - $\text{NHCOOCH}_3$ ). The  $^{13}\text{C}$  NMR spectrum of 1 (Table I) revealed 67 carbon atoms of which five were anomeric carbons, indicating that 1 contains five sugars.<sup>1</sup> Although a satisfactory analysis (C, H, N) for 1 for  $\text{C}_{67}\text{H}_{100}\text{N}_2\text{O}_{24}$  was obtained, it could not be used to unambiguously establish the molecular composition. An EIMS of 1 gave the highest mass fragment ion at  $m/e$  552 (2.1%). Kijanimicin (1) was therefore converted into 26-O-methylkijanimicin (2) by treatment with diazomethane. The 26-O-methyl group occurred as a singlet at  $\delta_{\text{H}}$  ( $\text{CDCl}_3$ ) (600 MHz) 4.12.<sup>3</sup> The EIMS, CIMS, and FDMS

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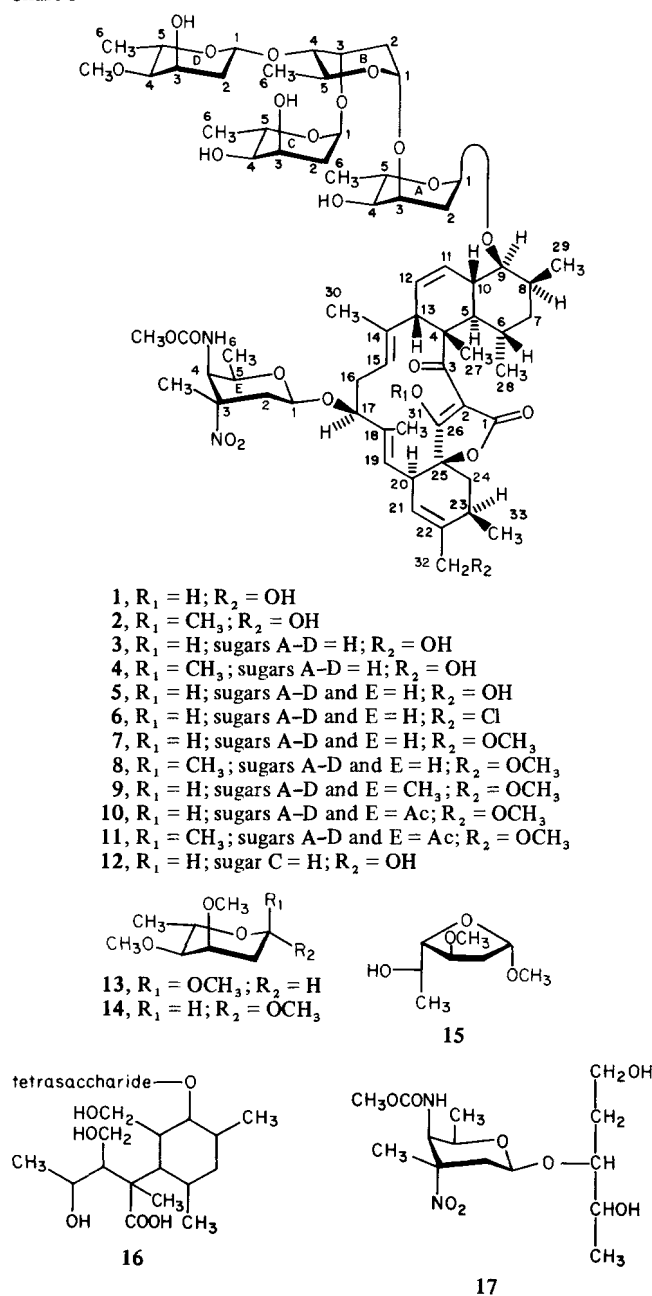
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(3) Full details will be published in *J. Chem. Soc., Perkin Trans. 1*.

Chart I



on **2** all failed to give the molecular ion, and we therefore turned to  $^{252}\text{Cf}$ -plasma desorption mass spectrometry ( $^{252}\text{Cf}$ -PDMS)<sup>2</sup> which gave a molecular weight for **2** of  $1331.1 \pm 0.4$  which was in excellent agreement with a molecular composition of  $\text{C}_{68}\text{H}_{102}\text{N}_2\text{O}_{24}$  ( $M_r$  1330.68). The molecular weight of kijanimicin (**1**) was therefore unambiguously established, suggesting a molecular formula  $\text{C}_{67}\text{H}_{100}\text{N}_2\text{O}_{24}$ .

Mild acidic hydrolysis of **1** gave *O*- $\beta$ -D-kijanosyl-(1 $\rightarrow$ 17)-kijanolid (3),<sup>1</sup>  $[\alpha]_D -37.6^\circ$  ( $\text{CH}_3\text{OH}$ );  $pK_a$  4.8;  $\lambda_{\text{max}}$  ( $\text{CH}_3\text{OH}$ ) 204 nm ( $\epsilon$  27 738), 240 (10 366), 266 (8244), 276 (7625);  $\nu_{\text{max}}$  3680, 3615, 3440, 2940, 1755, 1730, 1605, 1545, 1510, 1235, and 1060  $\text{cm}^{-1}$ . The highest mass ion in the EIMS of **3** was at  $m/e$  552. The  $^{13}\text{C}$  NMR data for **3** are given in Table I, and it is evident from the  $\Delta\delta_C$  values in going from **3** to **1** that the four digitoxose units are part of a tetrasaccharide moiety that is glycosidically linked to a secondary hydroxyl group at C<sub>9</sub> of the aglycone. Treatment of **3** with diazomethane gave the methyl ether **4**. The  $^{252}\text{Cf}$ -PDMS of **4** indicated a  $M_r$  796.8  $\pm$  0.2 which was in agreement with a composition of  $\text{C}_{43}\text{H}_{60}\text{N}_2\text{O}_{12}$  ( $M_r$  797.0). The differences in composition between **4** and **2**, and between their  $^{13}\text{C}$  NMR spectra, indicated that the tetrasaccharide was comprised of one 2,6-dideoxy-4-*O*-methyl-L-ribo-hexopyranose unit

and three 2,6-dideoxy-L-ribo-hexopyranose units. The 600 MHz  $^1\text{H}$  NMR spectrum<sup>4</sup> of **4** in  $\text{CDCl}_3$  revealed a doublet of doublets at  $\delta_H$  4.46 ( $J_{1^{\text{Eax}},2^{\text{Eax}}} = 9.5$  Hz,  $J_{1^{\text{Eax}},2^{\text{Eq}}} = 1.9$  Hz) due to  $H_{1^{\text{Eax}}}$ , indicating that the D-kijanosyl unit has a  $\beta$ -glycosidic linkage to the aglycone. The 600 MHz  $^1\text{H}$  NMR spectrum<sup>4</sup> of **2** in  $\text{CD}_3\text{CN}$  revealed three additional doublet of doublet signals at  $\delta_H$  4.72 ( $J_{1^{\text{Eq}},2^{\text{ax}}} = 4.5$  Hz,  $J_{1^{\text{Eq}},2^{\text{Eq}}} < 0.5$  Hz), 5.06 ( $J_{1^{\text{Eq}},2^{\text{ax}}} = 4.0$  Hz,  $J_{1^{\text{Eq}},2^{\text{Eq}}} < 0.5$  Hz) and 5.13 ( $J_{1^{\text{Eq}},2^{\text{ax}}} = 3.3$  Hz,  $J_{1^{\text{Eq}},2^{\text{Eq}}} < 0.5$  Hz) arising from the three equatorial anomeric protons of the L-digitoxose units, indicating that they were all present as  $\alpha$ -glycosides. A doublet of occurred at  $\delta_H$  4.88 ( $J_{1^{\text{Dax}},2^{\text{Dax}}} = 10.0$  Hz,  $J_{1^{\text{Dax}},2^{\text{Eq}}} = 1.9$  Hz) due to  $H_{1^{\text{Dax}}}$  indicated that the 2,6-dideoxy-4-*O*-methyl-L-ribo-hexopyranose was present as a  $\beta$ -glycoside.

Acidic hydrolysis<sup>1</sup> of **3** gave methyl  $\alpha$ - and  $\beta$ -D-kijanoside,<sup>1</sup> traces of kijanolide **5**, and also 32-chloro-32-deoxykijanolid (**6**).<sup>3</sup> Extensive decomposition of the aglycone occurred. Kijanimicin (**1**) was therefore exhaustively permethylated by using sodium hydride and methyl iodide in DMF, and the resulting per-N,O-methylated product was subjected to acidic hydrolysis by using 0.5 N hydrogen chloride in methanol at 25  $^\circ\text{C}$  for 20 h. The latter afforded **13**,<sup>3</sup> the  $\beta$  anomer **14**,<sup>3</sup> methyl 2,6-dideoxy-4-*O*-methyl- $\beta$ -L-ribo-hexopyranoside,<sup>1</sup> the  $\alpha$  anomer,<sup>1</sup> methyl 2,6-dideoxy- $\alpha$ -L-ribo-hexopyranoside,<sup>1</sup> the  $\beta$  anomer,<sup>1</sup> methyl 2,6-dideoxy- $\alpha$ -L-ribo-hexofuranoside,<sup>1</sup> and the  $\beta$  anomer,<sup>1</sup> which indicated that the tetrasaccharide moiety has a branch point. The acidic hydrolysis also produced *O*-(4<sup>E</sup>-*N*-methyl- $\beta$ -D-kijanosyl)-(1 $\rightarrow$ 17)-32-*O*-methylkijanolid<sup>3</sup> and some 32-*O*-methylkijanolid (**7**). By increasing the reaction time to 43 h the yield of **7** was greatly improved,  $[\alpha]_D -11.6^\circ$  ( $\text{CH}_3\text{OH}$ );  $\lambda_{\text{max}}$  ( $\text{CF}_3\text{CH}_2\text{OH}$ ) 262 nm ( $\epsilon$  7090), 262 (7976);  $\nu_{\text{max}}$  3610, 2960, 2920, 2880, 1748, 1058  $\text{cm}^{-1}$ ;  $m/e$  566 ( $M^+$ ). The  $^{13}\text{C}$  NMR data for **7** (Table I) indicated that no rearrangements had occurred during the acidic hydrolysis. The isolation of **7** enabled us to prepare 26,32-di-*O*-methylkijanolid **8**,<sup>3</sup> 9,17,32-tri-*O*-methylkijanolid (**9**),<sup>3</sup> 9,17-di-*O*-acetyl-32-*O*-methylkijanolid (**10**),<sup>3</sup> and 9,17-di-*O*-acetyl-26,32-di-*O*-methylkijanolid (**11**).<sup>3</sup> Analysis of the  $^{13}\text{C}$  NMR data<sup>3</sup> and the fully decoupled 600-MHz  $^1\text{H}$  NMR data<sup>3,4</sup> for **7**–**11** enabled us to assign all but two of the protons in kijanolide and revealed the structures of all of the proton containing fragments of the aglycone.

The diacetate **10** crystallized from chloroform-methanol.<sup>5</sup> The crystals belong to the orthorhombic system, space group  $P2_12_12_1$ , with  $a = 11.897$  (5),  $b = 28.423$  (11),  $c = 10.282$  (4)  $\text{\AA}$ ;  $U = 3447$   $\text{\AA}^3$ ;  $Z = 4$ ;  $d_{\text{calcd}} = 1.243$   $\text{g cm}^{-3}$ . The structure was solved by use of the MULTAN 76<sup>6</sup> program package incorporating the magic integer approach.<sup>7</sup> Approximate positions for 34 nonhydrogen atoms were obtained from an *E* map evaluated by use of the 400 largest  $|E|$  values and a set of phase angles which yielded one of the highest combined figures of merit. The remaining 13 nonhydrogen atoms were then located in an  $F_o$  Fourier synthesis phased by this 34-atom fragment. Full-matrix least-squares refinement of positional and thermal parameters for all nonhydrogen atoms<sup>8,9</sup> converged at  $R = 0.067$ <sup>10</sup> over 1871 statistically significant [ $I > 2.0\sigma(I)$ ] reflections measured<sup>11</sup> on an Enraf-Nonius CAD-3 automated diffractometer (Ni-filtered  $\text{Cu K}\alpha$  radiation,  $= 1.5418$   $\text{\AA}$   $\theta$ -2 $\theta$  scans). A view of the structure and solid-state conformation

(4) The 600-MHz  $^1\text{H}$  NMR spectra were recorded at Carnegie-Mellon University, Pittsburgh, PA, and NIH Grant RR00292 is gratefully acknowledged.

(5) A variety of salts, metal complexes, and iodobenzoyl derivatives of **1** and **3** had been prepared, and although some were crystalline, the crystals were not acceptable for X-ray analysis.

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(7) L. Lessinger and T. N. Margulis, *Acta Crystallogr., Sect. B* **B34**, 578 (1978).

(8) See paragraph at end of paper regarding supplementary material.

(9) Hydrogen atoms, save those on methyl carbons C(30), C(31), C(38), C(42), and C(45), were included at their calculated positions, but were not refined.

(10)  $R = \sum |F_o| - |F_c| / \sum |F_o|$ .

(11) R. W. Miller and A. T. McPhail, *J. Chem. Soc., Perkin Trans. 2*, 1527 (1979).

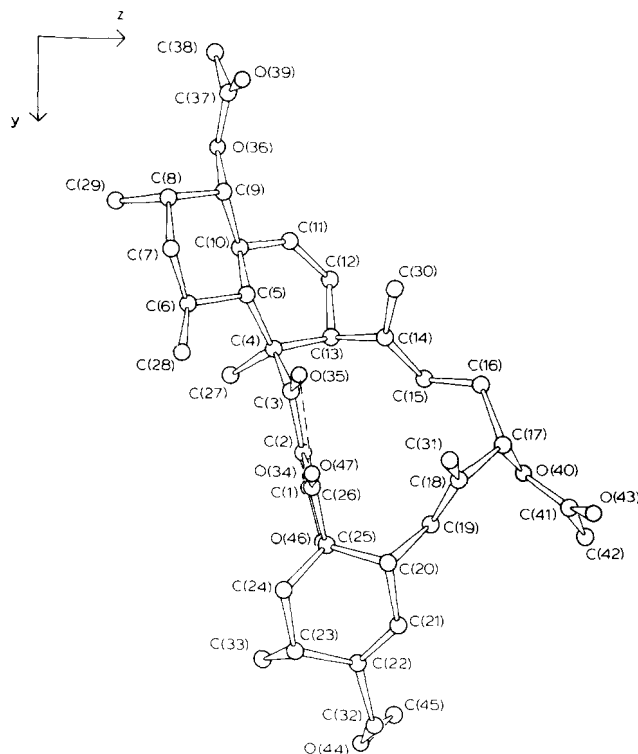


Figure 1. Structure and solid-state conformation of 10.

is shown in Figure 1. The basic ring system in 10 differs from that found recently in tetronolide<sup>12</sup> only in the location of the double bond in one of the cyclohexene rings. The 600-MHz <sup>1</sup>H NMR data<sup>3,4</sup> indicate that a similar conformation exists in solution.

It remained now to complete the structure of the tetrasaccharide moiety and to determine the absolute stereochemistry of kijanolide. Controlled sodium metaperiodate oxidation of 1 afforded the monodesdigitoxosyl derivative 12 after treatment with Dowex 50W-X8 (H<sup>+</sup>) resin. The <sup>13</sup>C NMR data for 12 are given in Table I. Exhaustive permethylation of 12 followed by acidic hydrolysis using 0.5 N hydrogen chloride in methanol afforded 13,<sup>3</sup> the β anomer (14),<sup>3</sup> methyl 2,6-dideoxy-4-O-methyl-β-L-ribo-hexopyranoside,<sup>1</sup> the α anomer,<sup>1</sup> and 15.<sup>3</sup> This hydrolytic data, coupled with that obtained when per-N,O-methylkijaninimicin was subjected to acidic hydrolysis, could only be accommodated with two possible branched chain tetrasaccharide structures. One of these did not fit the <sup>13</sup>C NMR data (Table I) and was therefore excluded, leaving us with only one possible structure. The carbons of the terminal sugar D were readily assignable. The other terminal sugar C showed the expected chemical shifts for C<sub>2</sub>–C<sub>6</sub>. The anomeric carbon, however, was strongly shielded (δ<sub>C</sub> 92.2). It was also apparent in going from 1 to 12 that almost no change was observed in δ<sub>C</sub> for C<sub>3</sub> of the sugar B, in spite of the fact that the sugar C had been removed. The marked shielding of C<sub>2</sub> in sugar B in 1 was also removed when sugar C was removed to give 12. These results are in excellent agreement with what one would expect from previous <sup>13</sup>C NMR studies carried out on 1-, 3-, and 5-*epi*-aminoglycoside antibiotics.<sup>13,14</sup> It is evident in 1 that the deshielding at C<sub>3</sub> of the sugar B resulting from the glycosidic linkage of sugar C is being cancelled out by the shielding produced by sugar C due to its conformation about the glycosidic linkage.<sup>13,14</sup> It is also apparent that similar shieldings are present at C<sub>1</sub> in the sugar B as well as at C<sub>3</sub> and C<sub>2</sub> in the sugar A in 1 and 12. From

Table I. <sup>13</sup>C NMR Data<sup>d</sup>

carbon	1	3	7	12
C <sub>1</sub>	167.1 (s)	167.1 (s)	167.3 (s)	167.2
C <sub>2</sub>	101.9 (s)	102.0 (s)	102.2 (s)	101.8
C <sub>3</sub>	206.2 (s)	206.5 (s)	206.5 (s)	206.3
C <sub>4</sub>	51.0 (s)	51.1 (s)	51.1 (s)	51.0
C <sub>5</sub>	31.3 (d) <sup>a,e</sup>	31.2 (d) <sup>a,e</sup>	31.2 (d) <sup>a,e</sup>	31.3 <sup>a,e</sup>
C <sub>6</sub>	27.9 (d) <sup>a</sup>	28.0 (d) <sup>a</sup>	27.7 (d) <sup>a</sup>	27.9 <sup>a</sup>
C <sub>7</sub>	41.6 (t)	41.9 (t)	41.8 (t)	41.8
C <sub>8</sub>	38.5 (d)	39.3 (d)	39.2 (d)	38.5
C <sub>9</sub>	84.5 (d)	76.1 (d)	76.1 (d)	83.9
C <sub>10</sub>	34.8 (d)	34.8 (d)	34.8 (d)	34.3
C <sub>11</sub>	125.8 (d)	125.8 (d)	125.6 (d)	126.1
C <sub>12</sub>	126.7 (d)	126.5 (d)	126.6 (d)	126.3
C <sub>13</sub>	53.2 (d)	53.3 (d)	53.3 (d)	53.3
C <sub>14</sub>	135.7 (s)	135.9 (s)	135.8 (s)	135.8
C <sub>15</sub>	123.6 (d)	123.4 (d)	123.1 (d)	123.5
C <sub>16</sub>	31.1 (t)	31.2 (t)	32.1 (t)	31.1
C <sub>17</sub>	78.4 (d)	78.6 (d)	72.9 (d)	78.1
C <sub>18</sub>	137.1 (s)	137.0 (s)	141.3 (s)	137.1
C <sub>19</sub>	121.5 (d)	121.5 (d)	123.9 (d)	121.6
C <sub>20</sub>	43.1 (d) <sup>e</sup>	42.9 (d) <sup>e</sup>	42.9 (d) <sup>e</sup>	43.2 <sup>e</sup>
C <sub>21</sub>	119.3 (d)	119.4 (d)	117.6 (d)	119.3
C <sub>22</sub>	141.5 (s)	141.5 (s)	138.6 (s)	141.5
C <sub>23</sub>	40.2 (d)	40.3 (d)	40.1 (d)	40.3
C <sub>24</sub>	35.5 (t)	35.4 (t)	35.3 (t)	35.6
C <sub>25</sub>	83.3 (s)	83.3 (s)	83.3 (s)	83.4
C <sub>26</sub>	201.5 (s)	201.5 (s)	201.0 (s)	201.7
C <sub>27</sub>	20.2 (q)	20.2 (q)	20.0 (q)	20.2
C <sub>28</sub>	22.2 (q)	22.3 (q)	22.3 (q)	22.2
C <sub>29</sub>	14.0 (q)	13.0 (q)	13.1 (q)	14.2
C <sub>30</sub>	15.1 (q)	15.2 (q)	14.8 (q)	15.1
C <sub>31</sub>	13.7 (q)	13.7 (q)	13.7 (q)	13.8
C <sub>32</sub>	64.4 (t)	64.9 (t)	74.7 (t)	64.0
C <sub>33</sub>	15.1 (q)	15.0 (q)	15.2 (q)	15.1
32-OCH <sub>3</sub>			58.0 (q)	
C <sub>1</sub> A	98.2 (d)			98.1
C <sub>2</sub> A	29.9 (t) <sup>b</sup>			30.2
C <sub>3</sub> A	66.8 (d) <sup>c</sup>			66.0 <sup>b</sup>
C <sub>4</sub> A	71.8 (d)			71.5
C <sub>5</sub> A	65.1 (d) <sup>c</sup>			65.0 <sup>b</sup>
C <sub>6</sub> A	17.9 (q)			17.9 <sup>c</sup>
C <sub>1</sub> B	90.8 (d)			91.1
C <sub>2</sub> B	29.7 (t) <sup>b</sup>			34.4
C <sub>3</sub> B	62.6 (d)			62.1
C <sub>4</sub> B	79.6 (d)			77.3
C <sub>5</sub> B	67.1 (d) <sup>c</sup>			65.5 <sup>b</sup>
C <sub>6</sub> B	17.9 (q)			17.8 <sup>c</sup>
C <sub>1</sub> C	92.2 (d)			
C <sub>2</sub> C	34.4 (t)			
C <sub>3</sub> C	67.5 (d)			
C <sub>4</sub> C	72.4 (d)			
C <sub>5</sub> C	64.9 (d)			
C <sub>6</sub> C	17.9 (q)			
C <sub>1</sub> D	99.8 (d)			98.4
C <sub>2</sub> D	36.8 (t)			36.9
C <sub>3</sub> D	63.8 (d)			64.0
C <sub>4</sub> D	82.6 (d)			82.3
C <sub>5</sub> D	68.1 (d)			68.4
C <sub>6</sub> D	18.4 (q)			18.3
4D-OCH <sub>3</sub>	57.3 (q)			57.3
C <sub>1</sub> E	97.1 (d)	97.1 (d)		96.9
C <sub>2</sub> E	35.7 (t)	35.8 (t)		35.6
C <sub>3</sub> E	91.0 (s)	91.2 (s)		91.1
C <sub>4</sub> E	53.8 (d)	53.8 (d)		53.8
C <sub>5</sub> E	69.1 (d)	69.2 (d)		69.1
C <sub>6</sub> E	17.0 (q)	17.0 (q)		17.0
3E-CH <sub>3</sub>	25.3 (q)	25.3 (q)		25.3
4E-NHCOOCH <sub>3</sub>	157.4 (s)	157.4 (s)		157.7
4E-NHCOOCH <sub>3</sub>	52.7 (q)	52.6 (q)		52.7

<sup>a</sup> May be interchanged in any vertical column. <sup>b</sup> Same as footnote a. <sup>c</sup> Same as footnote a. <sup>d</sup> Spectra recorded in CDCl<sub>3</sub>. SFOR multiplicities are indicated in parentheses. <sup>e</sup> Same as footnote a.

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the data in Table I it is evident that the tetrasaccharide moiety is glycosidically linked to kijanolide at C<sub>9</sub>, while the D-kijanose moiety is located at C<sub>17</sub>. Chemical proof for the location of the

glycosidic linkages to the aglycon was obtained by subjecting 1 to ozonolysis followed by reduction with sodium borohydride to give 16 and 17.<sup>3</sup>

Finally as a consequence of knowing the absolute stereochemistry of sugar A (L) and the nature of the glycosidic linkage to the aglycone ( $\alpha$ ),<sup>15</sup> as well as the relative stereochemical environment at C<sub>8</sub>, C<sub>9</sub> and C<sub>10</sub>,<sup>14</sup> we were able to deduce the absolute stereochemistry of kijanimicin (1) from the observed glycosylation shifts in the <sup>13</sup>C NMR spectra in going from 3 to 1. Thus the observed deshielding at C<sub>9</sub> (+8.4) coupled with the shielding at C<sub>8</sub> (-0.7) and absence of any shielding at C<sub>10</sub> were in excellent agreement with the expected glycosylation shifts for an  $\alpha$ -L-deoxysugar glycosidically attached to an aglycone having the stereochemical features present at C<sub>8</sub>, C<sub>9</sub>, and C<sub>10</sub> in 1.<sup>14</sup> It therefore follows that C<sub>9</sub> has the S configuration. Had the configuration at C<sub>9</sub> been R, then marked shielding would have been predicted at C<sub>8</sub>, C<sub>9</sub>, and at C<sub>1</sub> for sugar A<sup>13,14</sup> which was not the case.

It is therefore concluded that the total structure and absolute stereochemistry of kijanimicin may be represented by 1. Kijanimicin (1) has antitumor activity and is active against *P. acnes* and is a member of a new class of tetroneic acid containing antibiotics of which the tetrocarcins<sup>12,16-18</sup> and antlermicins<sup>19,20</sup> are the only other known members. The latter differ in the structure of the aglycon as well as in the structures of some of the glycosidic components, and full structures for these antibiotics have not yet been published.

**Supplementary Material Available:** Table of positional parameters for the nonhydrogen atoms in 10 (2 pages). Ordering information is given on any masthead page.

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## Electron-Impact-Induced Fragmentation of Quaternary Ammonium Cations

Mamoru Ohashi,<sup>†</sup> Robert P. Barron,\* and Walter R. Benson

Food and Drug Administration, Division of Drug Chemistry  
 Washington, D.C. 20204

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Recently Stoll and Röllgen as well as Cotter and Yergey have shown that tetraalkylammonium salts are evaporated and dissociated to yield the corresponding quaternary ammonium cation by simply heating the sample,<sup>1</sup> whereas Lee et al. proposed that electron impact is necessary to cause dissociation of gaseous quaternary ammonium salts to give the corresponding ammonium cation.<sup>2</sup> These reports prompted us to present our recent results on in-beam EI (desorption from extended probes) mass spectrometry<sup>3</sup> of these compounds. The series tetraethyl-, tetrapropyl-,

Table I. MIKES and IKES of Tetraalkylammonium Cation

R	transition <sup>a</sup>	fragment ion ( <i>m/z</i> )	<i>E/E</i> <sub>0</sub> <sup>b</sup>	<i>V/V</i> <sub>0</sub> <sup>c</sup>
Et	1	102 (a)	0.781	1.275
	2	101 (b)	0.776	1.288
	3	100 (c)	0.766	1.301
	4	86 (d)	0.659	<i>e</i>
Pr	1	144 (a)	0.774	1.290
	2	143 (b)	<i>d</i>	1.300
	3	142 (c)	0.762	1.309
	4	114 (d)	0.611	1.633
	5	43 (f)	0.229	<i>e</i>
Bu	1	186 (a)	0.768	1.302
	2	185 (b)	<i>d</i>	1.306
	3	184 (c)	0.761	1.314
	4	142 (d)	0.588	1.703
	5	57 (f)	0.233	<i>e</i>
Pn	1	228 (a)	0.765	1.307
	2	227 (b)	<i>d</i>	1.311
	3	226 (c)	0.757	1.317
	4	170 (d)	0.570	<i>e</i>

<sup>a</sup> See Figure 1. <sup>b</sup> MIKES of tetraalkylammonium cation.

<sup>c</sup> IKES obtained by acceleration voltage scanning (*V*<sub>0</sub> = 1 kV).

<sup>d</sup> Overlapping peaks. <sup>e</sup> No data obtained.

tetrabutyl-, and tetrapentylammonium bromide was selected as typical and studied by the techniques of in-beam EI mass spectrometry and mass-analyzed ion kinetic energy spectrometry (MIKES) or the direct analysis of daughter ions (DADI).<sup>4</sup> Our results are summarized as follows:

(i) When the compound was loaded on a metal tip and inserted into the hot ion source (ca. 280 °C) and heated to 350 °C by the sample heater, or the compound was deposited on an unactivated FD wire of 10-μm diameter tungsten and quickly heated, only the corresponding tetraalkylammonium cation was recorded. Without an electron beam, no fragment ions were detected. This observation is consistent with that of Röllgen or Cotter and with the results of Lee et al.

(ii) Then the electron beam (filament) was turned on and the in-beam EI spectra were recorded. A significant increase in the abundance of the quaternary ammonium cation was observed, which appears to be consistent with the observation of Lee et al. Two possible explanations are proposed for this increase: (a) thermal dissociation of gaseous neutral molecules such as (R<sub>4</sub>N<sup>+</sup>X<sup>-</sup>)<sub>n</sub> to the cation R<sub>4</sub>N<sup>+</sup> on the hot surface<sup>5</sup> or (b) dissociation induced by electron bombardment.<sup>2</sup> Both processes are conceivable, but from observations at different positions of the tip relative to the electron beam, process b appears to be the major contributing factor. The in-beam EI spectrum of tetrabutylammonium bromide is shown in Figure 1 as a typical example. In addition to these peaks, weak but remarkable cluster ions were observed at *m/z* 563, 565 [(R<sub>4</sub>N<sup>+</sup>)<sub>2</sub>Br]<sup>+</sup> and *m/z* 320, 322 (R<sub>4</sub>NBr - H)<sup>+</sup>. The major spectral features are very similar to those obtained by field desorption,<sup>6</sup> <sup>252</sup>Cf plasma desorption,<sup>7</sup> laser desorption,<sup>7</sup> and flash desorption.<sup>2</sup> If quaternary ammonium salts undergo thermal decomposition prior to electron impact<sup>8</sup> or chemical ionization<sup>9</sup> in the ion source as hitherto believed, fragment ions observed in this study are considered to be produced by electron-impact ionization of thermally degraded products such as tributylamine. However, the in-beam EI spectra exhibit very weak peaks corresponding to the molecular ion of butyl bromide,

(4) A Varian 311A double focusing mass spectrometer or a Finnigan 4023T GC/MS/DS quadrupole instrument was used.

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<sup>†</sup> Visiting scientist on leave from The University of Electro-Communications, Chofu, Tokyo 182, Japan.

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