Kijanimicin. Part 3.[1] Structure and Absolute Stereochemistry of Kijanimicin

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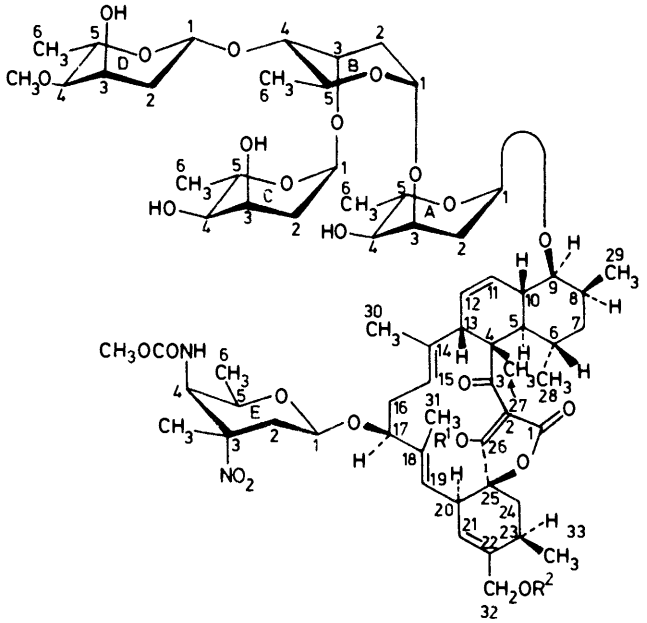
Kijanimicin, a novel antibiotic from *Actinomadura kijaniata* nov. sp. SCC1256 (ATCC 31588), has been shown by chemical degradation, spectroscopic studies, and *X*-ray crystallographic studies to have a unique tetronic acid structure. The molecule contains a branched chain tetrasaccharide moiety consisting of three units of 2,6-dideoxy-α-L-*ribo*-hexopyranose and one unit of 2,6-dideoxy-4-*O*-methyl-β-L-*ribo*-hexopyranose. The molecule also contains a novel nitrosugar, namely 2,3,4,6-tetradeoxy-4-methoxycarbonylamino-3-*C*-methyl-3-nitro-β-D-*xylo*-hexopyranose (D-kijanose), which is the third nitrosugar to be isolated from an antibiotic. The structure of L-rubranitrose is revised to D-rubranitrose. Evidence for the total structure, the absolute stereochemistry, and the solution conformation of kijanimicin is presented.

Kijanimicin,† the major component of a complex of antibiotics produced by *Actinomadura kijaniata* nov. sp. SCC1256 (ATCC 31588),[2,3] has been shown by chemical degradation, spectroscopic studies, and *X*-ray crystallographic studies to have a novel tetronic acid structure (1).[1,4] Kijanimicin (1) was separated from the crude antibiotic complex [3] by preparative h.p.l.c. on silica gel using dichloromethane–methanol–triethylamine (98 : 1 : 1) as the eluant. The resulting material was rechromatographed using preparative h.p.l.c. on C_{18} reversed phase silica gel using acetonitrile–aqueous ammonium acetate (3 : 5) (pH 6.8) as the eluant to give (1) as a colourless amorphous solid.‡ Kijanimicin was strongly laevorotatory and was weakly acidic (pK_a 5.0). The u.v. spectrum of (1) was highly complex and sensitive to acid and base suggesting the presence of an acidic enol group. The i.r. spectrum of (1) indicated the presence of hydroxy, carbonyl, lactone, carbamate, nitro, and ether functions in the molecule. The ^{1}H n.m.r. spectrum of (1) at 220 MHz revealed the presence of one *O*-methyl group (δ_H 3.45) and one methyl carbamate group (δ_H 3.76) as well as numerous methyl, anomeric, and olefinic protons. The ^{13}C n.m.r. spectrum of (1) revealed a wealth of structural information and the data are given in Table 1. The data indicate that (1) contains 67 carbon atoms. An SFOR experiment revealed the multiplicities of the various signals and in order to further clarify the methyl/methylene region of the spectrum, an inversion-recovery experiment was performed. This revealed 13 of the 14 methyl groups present in the molecule. The relaxation properties of the C-28 methyl group (δ_C 22.2) were such that it was not detected in this experiment. Of the methyl groups, two were clearly OCH_3 groups, namely δ_C 57.3 (4^D-OCH_3) and δ_C 52.7 (4^E-$NHCOOCH_3$). One of the methyl groups (δ_C 25.3)



(1) $R^1 = R^2 = H$
(2) $R^1 = CH_3, R^2 = H$
(3) $R^1 = H, R^2 = 4\text{-}IC_6H_4CO$

was also located on a carbon bearing a hetero-substituent. These experiments also revealed the presence of 11 quaternary carbons in kijanimicin (1). Of these, the four signals at δ_C 101.9, 167.1, 201.5, and 206.2 were assigned to a 1,3,3′-diketolactone fragment. One of the signals at δ_C 157.4 was consistent with that expected for a carbamate (4^E-$NHCOOCH_3$) carbonyl. Three quaternary olefinic carbons were also apparent at δ_C 141.5, 137.1, and 135.7. A quaternary carbon at δ_C 91.0 was consistent with a carbon bearing a nitro-group. A quaternary carbon bearing an oxygen atom was also present at δ_C 83.3. The signal at δ_C 51.0 was assigned to a quaternary carbon bearing only carbon substituents and from its chemical shift it was in a deshielded environment. The ^{13}C

† Isolated from a culture of a soil sample collected at Hippo Point on the Galana River, Tsavo West, Kenya, and named after the Swahili word 'kijani' for green, which is the colour of the fermentation broth. Kijanimicin is also referred to as SCH 25663.

‡ Kijanimicin showed a marked tendency to form chelates/salts with inorganic metal cations and with organic bases due to the presence of the tetronic acid moiety. All samples containing the underivatized tetronic acid moiety were therefore treated with hydrogen sulphide prior to the final chromatography to remove metal cations.

Table 1. ^{13}C N.m.r. data for kijanimicin and derivatives [δ(CDCl$_3$, SFOR)][1]

Carbon	(1)	(2)	Δδc-(1)→(2)	(3)	Δδc-(1)→(3)	(14)	(15)	Δδc-(14)→(15)	Δδc-(14)→(1)	Δδc-(15)→(2)	(16)	Δδc-(14)→(16)	(17)
C-1	167.1(s)	169.0	+1.9	167.0		167.1(s)	169.0	+1.9			167.0		168.9(s)
C-2	101.9(s)	106.9	+5.0	101.9		102.0(s)	106.8	+4.8			102.0		106.9(s)
C-3	206.2(s)	199.0	−7.2	206.2		206.5(s)	199.3	−7.2	−0.3	−0.3	206.1	−0.4	198.7(s)
C-4	51.0(s)	53.9	+2.9	51.0		51.1(s)	53.9	+2.8			51.1		53.9(s)
C-5	31.3(d) [a]	31.6 [a]	+0.3	31.3 [a]		31.2(d) [a]	31.4 [a]				31.3 [a]		31.4(d) [a]
C-6	27.9(d) [a]	28.0 [a]		28.5 [a]	+0.6	28.0(d) [a]	27.9 [a]				28.2 [a]		28.1(d) [a]
C-7	41.6(t)	41.6		41.6		41.9(t)	41.8		−0.3		41.4	−0.5	41.3(t)
C-8	38.5(d)	38.9	+0.4	38.5		39.3(d)	39.6	+0.3	−0.8	−0.7	36.9	−2.4	37.3(d)
C-9	84.5(d)	84.8	+0.3	84.6		76.1(d)	76.3		+8.4	+8.5	78.2	+2.1	78.4(d)
C-10	34.8(d)	34.9		34.9		34.8(d)	34.6			+0.3	31.6	−3.2	31.5(d)
C-11	125.8(d)	125.3	−0.5	125.8		125.8(d)	124.9	−0.9		+0.4	124.7	−1.1	124.9(d)
C-12	126.7(d)	127.5	+0.8	126.7		126.5(d)	127.3	+0.8			127.1	+0.6	127.8(d)
C-13	53.2(d)	51.0	−2.2	53.3		53.3(d)	50.8	−2.5			53.1		50.7(d)
C-14	135.7(s)	137.9	+2.2	135.7		135.9(s)	138.0	+2.1			135.7		138.0(s)
C-15	123.6(d)	122.1	−1.5	123.6		123.4(d)	121.9	−1.5			123.6		121.9(d)
C-16	31.1(t)	31.3		31.3		31.2(t)	31.4				31.3		31.5(t)
C-17	78.4(d)	78.7	+0.3	78.7	+0.3	78.6(d)	78.7				78.7		79.1(d)
C-18	137.1(s)	137.3		137.8	+0.7	137.0(s)	137.1				137.8	+0.8	138.0(s)
C-19	121.5(d)	121.8	+0.3	124.8	+3.3	121.5(d)	121.6				124.3	+2.8	124.1(d)
C-20	43.1(d)	44.8	+1.7	43.2		42.9(d)	44.4	+1.5		+0.4	43.0		44.5(d)
C-21	119.3(d)	119.8	+0.5	118.8	−0.5	119.4(d)	119.8	+0.4			118.8	−0.6	119.2(d)
C-22	141.5(s)	141.1	−0.4	136.8	−4.7	141.5(s)	141.0	−0.5			136.7	−4.8	136.2(s)
C-23	40.2(d)	40.1		40.2		40.3(d)	40.0	−0.3			40.2		40.0(d)
C-24	35.5(t)	36.2	+0.7	35.5		35.4(t)	36.1	+0.7	+0.1	+0.1	35.4		36.1(t)
C-25	83.3(s)	83.2		83.0	−0.3	83.3(s)	83.1				83.0	−0.3	83.0(s)
C-26	201.5(s)	190.2	−11.3	201.4		201.5(s)	190.1	−11.4			201.3		190.0(s)
C-27	20.2(q)	20.0		20.2		20.2(q)	20.0				20.1		19.8(q)
C-28	22.2(q)	24.5	+2.3	22.2		22.3(q)	24.6	+2.3			22.2		24.3(q)
C-29	14.0(q)	14.1		14.0		13.0(q)	13.1		+1.0	+1.0	13.8	+0.8	13.9(q)
C-30	15.1(q)	14.1	−1.0	15.1		15.2(q)	14.0	−1.2			15.1		14.1(q)
C-31	13.7(q)	14.4	+0.7	13.7		13.7(q)	14.4	+0.7			13.8		14.5(q)
C-32	64.4(t)	64.5		66.8	+2.4	64.9(t)	65.0		−0.5	−0.5	66.0	+1.1	66.1(t)
C-33	15.1(q)	14.9		15.1		15.0(q)	14.8				15.0		14.7(q)
9-COO−													
9-Ar-C-1													
9-Ar-C-2													
9-Ar-C-3													
9-Ar-C-4													
9-Ar-C-5													
9-Ar-C-6													
32-COO−				165.8									
32-Ar-C-1				129.7									
32-Ar-C-2				131.2									
32-Ar-C-3				137.8									
32-Ar-C-4				100.8									
32-Ar-C-5				137.8									
32-Ar-C-6				131.2									
9-OCOCH$_3$											170.4		170.5(s)
9-OCOCH$_3$											21.1		21.1(q)
17-OCOCH$_3$													
17-OCOCH$_3$													
32-OCOCH$_3$											170.8		170.9(s)
32-OCOCH$_3$											20.9		20.9(q)
9-OCH$_3$													
17-OCH$_3$													
26-OCH$_3$		63.7					63.7						63.8(q)
32-OCH$_3$													
C-1A	98.2(d)	98.3		98.3									
C-2A	29.9(t) [b]	30.0 [b]		30.0 [b]									
C-3A	66.8(d) [c]	67.0 [c]		66.8 [c]									
C-4A	71.8(d)	71.9		71.8									
C-5A	65.1(d) [c]	65.1 [c]		65.2 [c]									
C-6A	17.9(q)	18.0		17.9									
C-1B	90.8(d)	90.9		90.9									
C-2B	29.7(t) [b]	29.8 [b]		29.5 [b]									
C-3B	62.6(d)	62.7		62.7									
C-4B	79.6(d)	79.7		79.6									
C-5B	67.1(d) [c]	67.2 [c]		67.1 [c]									
C-6B	17.9(q)	18.0		17.9									
C-1C	92.2(d)	92.2		92.2									
C-2C	34.4(t)	34.4		34.4									
C-3C	67.5(d)	67.6		67.5									
C-4C	72.4(d)	72.6		72.5									
C-5C	64.9(d)	65.0		64.5	−0.4								
C-6C	17.9(q)	18.0		17.9									
C-1D	99.8(d)	99.8		99.8									
C-2D	36.8(t)	36.9		36.8									
C-3D	63.8(d)	63.9		63.9									
C-4D	82.6(d)	82.7		82.7									
C-5D	68.1(d)	68.1		68.1									
C-6D	18.4(q)	18.4		18.4									
4D-OCH$_3$	57.3(q)	57.3		57.3									
C-1E	97.1(d)	97.3		97.4	+0.3	97.1(d)	97.2				97.4	+0.3	97.6(d)
C-2E	35.7(t)	35.9		35.8		35.8(t)	35.7				35.8		35.8(t)
C-3E	91.0(s)	91.1		90.9		91.2(s)	91.2				90.9		90.9(s)
C-4E	53.8(d)	53.9		53.9		53.8(d)	53.7				53.8		53.8(d)
C-5E	69.1(d)	69.1		69.0		69.2(d)	69.2				69.0		68.9(d)
C-6E	17.0(q)	17.0		17.0		17.0(q)	17.0				17.0		17.0(q)
3E-CH$_3$	25.3(q)	25.4		25.2		25.3(q)	25.4				25.3		25.3(q)
4E-NHCOOCH$_3$	157.4(s)	157.4		157.3		157.4(s)	157.5				157.3		157.3(s)
4E-NHCOOCH$_3$	52.7(q)	52.7		52.7		52.6(q)	52.6				52.7		52.7(q)

Table 1 (*continued*)

Carbon	Δδc-(15)→(17)	Δδc-(16)→(17)	(18)	Δδc-(14)→(18)	(19)	Δδc-(15)→(19)	Δδc-(18)→(19)	(20)	Δδc-(14)→(20)	(21)	Δδc-(15)→(21)	Δδc-(20)→(21)
C-1		+1.9	167.0		168.8		+1.8	167.0		168.9		+1.9
C-2		+4.9	102.0		107.0		+5.0	102.0		106.8		+4.8
C-3	−0.6	−7.4	206.5		199.2		−7.3	206.0	−0.5	198.7	−0.6	−7.3
C-4		+2.8	51.1		53.9		+2.8	51.1		53.8		+2.7
C-5			31.2 [a]		31.4 [a]			31.2 [a]		31.5 [a]		+0.3
C-6			28.4 [a]	+0.4	28.4 [a]	+0.5		28.5 [a]	+0.5	28.3 [a]	+0.4	
C-7	−0.5		41.8		41.7			41.4	−0.5	41.3	−0.5	
C-8	−2.3	+0.4	39.3		39.7		+0.4	37.2	−2.1	37.5	−2.1	+0.3
C-9	+2.1		76.1		76.3			79.1	+3.0	79.1	+2.8	
C-10	−3.1		34.8		34.6			31.9	−2.9	31.7	−2.9	
C-11			125.6		125.6	+0.7		124.5	−1.3	125.5	+0.6	
C-12	10.5	+0.7	126.5		127.3		+0.8	127.4	+0.9	128.1	+0.8	+0.7
C-13		−2.4	53.3		50.8		−2.5	53.2		50.7		−2.5
C-14		+2.3	135.9		138.0		+2.1	135.6	−0.3	137.8		+2.2
C-15		−1.7	123.4		121.9		−1.5	123.7	+0.3	122.0		−1.7
C-16			31.2		31.4			31.3		31.4		
C-17	+0.4	+0.4	78.7		79.1	+0.4	+0.4	78.7		79.3	+0.6	+0.6
C-18	+0.9		137.8	+0.8	138.1	+1.0	+0.3	137.9	+0.9	138.2	+1.1	+0.3
C-19	+2.5		124.8	+3.3	125.0	+3.4		124.8	+3.3	123.9	+2.3	−0.9
C-20		+1.5	42.9		44.4		+1.5	43.1		44.5		+1.4
C-21	−0.6	+0.4	118.7	−0.7	119.2	−0.6	+0.5	118.7	−0.7	119.0	−0.8	+0.3
C-22	−4.8	−0.5	136.8	−4.7	136.3	−4.7	−0.5	136.8	−4.7	136.2	−4.8	−0.6
C-23			40.2		40.1			40.2		40.0		
C-24		+0.7	35.4		36.1		+0.7	35.5		36.1		+0.6
C-25			83.0	−0.3	82.0	−1.1	−1.0	83.0	−0.3	82.9		
C-26		−11.3	201.4		189.8		−11.6	201.2	−0.3	190.0		−11.2
C-27		−0.3	20.2		19.9		−0.3	20.2		19.9		−0.3
C-28	−0.3	+2.1	22.3		24.6		+2.3	22.2		24.3	−0.3	+2.1
C-29	+0.8		13.0		13.1			14.0	+1.0	14.0	+0.9	
C-30		−1.0	15.2		14.4	+0.4	−0.8	15.1		14.5	+0.5	−0.6
C-31		+0.7	13.8		14.1	−0.3	+0.3	13.8		14.1	−0.3	+0.3
C-32	+1.1		66.8	+1.9	67.0	+2.0		66.8	+1.9	66.9	+1.9	
C-33		−0.3	15.2		14.8		−0.4	15.1		14.7		−0.4
9-COO⁻								165.3		165.3		
9-Ar-C-1								130.0		130.0		
9-Ar-C-2								131.0		131.0		
9-Ar-C-3								137.9		137.8		
9-Ar-C-4								100.7		100.7		
9-Ar-C-5								137.9		137.8		
9-Ar-C-6								131.0		131.0		
32-COO⁻			165.9		165.8			165.8		165.9		
32-Ar-C-1			129.7		129.8			129.7		129.7		
32-Ar-C-2			131.2		131.2			131.2		131.2		
32-Ar-C-3			137.8		137.8			137.9		137.8		
32-Ar-C-4			100.8		100.8			100.8		100.8		
32-Ar-C-5			137.8		137.8			137.9		137.8		
32-Ar-C-6			131.2		131.2			131.2		131.2		
9-OCOCH$_3$												
9-OCOCH$_3$												
17-OCOCH$_3$												
17-OCOCH$_3$												
32-OCOCH$_3$												
32-OCOCH$_3$												
9-OCH$_3$												
17-OCH$_3$												
26-OCH$_3$					63.7					63.8		
C-1A												
C-2A												
C-3A												
C-4A												
C-5A												
C-6A												
C-1B												
C-2B												
C-3B												
C-4B												
C-5B												
C-6B												
C-1C												
C-2C												
C-3C												
C-4C												
C-5C												
C-6C												
C-1D												
C-2D												
C-3D												
C-4D												
C-5D												
C-6D												
4D-OCH$_3$												
C-1E	+0.4		97.4	+0.3	97.6	+0.4		97.4	+0.3	97.6	+0.4	
C-2E			35.8		35.8			35.8		35.8		
C-3E	−0.3		90.9	−0.3	91.0			90.9	−0.3	90.9	−0.3	
C-4E			53.8		53.9			53.8		53.8		
C-5E	−0.3		69.0		69.1			69.0		68.9	−0.3	
C-6E			17.0		17.0			17.0		17.0		
3E-CH$_3$			25.2		25.3			25.2		25.3		
4E-NHCOOCH$_3$			157.4		157.5			157.3		157.3		
4E-NHCOOCH$_3$			52.7		52.6			52.7		52.7		

Table 1 (*continued*)

Carbon	(22) [e]	(48)	Δδc-(48)→(1)	Δδc-(48)→(14)	(49)	Δδc-(48)→(49)	(50)	Δδc-(48)→(50)	(51)	Δδc-(48)→(51)	(52)
C-1	167.2	167.3(s)			169.2	+1.9	166.9	−0.4	167.1		169.1
C-2	102.0	102.2(s)	−0.3		107.0	+4.8	101.9	−0.3	102.2		106.5
C-3	206.5	206.5(s)	−0.3		199.3	−7.2	206.6		206.1	−0.4	198.8
C-4	51.1	51.1(s)			53.9	+2.8	51.2		51.1		53.8
C-5	31.3 [a]	31.2(d) [a]			31.5 [a]	+0.3	31.4 [a]		31.2 [a]		31.4 [a]
C-6	27.7/27.9 [a]	27.7(d) [a]		+0.3	27.7 [a]		27.9 [a]		27.7 [a]		27.6 [a]
C-7	41.8	41.8(t)			41.7		41.5	−0.3	41.4	−0.4	41.3
C-8	39.3	39.2(d)	−0.7		39.7	+0.5	38.3	−0.9	36.8	−2.4	37.2
C-9	76.2	76.1(d)	+8.4		76.3		85.0	+8.9	78.3	+2.2	78.4
C-10	34.8	34.8(d)			34.7		30.2	−4.6	31.5	−3.3	31.4
C-11	125.4/125.6	125.6(d)			125.0	−0.6	126.0	+0.4	124.8	−0.8	124.3
C-12	126.6/126.8	126.6(d)			127.4	+0.8	126.1	−0.5	127.0	+0.4	127.7
C-13	53.4	53.3(d)			51.1	−2.2	53.1		53.1		50.9
C-14	135.4/135.5	135.8(s)			138.2	+2.4	135.6		136.4	+0.6	138.2
C-15	123.8	123.1(d)	+0.5	+0.3	121.4	−1.7	123.6	+0.5	123.3		121.7
C-16	31.3	32.1(t)	−1.0	−0.9	32.4	+0.3	29.7	−2.4	29.9	−2.2	30.0
C-17	78.6	72.9(d)	+5.5	+5.7	73.1		82.1	+9.2	74.1	+1.2	74.3
C-18	137.4/137.6	141.3(s)	−4.2	−4.3	141.4		137.3	−4.0	136.9	−4.4	137.0
C-19	124.0/124.4	123.9(d)	−2.4	−2.4	124.4	+0.5	124.0		123.6	−0.3	124.2
C-20	42.9	42.9(d)			44.5	+1.6	43.0		43.0		44.5
C-21	118.5/119.0	117.6(d)	+1.7	+1.8	118.2	+0.6	118.5	+0.9	118.3	+0.7	118.7
C-22	136.8/139.8	138.6(s)	+2.9	+2.9	138.3	−0.3	138.7		138.8		138.6
C-23	40.2	40.1(d)			39.9		40.2		40.1		39.9
C-24	35.4	35.3(t)	+0.2		36.2	+0.9	35.5		35.3		36.2
C-25	82.1/83.3	83.3(s)			83.3		83.2		83.2		83.3
C-26	201.5	201.0(s)	+0.5	+0.5	189.9	−11.1	201.7	+0.7	200.9		190.0
C-27	20.0	20.0(q)			19.7	−0.3	20.0		20.0		19.7
C-28	24.9	22.3(q)			24.6	+2.3	22.3		22.2		24.4
C-29	13.0	13.1(q)	+0.9		13.1		13.0		13.9	+0.8	13.9
C-30	15.1	14.8(q)	+0.3	+0.4	14.4	−0.4	15.1	+0.3	14.9		14.4
C-31	13.7/13.9	13.7(q)			13.8		13.9		13.9		13.9
C-32	74.9	74.7(t)	−10.3	−9.8	74.8		74.8		74.7		74.8
C-33	15.1	15.2(q)			14.9	−0.3	15.1		15.2		14.7
9-COO⁻											
9-Ar-C-1											
9-Ar-C-2											
9-Ar-C-3											
9-Ar-C-4											
9-Ar-C-5											
9-Ar-C-6											
32-COO⁻											
32-Ar-C-1											
32-Ar-C-2											
32-Ar-C-3											
32-Ar-C-4											
32-Ar-C-5											
32-Ar-C-6											
9-OCOCH$_3$									170.5		170.5
9-OCOCH$_3$									21.1		21.1
17-OCOCH$_3$									170.0		170.2
17-OCOCH$_3$									21.4		21.4
32-OCOCH$_3$											
32-OCOCH$_3$											
9-OCH$_3$							56.8 [b]				
17-OCH$_3$							56.4 [b]				
26-OCH$_3$					63.6						63.7
32-OCH$_3$		58.0(q)			57.9		58.1		58.0		57.9
C-1A											
C-2A											
C-3A											
C-4A											
C-5A											
C-6A											
C-1B											
C-2B											
C-3B											
C-4B											
C-5B											
C-6B											
C-1C											
C-2C											
C-3C											
C-4C											
C-5C											
C-6C											
C-1D											
C-2D											
C-3D											
C-4D											
C-5D											
C-6D											
4D-OCH$_3$											
C-1E	97.4										
C-2E	35.5/35.6										
C-3E	91.7										
C-4E	53.2										
C-5E	69.9										
C-6E	17.2/18.0										
3E-CH$_3$	20.0/25.4/25.9										
4E-NHCOOCH$_3$	157.9/159.8										
4E-NHCOOCH$_3$	55.3/55.6/56.8										

Table 1 (*continued*)

Carbon	Δδc-(49)→ (52)	(53)	Δδc-(53)→ (1)	(54)	Δδc-(54)→ (2)	Δδc-(53)→ (54)	(55) [f]	(14) [g]	(46) [g]	(47) [g]
C-1		167.2		169.1		+1.9		170.2(s)	177.3	170.3(s)
C-2	−0.5	101.8		106.8		+5.0		103.1(s)	100.1	103.3(s)
C-3	−0.5	206.3		199.1		−7.2	182.3(s)	206.0(s)	203.6	206.1(s)
C-4		51.0		53.7		+2.7	50.0(s)	52.3(s)	52.6	52.4(s)
C-5		31.3 [a]		31.5 [a]			33.7 [a]	32.1(d) [a]	32.6 [a]	32.4(d) [a]
C-6		27.9 [a]		28.0 [a]			26.6 [a]	28.5(d) [a]	28.9 [a]	28.7(d) [a]
C-7	−0.4	41.8		41.7			41.2	43.0(t)	43.4	43.1(t)
C-8	−2.5	38.5		38.9		+0.4	44.6(d)	40.2(d)	40.6	40.2(d)
C-9	+2.1	83.9	+0.6	84.1	+0.7		74.8(d)	76.7(d)	77.4	76.8(d)
C-10	−3.3	34.3	+0.5	34.4	+0.5		25.9	36.4(d)	36.3	36.2(d)
C-11	−0.7	126.1	−0.3	125.5		−0.6	66.0 [b]	127.0(d)	126.6	127.3(d)
C-12	+0.3	126.3	+0.4	127.1	+0.4	+0.8	66.2 [b]	127.4(d)	128.9	127.9(d)
C-13		53.3		50.8		−2.5	47.6(d)	54.1(d)	53.0	54.3(d)
C-14		135.8		138.2	−0.3	+2.4	66.6(d) [b]	138.6(s)	138.3	136.9(s)
C-15	+0.3	123.5		121.9		−1.6		124.4(d)	124.9	124.5(d)
C-16	−2.4	31.1		31.0	+0.3			32.3(t)	33.3	33.0(t)
C-17	+1.2	78.1	+0.3	78.3	+0.4			80.0(d)	74.3	73.5(d)
C-18	−4.4	137.1		137.2				137.2(s)	140.5	139.3(s)
C-19		121.6		121.9		+0.3		123.4(d)	122.7	127.3(d)
C-20		43.2		44.7		+1.5		44.3(d)	45.2	44.4(d)
C-21	+0.5	119.3		119.7		+0.4		120.3(d)	120.4	118.4(d)
C-22	+0.3	141.5		141.0		−0.5		142.5(s)	141.7	142.5(s)
C-23		40.3		40.1				41.4(d)	41.4	41.7(d)
C-24		35.6	−0.1	36.1	+0.1	+0.5		36.1(t)	36.9	36.2(t)
C-25		83.4		83.1		−0.3		84.7(s)	85.4	84.4(s)
C-26		201.7		190.2		11.5		201.2(s)	201.9	200.8(s)
C-27		20.2		20.0			21.3	20.3(q)	20.6	20.1(q)
C-28		22.2		24.5		+2.3	25.3	23.0(q)	23.3	23.0(q)
C-29	+0.8	14.2		14.1			16.5(q)	13.6(q)	13.8	13.7(q)
C-30		15.1		14.3		−0.8	18.1(q)	15.6(q)	16.0	15.7(q)
C-31		13.8		14.4		+0.6		14.1(q)	14.6	14.2(q)
C-32		64.0	+0.4	64.0	+0.5			65.1(t)	65.4	*h*
C-33		15.1		14.8		0.3		15.3(q)	15.5	15.3(q)
9-COO⁻										
9-Ar-C-1										
9-Ar-C-2										
9-Ar-C-3										
9-Ar-C-4										
9-Ar-C-5										
9-Ar-C-6										
32-COO⁻										
32-Ar-C-1										
32-Ar-C-2										
32-Ar-C-3										
32-Ar-C-4										
32-Ar-C-5										
32-Ar-C-6										
9-OCOCH$_3$										
9-OCOCH$_3$										
17-OCOCH$_3$										
17-OCOCH$_3$										
32-OCOCH$_3$										
32-OCOCH$_3$										
9-OCH$_3$										
17-OCH$_3$										
26-OCH$_3$				64.0	−0.3					
32-OCH$_3$										
C-1A		98.1		98.1			92.9(d)			
C-2A		30.2	−0.3	30.1			30.2(t) [c]			
C-3A		66.0 [b]	+0.8	66.5 [b]	+0.5	+0.5	66.8(d) [b]			
C-4A		71.5	+0.3	71.5	+0.4		71.7(d)			
C-5A		65.0 [b]		65.0 [b]			62.2(d) [b]			
C-6A		17.9 [c]		17.9 [c]			17.8(q) [d]			
C-1B		91.1	−0.3	91.0			91.0(d)			
C-2B		34.4	−4.7	34.4	−4.9	+0.5	29.5(t) [c]			
C-3B		62.1	+0.5	62.1	+0.6		62.7(d)			
C-4B		77.3	+2.3	77.2	+2.5		79.5(d)			
C-5B		65.5 [b]	+1.6	65.5 [b]	+1.7		67.2(d) [b]			
C-6B		17.8 [c]		17.8 [c]			17.9(q) [d]			
C-1C							92.2(d)			
C-2C							34.8(t)			
C-3C							67.5(d)			
C-4C							72.4(d)			
C-5C							64.4(d)			
C-6C							17.9(q)			
C-1D		98.4	+1.4	98.4	+1.4		99.7(d)			
C-2D		36.9		36.8			36.8(t)			
C-3D		64.0		63.7		−0.3	63.8(d)			
C-4D		82.3	+0.3	82.3	+0.4		82.6(d)			
C-5D		68.4	−0.3	68.4	−0.3		68.0(d)			
C-6D		18.3		18.3			18.4(q)			
4D-OCH$_3$		57.3		57.3			57.3(q)			
C-1E		96.9		97.0	+0.3			98.8(d)		
C-2E		35.6		35.8				36.4(t)		
C-3E		91.1		91.2				92.2(s)		
C-4E		53.8		53.9				55.1(d)		
C-5E		69.1		69.1				70.1(d)		
C-6E		17.0		17.0				17.2(q)		
3E-CH$_3$		25.3		25.4				25.7(q)		
4E-NHCOOCH$_3$		157.7	−0.3	157.7	−0.3			159.9(s)		
4E-NHCOOCH$_3$		52.7		52.7				53.0(q)		

a–d May be interchanged in any vertical column. *e* Mixture of rotamers at ambient temperature. *f* Not all multiplicities could be determined from the SFOR spectrum. *g* Run in CD_3OD. *h* Obscured by CD_3OD signal. *i* Due to the structural complexity of these molecules, all of the above assignments must of necessity be regarded as tentative assignments pending confirmation by δ_H/δ_C correlation techniques.

n.m.r. spectrum also revealed five olefinic carbon atoms each bearing a hydrogen atom. It was therefore concluded that the molecule contained four double bonds, three of which were trisubstituted and one of which was disubstituted, in addition to the enolic double bond. The molecule also contained five anomeric carbon atoms at δ_C 99.8, 98.2, 97.1, 92.2, and 90.8 of which two were strongly shielded, indicating that (1) contains five glycosidic moieties. It was also clear from the chemical shifts of these anomeric carbons, that none of the sugars were present as furanosides. The $J_{^{13}C-^1H}$ values for the five anomeric carbons were determined and found to be δ_C 97.1 ($J_{^{13}C-^1H}$ 160 Hz), 99.8 ($J_{^{13}C-^1H}$ 160 Hz), 98.2 ($J_{^{13}C-^1H}$ 160 Hz), 90.8 ($J_{^{13}C-^1H}$ 166 Hz) and 92.2 ($J_{^{13}C-^1H}$ 164 Hz). The ^{13}C n.m.r. data also revealed the presence of an additional 23 methine groups in (1). Of these four where strongly deshielded (δ_C 84.5, 82.6, 79.6, and 78.4) suggesting that they belonged to ether carbons. From the chemical shifts, 11 of the remaining methine carbons bore oxygen substituents, one bore a nitrogen substituent (δ_C 53.8), and the remainder bore only carbon substituents. Two of these carbon-bearing methine groups experienced strong deshielding (δ_C 53.2 and 43.1). Of the remaining signals, eight were attributed to the carbons of methylene groups, while one obviously belonged to a primary allylic alcohol group (δ_C 64.4).

Although a satisfactory analysis (C,H,N) for (1) $C_{67}H_{100}N_2O_{24}$ was obtained, it could not be used to establish unambiguously the molecular composition. An electron impact mass spectrum (e.i.m.s.) of (1) failed to give a molecular ion, the highest mass fragment ion being observed at *m/z* 552 due to ion D_1 (Figure 1) which suffered two successive losses of water. The only other prominent ion arising from the aglycone was at *m/z* 374 and this was thought to arise from the ion D_9, which also then lost one molecule of water. Several ions arising from the sugar fragments are listed in the Experimental section. The use of f.d., or c.i. mass spectrometry also failed to give a molecular ion for (1). The presence of an acidic enol grouping in (1) made it possible to convert (1) into the 26-*O*-methyl ether (2) using diazomethane under mild conditions. The methyl ether (2) showed only two bands in the u.v. region at 200 and 254 nm. The i.r. spectrum of (2) also revealed additional absorption at 1 662 and 1 570 cm^{-1} due to the vinylic ether group. The ^1H n.m.r. spectrum of (2) at 600 MHz * ($CDCl_3$) revealed the presence of 12 *C*-methyl groups, of which four were substituted on quaternary carbons. The newly introduced 26-*O*-methyl group gave rise to a singlet at δ_H 4.12. The downfield signals were ultimately assigned as indicated in Table 19 using 2D *J* spectroscopy and n.O.e. difference spectroscopy and arose from five olefinic protons, one carbamate NH-proton, and five anomeric proton signals. Of these latter signals, the doublet of doublets at δ_H 4.45 ($J_{1^E_{ax},2^E_{ax}}$ 10.0 Hz, $J_{1^E_{ax},2^E_{eq}}$ 2.2 Hz) was assigned to the kijanose moiety (see later) indicating that the latter was an equatorially linked, 2-deoxysugar. Some overlap of signals was observed with the remaining anomeric protons and the lowfield region of the spectrum was therefore re-run in CD_3CN, which resulted in clear resolution of the remaining four anomeric proton signals. Three doublet of doublet signals at δ_H 4.72 ($J_{1eq,2ax}$ 4.5 Hz, $J_{1eq,2eq} < 0.5$ Hz), 5.06 ($J_{1eq,2ax}$ 4.0 Hz, $J_{1eq,2eq} < 0.5$ Hz) and 5.13 ($J_{1eq,2ax}$ 3.3 Hz, $J_{1eq,2eq} < 0.5$ Hz) indicated the presence of three axially linked 2-deoxysugars. A doublet of doublets at δ_H 4.88 ($J_{1^D_{ax},2^D_{ax}}$ 10.0 Hz, $J_{1^D_{ax},2^D_{eq}}$ 1.9 Hz) revealed the presence of one equatorially linked 2-deoxysugar. The ^{13}C n.m.r. data for (2) are given in Table 1 and it is evident from the observed δ_C values in going from

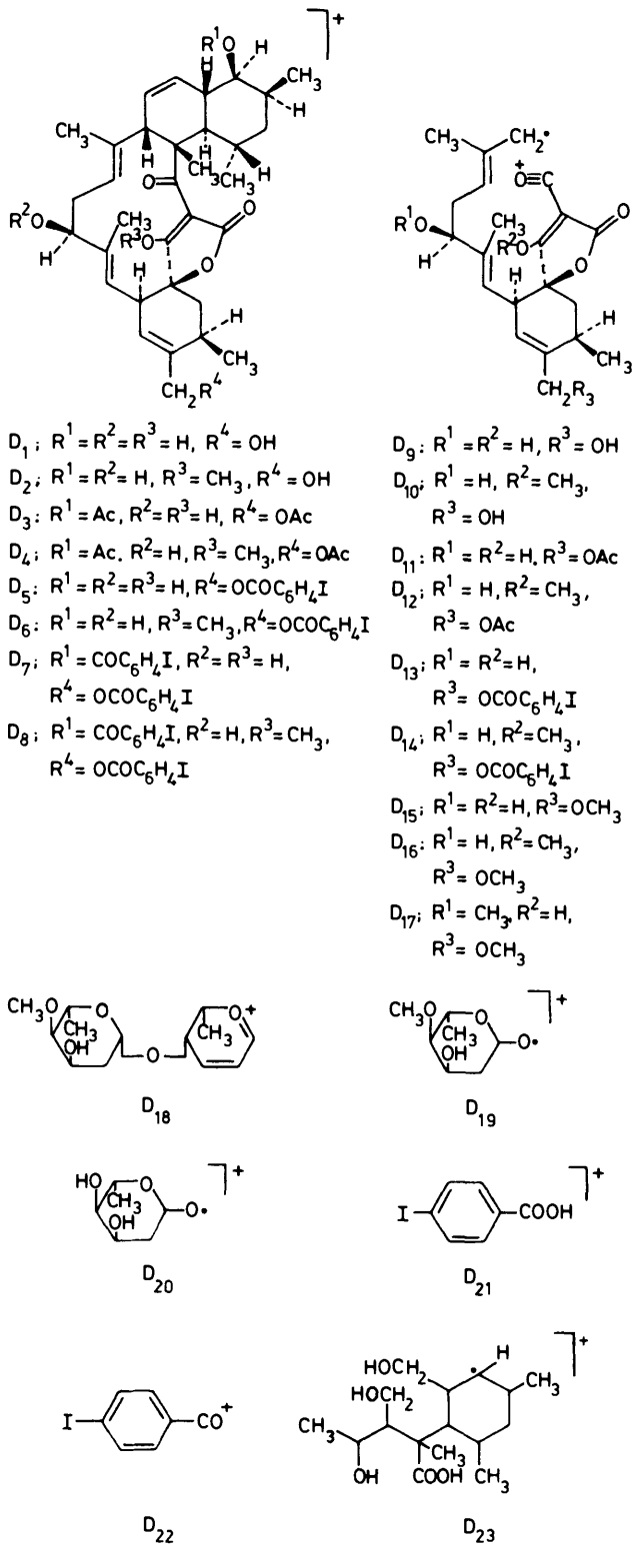


Figure 1. Mass spectral fragment ions for kijanimicin and kijanolide derivatives

(1) to (2), that the introduction of the 26-*O*-methyl group affects most of the proximal carbons around the macrocyclic portion of the aglycone. The ^1H and ^{13}C n.m.r. data gave no evidence for mixtures of tautomers and we tentatively conclude that in solution the tautomer is as shown in (2) which is in good agreement with what has been observed in similarly

* The 600 MHz ^1H n.m.r. spectra were recorded at Carnegie-Mellon University, Pittsburgh, Pennsylvania, and NIH GRANT RR00292 is gratefully acknowledged.

substituted simple acyl tetronic acids.[5] A radiochemical molecular weight determination [6] using [^{14}C]diazomethane to prepare (2), indicated a molecular weight for (2) of 1 301.4 and 1 291.7 from duplicate determinations. The e.i.m.s. of (2) again showed ion D_1 as the highest mass ion (Figure 1). The f.d. and c.i. mass spectra also failed to give a molecular ion and we therefore turned to ^{252}Cf-plasma desorption mass spectrometry (^{252}Cf-p.d.m.s.) [7] in order to establish the molecular composition of kijanimicin (1).

The ^{252}Cf-p.d.m.s. positive ion spectrum of 26-*O*-methylkijanimicin (2) exhibited a strong peak at *m/z* 1 354 which we assigned to an ion of the type $(M + Na)^+$. No other ions were observed above this mass. The negative ion spectrum in the same mass region showed an intense ion at *m/z* 1 317 and an additional ion with *ca.* 0.4 the intensity at *m/z* 1 330. The latter ion can be correlated with the positive ion results if it is assigned to $(M - H)^-$, implying that the *m/z* 1 315 species is a fragment ion. It may arise from loss of CH_4 from the 26-*O*-methyl group. From these results we deduce that the molecular weight of (2) is 1 331.07 ± 0.4 which is in excellent agreement with a molecular composition of $C_{68}H_{102}N_2O_{24}$ (*M* 1 330.68). The molecular formula of kijanimicin (1) was therefore established as $C_{67}H_{100}N_2O_{24}$ (*M* 1 317.5). The major fragment ions in the positive ion spectrum occur at *m/z* 258, 532, 549, and 566. The *m/z* 258 peak corresponds in mass to a fragment ion containing sugars B and D resulting from cleavage of the C(1^B)–O and C(3^B)–O bonds. The fragment ion at *m/z* 532 results from cleavage of the C(9)–O and C(17)–O bonds and arises from the aglycone. A fragment ion at *m/z* 549 was also observed and this may arise from the aglycone either by cleavage of the O–C(1^A) bond with a hydrogen transfer and the C(17)–O bond, or by cleavage of the O–C(1^E) bond with a hydrogen transfer and the C(9)–O bond. The fragment at *m/z* 566 is due to the aglycone in which both the O–C(1^A) and O–C(1^E) bonds have cleaved with hydrogen transfers in each case.

With the molecular composition established it was possible to calculate the number of elements of unsaturation in kijanimicin (1) as 19. Of these, four may be attributed to the tetrasaccharide unit, three may be attributed to the 1,3,3′-diketolactone group, four may be attributed to olefinic double bonds, one may be attributed to a nitro-group, one may be attributed to a methyl carbamoyl group, and one may be assigned to the kijanose ring. The remaining five elements of unsaturation indicate that the aglycone is comprised of five rings.

The acidic properties of the enolic group in (1) were used to prepare a number of water soluble salts (sodium, potassium, rubidium and *N*-methylglucamine) of (1). Unfortunately all of the salts showed a marked tendency to form gels in aqueous organic solvents and consequently none were obtained crystalline. Due to the enolic group present in (1), the molecule could also be converted into a metal chelate. Thus the copper and zinc chelates were prepared, both of which were soluble in organic solvents. Although the copper complex was obtained as fine crystalline needles from ethyl acetate–hexane, the crystals were not acceptable for *X*-ray analysis. We therefore turned to a second approach for obtaining a heavy atom derivative and this involved the selective preparation of a monoiodobenzoyl derivative of (1). The recognition that the molecule contained a primary allylic alcohol group led us to believe that it might be possible selectively to prepare a 4-iodobenzoyl derivative of (1). Thus kijanimicin (1), under controlled conditions using 4-iodobenzoyl chloride in pyridine, was converted into the 32-*O*-(4-iodobenzoyl) derivative (3). Although the ^{13}C n.m.r. data for (3) (Table 1) confirmed the presence of one iodobenzoyl group on the primary allylic alcohol at C-32, all attempts to crystallize (3) failed. Further

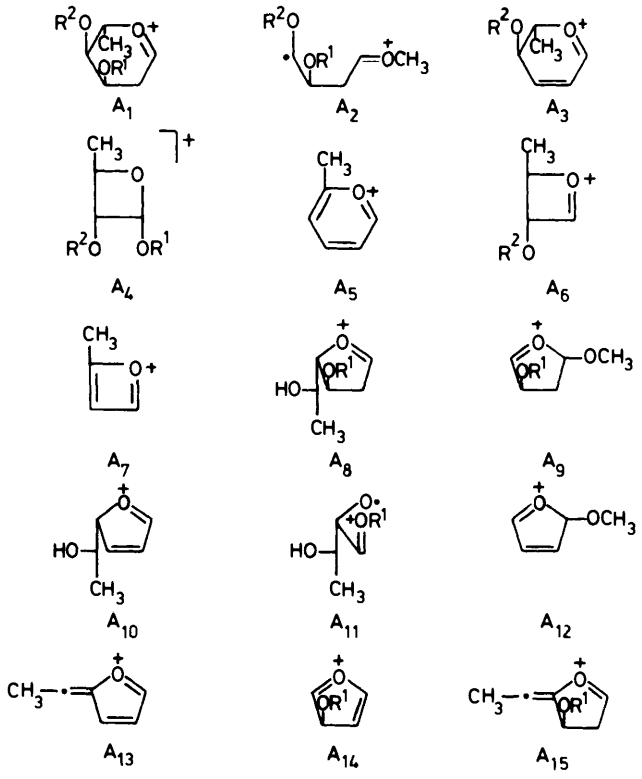


Figure 2. Mass spectral fragment ions for L-digitoxose derivatives (Table 2)

chemical studies were therefore undertaken in order to elucidate the structure of (1).

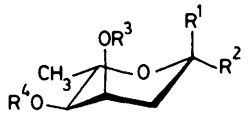
Methanolysis of kijanimicin (1) using 0.5M-methanolic hydrogen chloride at 25 °C for 16.5 h afforded, after extensive chromatography, methyl 2,6-dideoxy-4-*O*-methyl-α-L-*ribo*-hexopyranoside (4), the β-anomer (5), methyl 2,6-dideoxy-α-L-*ribo*-hexopyranoside (methyl α-L-digitoxoside) (6), the β-anomer (7), methyl 2,6-dideoxy-α-L-*ribo*-hexofuranoside (11), the β-anomer (12), and *O*-β-D-kijanosyl-(1→17)-kijanolide (14). At the time the structures were elucidated both of the 2-deoxysugars were novel compounds not previously described in the literature. Subsequently, L-digitoxose was found to occur in three polyene antibiotics,[8] as well as in the tetrocarcins [9-12] and antlermicins [13,14] which are closely related to kijanimicin (1). The e.i. mass spectra of methyl 2,6-dideoxy-4-*O*-methyl-α-L-*ribo*-hexopyranoside (4) and the β-anomer (5) (Table 2) both showed molecular ions at *m/z* 176 and gave rise to the fragment ions A_1—A_7 (Figure 2) which were consistent with the proposed structure. The occurrence of ion A_6 at *m/z* 101 indicated that the *O*-methyl group was located at C-4. The ^{1}H n.m.r. data for (4) and (5) (Table 3) confirmed the fact that these sugars were 2,6-dideoxyhexopyranosides having the *ribo*-configuration. The *O*-methyl group was clearly located at C-4 from the observed shielding of 4_{ax}-H in (4) and (5) relative to the corresponding digitoxosides (6) and (7). The ^{13}C n.m.r. data for (4) and (5) were consistent with the assigned structures (Table 4). The observed deshielding of C-4 in (4) and (5), relative to (6) and (7) respectively, was consistent with the presence of a 4-*O*-methyl group in (4) and (5). The observed shieldings of C-3 and C-5 in (4) and (5) relative to (6) and (7), due to interactions between the 4-*O*-methyl group and the protons at C-3 and C-5 [15] that are vicinal *cis* to it, were also consistent with a *ribo*-configuration for these sugars. The molecular rotations of (4) and (5) are given in Table 5 and the application of Hudson's Rules of Isorotation [16] gave a 2A

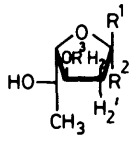
Table 2. Mass spectra of the L-digitoxose derivatives [*m/z* (%)]

Compd. Pyranosides:	$M^{+\cdot}$	$(M-H)^+$	A_1	A_2	A_3	A_4	A_5	A_6	A_7
(6)	162 (1.4)	161 (0.1)	131 (15.0) [a,b]	118 (16.5) [a,b]	113 (14.3) [a,b]	104 (72.7) [a,b]	95 (4.8) [a]	87 (9.1) [a,b]	69 (30.5)
(7)	162 (1.8)	161 (1.9)	131 (11.0) [a,b]	118 (37.1) [a,b]	113 (9.1) [a,b]	104 (99.5) [a,b]	95 (2.7) [a]	87 (16.3) [a,b]	69 (27.6)
(4)	176 (2.1)	175 (0.9)	145 (30.0) [a,c]	132 (30.2) [c]	127 (94.8) [a,c]	118 (99.6) [c]	95 (15.3) [a]	101 (11.5) [c]	69 (25.9)
(5)	176 (1.4) [a]	175 (2.8) [a]	145 (5.5) [a,c]	132 (35.0) [a,c]	127 (26.8) [a,c]	118 (92.9) [a,c]	95 (4.9)	101 (9.9) [c]	69 (20.8)
(8) [h]	280 (0.1)	279 (0.5)	249 (0.3) [d]	236 (12.9) [d]	127 (45.8) [d]	222 (0.8) [d]	95 (10.4)	101 (2.5) [d]	69 (16.2)
(9) [i]	190 (0.2)	189 (0.2)	159 (2.3) [e]	146 (0.8) [e]	127 (18.5) [e]	132 (3.7) [e]	95 (4.1)	101 (7.6) [e]	69 (3.1)
(10) [j]	190 (0.1)	189 (0.1)	159 (1.9) [e]	146 (0.6) [e]	127 (15.1) [e]	132 (3.1) [e]	95 (3.5)	101 (6.6) [e]	69 (2.6)

Furanosides:	$M^{+\cdot}$	$(M-H)^+$	A_8	A_9	A_{10}	A_{11}	A_{12}	A_{13}	A_{14}	A_{15}
(11)	162 (0.3)	161 (0.8)	131 (86.7) [a,f]	117 (91.1) [a,f]	113 (38.0)	104 (35.1) [f]	99 (92.7) [a]	95 (15.4) [a]	85 (67.9) [a,f]	113 (38.0) [f]
(12)	162 (0.2)	161 (1.2)	131 (31.6) [a,f]	117 (92.4) [a,f]	113 (15.0) [a]	104 (26.3) [a,f]	99 (96.4) [a]	95 (6.5) [a]	85 (42.3) [a,f]	113 (15.0) [a,f]
(13)		175 (0.1)	145 (9.0) [g]	131 (32.8) [g]	113 (3.8)	118 (2.4) [g]	99 (67.8)	95 (4.4)	99 (67.8) [g]	127 (2.1) [g]

[a] Composition confirmed by high resolution mass spectrometry. [b] $R^1 = R^2 = H$. [c] $R^1 = H$, $R^2 = CH_3$. [d] $R^1 = Bz$, $R^2 = CH_3$. [e] $R^1 = R^2 = CH_3$. [f] $R^1 = H$. [g] $R^1 = CH_3$. [h] Also showed fragment ions at: *m/z* 216 (16) ($M - CH_3OH{-}CH_3OH$) and *m/z* 105 (98.7) ($C_6H_5CO^+$). [i] Also showed a fragment ion at: *m/z* 88 (100) $CH_3OCH{=}CHOCH_3^{+\cdot}$.





(4) $R^1 = OCH_3$, $R^2 = R^3 = H$, $R^4 = CH_3$

(5) $R^1 = R^3 = H$, $R^2 = OCH_3$, $R^4 = CH_3$

(6) $R^1 = OCH_3$, $R^2 = R^3 = R^4 = H$

(7) $R^1 = R^3 = R^4 = H$, $R^2 = OCH_3$

(8) $R^1 = H$, $R^2 = OCH_3$, $R^3 = Bz$, $R^4 = CH_3$

(9) $R^1 = OCH_3$, $R^2 = H$, $R^3 = R^4 = CH_3$

(10) $R^1 = H$, $R^2 = OCH_3$, $R^3 = R^4 = CH_3$

(11) $R^1 = OCH_3$, $R^2 = R^3 = H$

(12) $R^1 = R^3 = H$, $R^2 = OCH_3$

(13) $R^1 = H$, $R^2 = OCH_3$, $R^3 = CH_3$

value of $-346°$, which was in excellent agreement with an L-configuration for (4) and (5).

The e.i. mass spectra of methyl 2,6-dideoxy-α-L-*ribo*-hexopyranoside (6) and the β-anomer (7) showed molecular ions at *m/z* 162 (Table 2) and fragment ions A_1—A_7 (Figure 2) consistent with a hexopyranoside structure in each instance. The ^1H n.m.r. data for (6) and (7) (Table 3) were in excellent agreement with data published earlier for the D-enantiomers [17] and were consistent with the proposed structures. The ^{13}C n.m.r. data for (6) and (7) are given in Table 4 and the data are in agreement with what would be expected for a *ribo*-hexopyranosyl structure. The molecular rotations of (6) and (7) are given in Table 5 and the application of Hudson's Rules of Isorotation [16] gave a 2A value of $-331°$ which clearly in-indicated an L-configuration for (6) and (7). The corresponding value for the D-enantiomers [17] is $+340°$. The e.i. mass spectra of the furanosides (11) and (12) also showed molecular ions at *m/z* 162 (Table 2) and gave fragment ions corresponding to A_8—A_{15} (Figure 2), the ion A_9 being diagnostic for a furanoside structure. The ^1H n.m.r. data (Table 3) also revealed characteristic lowfield signals for the anomeric protons and were consistent with furanoside structures. The data were also in good agreement with those published earlier for the D-furanosides.[17] The ^{13}C n.m.r. data were also characteristic of a *ribo*-hexofuranoside structure (Table 4). The molecular rotations of (11) and (12) are given in Table 5 and the application of Hudson's Rules of Isorotation [16] gave a 2A value of $-379°$ consistent with an L-configuration. In contrast, the D-furanosides gave a value of $+399°$.[17] No other sugars were isolated indicating that kijanimicin (1) contained a tetrasaccharide unit comprised of one 2,6-dideoxy-4-*O*-methyl-L-*ribo*-hexopyranosyl unit and three 2,6-dideoxy-L-*ribo*-hexopyranosyl units. The only other product isolated from the methanolysis of (1) was *O*-β-D-kijanosyl-(1→17)-kijanolide (14). The latter was laevorotatory and gave rise to a u.v. spectrum similar to that of (1). The i.r. spectrum of (14) showed that the molecule had retained the nitro, methylcarbamate, ketone, and lactone groups. The ^1H n.m.r. spectrum of (14) at 220 MHz confirmed the presence of the methylcarbamate group at δ_H 3.69 and exhibited a doublet of doublets at δ_H 4.43 ($J_{1^E_{ax},2^E_{ax}}$ 10 Hz, $J_{1^E_{ax},2^E_{eq}}$ 2.5 Hz) due to 1^E_{ax}-H, clearly indicating that the sugar was an equatorially linked 2-deoxysugar. The ^{13}C n.m.r. data for (14) (Table 1) revealed that no rearrangements had occurred in the aglycone during the methanolysis. The $\Delta\delta_C$ values in going from (14) to (1) revealed deshielding at C-9 (+8.4) and shielding at C-8 (−0.8) due to the introduction of a tetrasaccharide unit at C-9. Some deshielding was also observed at C-29, and C-24 was shielded in going from (14) to (1). The e.i. mass spectrum of (14) again showed no molecular ion, the highest fragment ion being observed at *m/z* 552 due to ion D_1 (Figure 1).

Methyl 2,6-dideoxy-4-*O*-methyl-β-L-*ribo*-hexopyranoside

Table 3. ^{1}H N.m.r. data for L-digitoxose derivatives [δ_H($CDCl_3$), multiplicity, J in Hz]

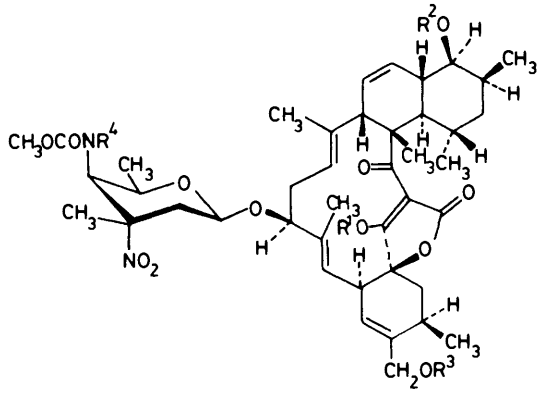
Compd. Pyranosides:	1-H	2eq-H	2ax-H	3eq-H	4ax-H	5ax-H	6-CH_3	Substituents at C-1	C-3	C-4
(6)	4.75dd $J_{1eq,2eq}$ 1.5 $J_{1eq,2ax}$ 3.5 1eq-H	2.17ddd $J_{1eq,2eq}$ 1.5 $J_{2eq,2ax}$ 14.5 $J_{2eq,3eq}$ 3.5	1.87ddd $J_{1eq,2ax}$ 3.5 $J_{2eq,2ax}$ 14.5 $J_{2ax,3eq}$ 3.5	3.90ddd $J_{2eq,3eq}$ 3.5 $J_{2ax,3eq}$ 3.5 $J_{3eq,4ax}$ 3	3.09dd $J_{3eq,4ax}$ 3 $J_{4ax,5ax}$ 10	3.71dq $J_{4ax,5ax}$ 10 $J_{5ax,6}$ 6	1.33d $J_{5ax,6}$ 6	3.37s OCH_3		
(7)	4.74dd $J_{1ax,2eq}$ 2.5 $J_{1ax,2ax}$ 9.5 1ax-H	2.13ddd $J_{1ax,2eq}$ 2.5 $J_{2eq,2ax}$ 14 $J_{2eq,3eq}$ 3.5	1.70ddd $J_{1ax,2ax}$ 9.5 $J_{2eq,2ax}$ 14 $J_{2ax,3eq}$ 3	4.14ddd $J_{2eq,3eq}$ 3.5 $J_{2ax,3eq}$ 3 $J_{3eq,4ax}$ 3	3.33dd $J_{3eq,4ax}$ 3 $J_{4ax,5ax}$ 9.5	3.77dq $J_{4ax,5ax}$ 9.5 $J_{5ax,6}$ 6	1.32d $J_{5ax,6}$ 6	3.48s OCH_3	3.07s OH	3.07s OH
(4)	4.79dd $J_{1eq,2eq}$ 1.5 $J_{1eq,2ax}$ 3.5 1eq-H	2.21ddd $J_{1eq,2eq}$ 1.5 $J_{2eq,2ax}$ 14 $J_{2eq,3eq}$ 3	1.87ddd $J_{1eq,2ax}$ 3.5 $J_{2eq,2ax}$ 14 $J_{2ax,3eq}$ 3.5	4.24ddd $J_{2eq,3eq}$ 3 $J_{2ax,3eq}$ 3.5 $J_{3eq,4ax}$ 3	2.87dd $J_{3eq,4ax}$ 3 $J_{4ax,5ax}$ 10	3.99dq $J_{4ax,5ax}$ 10 $J_{5ax,6}$ 6	1.33d $J_{5ax,6}$ 6	3.47s [a] OCH_3	1.61bs OH	3.40s [a] OCH_3
(5)	4.73dd $J_{1ax,2eq}$ 2.5 $J_{1ax,2ax}$ 9.5 1ax-H	2.19ddd $J_{1ax,2eq}$ 2.5 $J_{2eq,2ax}$ 14 $J_{2eq,3eq}$ 3.5	1.62ddd $J_{1ax,2ax}$ 9.5 $J_{2eq,2ax}$ 14 $J_{2ax,3eq}$ 3	4.28ddd $J_{2eq,3eq}$ 3.5 $J_{2ax,3eq}$ 3 $J_{3eq,4ax}$ 3	2.88dd $J_{3eq,4ax}$ 3 $J_{4ax,5ax}$ 9.5	3.77dq $J_{4ax,5ax}$ 9.5 $J_{5ax,6}$ 6	1.30d $J_{5ax,6}$ 6	3.48s OCH_3	2.34s OH	3.42s OCH_3
(8)	4.78dd $J_{1ax,2eq}$ 2.5 $J_{1ax,2ax}$ 9 1ax-H	2.24ddd $J_{1ax,2eq}$ 2.5 $J_{2eq,2ax}$ 14 $J_{2eq,3eq}$ 3.5	1.81ddd $J_{1ax,2ax}$ 9 $J_{2eq,2ax}$ 14 $J_{2ax,3eq}$ 3	5.76ddd $J_{2eq,3eq}$ 3.5 $J_{2ax,3eq}$ 3 $J_{3eq,4ax}$ 3	3.00dd $J_{3eq,4ax}$ 3 $J_{4ax,5ax}$ 9	3.94dq $J_{4ax,5ax}$ 9 $J_{5ax,6}$ 6.5	1.36d $J_{5ax,6}$ 6.5	3.49s OCH_3	7.40m OBz	3.37s OCH_3
(9)	4.60dd $J_{1eq,2eq}$ 2 $J_{1eq,2ax}$ 5 1eq-H	2.22ddd $J_{1eq,2eq}$ 2 $J_{2eq,2ax}$ 14 $J_{2eq,3eq}$ 4	1.68ddd $J_{1eq,2ax}$ 5 $J_{2eq,2ax}$ 14 $J_{2ax,3eq}$ 4	3.75ddd $J_{2eq,3eq}$ 4 $J_{2ax,3eq}$ 4 $J_{3eq,4ax}$ 3	2.91dd $J_{3eq,4ax}$ 3 $J_{4ax,5ax}$ 9	4.09dq $J_{4ax,5ax}$ 9 $J_{5ax,6}$ 6	1.24d $J_{5ax,6}$ 6	3.33s [a] OCH_3	3.44s [a] OCH_3	3.40s OCH_3
(10)	4.62dd $J_{1ax,2eq}$ 3 $J_{1ax,2ax}$ 9 1ax-H	2.21ddd $J_{1ax,2eq}$ 3 $J_{2eq,2ax}$ 14 $J_{2eq,3eq}$ 4	1.50ddd $J_{1ax,2ax}$ 9 $J_{2eq,2ax}$ 14 $J_{2eq,3eq}$ 4	3.77ddd $J_{2eq,3eq}$ 4 $J_{2ax,3eq}$ 4 $J_{3eq,4ax}$ 3	2.87dd $J_{3eq,4ax}$ 3 $J_{4ax,5ax}$ 9	3.91dq $J_{4ax,5ax}$ 9 $J_{5ax,6}$ 6	1.28d $J_{5ax,6}$ 6	3.47s [a] OCH_3	3.46s [a] OCH_3	3.40s OCH_3

Furanosides:	1-H	2'-H	2-H	3-H	4-H	5-H	6-CH_3	Substituents at C-1	C-3	C-5
(11)	5.18dd $J_{1,2}$ 1.5 $J_{1,2'}$ 4	2.16ddd $J_{1,2'}$ 4 $J_{2,2'}$ 14 $J_{2',3}$ 6	1.96ddd $J_{1,2}$ 1.5 $J_{2,2'}$ 14 $J_{2,3}$ 2.5	4.27ddd $J_{2',3}$ 6 $J_{2,3}$ 2.5 $J_{3,4}$ *ca.* 4	3.91dd $J_{3,4}$ *ca.* 4 $J_{4,5}$ 4	3.91dq $J_{4,5}$ 4 $J_{5,6}$ 6.5	1.27d $J_{5,6}$ 6.5	3.40s OCH_3		
(12)	5.12dd $J_{1,2}$ 5 $J_{1,2'}$ 2.5	2.34ddd $J_{1,2'}$ 2.5 $J_{2',2}$ 14 $J_{2',3}$ 7	2.11ddd $J_{1,2}$ 5 $J_{2,2'}$ 14 $J_{2,3}$ 5	4.63ddd $J_{2,3}$ 5 $J_{2',3}$ 7 $J_{3,4}$ 4	3.81dd $J_{3,4}$ 4 $J_{4,5}$ 4	3.92dq $J_{4,5}$ 4 $J_{5,6}$ 6.5	1.27d $J_{5,6}$ 6.5	3.40s OCH_3	1.79bs OH	1.79bs OH
(13)	5.02dd $J_{1,2}$ 4 $J_{1,2'}$ 3	2.02m	1.63m	2.02m	3.89m	3.93m	1.21d $J_{5,6}$ 6	3.37s [a] OCH_3	3.33s [a] OCH_3	

[a] May be interchanged in any horizontal column.

Table 4. ^{13}C N.m.r. data for L-digitoxose derivatives [$\delta_C(CDCl_3)$, SFOR]

	Pyranosides										Furanosides			
Carbon	(6)	(7)	$\Delta\delta_C$(7) → (6)	(4)	(5)	$\Delta\delta_C$(5) → (4)	(8)	(9)	(10)	$\Delta\delta_C$(10) → (9)	(11)	(12)	$\Delta\delta_C$(12) → (11)	(13)
C-1	98.4	99.0 (d)	−0.6	98.4	99.0	−0.6	99.2	97.6	98.9	−1.3	105.3	105.5	−0.2	105.1
C-2	35.2	37.7 (t)	−2.5	35.2	36.7	−1.5	35.9	32.1	34.1	−2.0	42.0	42.7	−0.7	38.8
C-3	67.4	68.0 (d)	−0.6	63.5	64.0	−0.5	66.4	72.4	73.3	−0.9	67.7	68.7	−1.0	79.4
C-4	72.6	73.2 (d)	−0.6	82.4	82.8	−0.4	81.4	82.7	82.9	−0.2	90.9	91.1	−0.2	86.4
C-5	64.4	69.6 (d)	−5.2	62.4	68.2	−5.8	69.7	63.1	69.1	−6.0	71.2	71.1	+0.1	67.2
C-6	17.8	18.1 (q)	−0.3	18.0	18.2	−0.2	18.3	17.9	18.3	−0.4	18.6	18.9	−0.3	18.0
1-OCH_3	55.1	56.5 (q)	−1.4	55.1	56.5	−1.4	56.4	55.2	56.4	−1.2	54.8	55.4	−0.6	55.1
3-OCH_3								57.6	57.1	+0.5				57.3
4-OCH_3				56.5	57.3	−0.8	57.5	57.0	57.5	−0.5				
3-$OCOC_6H_5$							165.7							
3-$OCOC_6H_5$							133.6							
							133.1							
							130.2							
							129.8							
							128.5							



(14) $R^1 = R^2 = R^3 = R^4 = H$

(15) $R^1 = CH_3, R^2 = R^3 = R^4 = H$

(16) $R^1 = R^4 = H, R^2 = R^3 = Ac$

(17) $R^1 = CH_3, R^2 = R^3 = Ac, R^4 = H$

(18) $R^1 = R^2 = R^4 = H, R^3 = 4\text{-}IC_6H_4CO$

(19) $R^1 = CH_3, R^2 = R^4 = H, R^3 = 4\text{-}IC_6H_4CO$

(20) $R^1 = R^4 = H, R^2 = R^3 = 4\text{-}IC_6H_4CO$

(21) $R^1 = CH_3, R^2 = R^3 = 4\text{-}IC_6H_4CO, R^4 = H$

(22) $R^1 = R^2 = H, R^3 = R^4 = CH_3$

(5) on treatment with benzoyl chloride in pyridine afforded the 3-*O*-benzoate (8). The data for (8) (Tables 2—4, Figure 2) clearly supported the presence of a benzoyl group at C-3, thus giving additional support to the assigned structures (4) and (5). Methyl 2,6-dideoxy-α-L-*ribo*-hexopyranoside (6) and the β-anomer (7) on methylation with sodium hydride and methyl iodide gave the corresponding 3,4-di-*O*-methyl derivatives (9) and (10). Data for the latter are given in Tables 2—4 and Figure 2.

O-β-D-Kijanosyl-(1→17)-kijanolide (14) on treatment with diazomethane gave the 26-*O*-methyl ether (15). The e.i. mass spectrum of (15) showed a molecular ion at *m/z* 796 and several fragment ions (see Experimental section and Figure 1). The ^{252}Cf-p.d.m.s. of (15) revealed positive molecular ions at *m/z* 798 and 820 which were attributed to the $(M + H)^+$ and $(M + Na)^+$ ions respectively. The negative ion spectrum showed peaks at *m/z* 782 and 797. The latter ion was assigned to $(M - H)^-$ and the former to loss of CH_4 from the 26-*O*-methyl group as was observed earlier for (2). From these results it was deduced that the molecular weight of (15) was 796.8 ± 0.2, which was consistent with a molecular formula $C_{43}H_{60}N_2O_{12}$ (*M* 797.0). The difference between the molecular formulae of (2) and (15) was in excellent agreement with the proposed tetrasaccharide composition mentioned earlier. Positive fragment ions were observed for (15) at *m/z* 532, 549, and 566 corresponding to similar cleavages to those discussed above for (2). The *m/z* 258 peak was not observed for (15) since the molecule does not contain the tetrasaccharide unit.

The ^1H n.m.r. spectrum of (15) was recorded at 600 MHz (see Experimental section) and a doublet of doublets at δ_H 4.46 ($J_{1^E_{ax},2^E_{ax}}$ 10.0 Hz, $J_{1^E_{ax},2^E_{eq}}$ 2.0 Hz) due to the 1^E_{ax}-H indicated that the kijanose was equatorially linked to the aglycone. These data allowed us to assign umambiguously 1^E_{ax}-H in (2). The ^{13}C n.m.r. data for (15) are given in Table 1 and the $\Delta\delta_C$ values in going from (14) to (15) were consistent with those observed in going from (1) to (2). The $\Delta\delta_C$ values observed in going from (15) to

Table 5. Molecular rotation data

Compound [a]	$[\alpha]_D$/(°)	$[M]_{D\alpha}$/(°)	$[M]_{D\alpha} - [M]_{D\beta} = 2A$ (°)	Absolute stereochemistry
Methyl α-L-digitoxoside (6)	−170.7 [b]	−277		
Methyl β-L-digitoxoside (7)	+33.2 [b]	+54	−331	L
Methyl α-D-digitoxoside [17]	+174.0 [d]	+282		
Methyl β-D-digitoxoside [17]	−36.0 [d]	−58	+340	D
Methyl α-L-digitoxoside (furanoside) (11)	−135.8 [d]	−220		
Methyl β-L-digitoxoside (furanoside) (12)	+98.4 [b]	+159	−379	L
Methyl α-D-digitoxoside (furanoside) [17]	+140.0 [d]	+227		
Methyl β-D-digitoxoside (furanoside) [17]	−106.0 [d]	−172	+399	D
Methyl 4-*O*-methyl-α-L-digitoxoside (4)	−209.2 [c]	−368		
Methyl 4-*O*-methyl-β-L-digitoxoside (5)	−12.4 [c]	−22	−346	L
Methyl 3,4-di-*O*-methyl-α-L-digitoxoside (9)	−211.0 [c]	−401		
Methyl 3,4-di-*O*-methyl-β-L-digitoxoside (10)	−26.6 [c]	−51	−350	L
Methyl 3-*O*-methyl-α-D-digitoxoside [g]	+212.0 [e]	+373		
Methyl α-D-kijanoside (23)	+130.0 [c]	+341		
Methyl β-D-kijanoside (24)	+34.1 [c]	+89	+252	D
Methyl 3-amino-2,3,4,6-tetradeoxy-4-[(methoxycarbonyl)amino]-3-*C*-methyl-α-D-*xylo*-hexopyranoside (25)	+121.4 [c]	+282		
Methyl 3-amino-2,3,4,6-tetradeoxy-4-[(methoxycarbonyl)amino]-3-*C*-methyl-β-D-*xylo*-hexopyranoside (27)	−4.9 [c]	−11	+293	D
Methyl α-L-mycaroside (30) [21]	−138.1 [f]	−243		
Methyl β-L-mycaroside (31) [21]	+20.8 [f]	+37	−280	L
Kijanimicin (1)	−124.2 [c]	−1 636		
3[B]-*O*-Dedigitoxosylkijanimicin (53)	−129.5 [c]	−1 537		
O-β-D-Kijanosyl-(1→17)-kijanolide (14)	−37.6 [c]	−294		
32-*O*-Methylkijanolide (48)	−11.6 [c]	−66		

[a] Unless otherwise stated all sugars are hexopyranosides. [b] Run at *c* 0.3 in $CHCl_3$. [c] Run at *c* 0.3 in CH_3OH. [d] Run at *c* 1.0 in $CHCl_3$. [e] Run at *c* 1.2 in CH_3OH. [f] Run at *c* 0.3 in EtOH. [g] H. R. Bolliger and P. Ulrich, *Helv. Chim. Acta*, 1952, **35**, 93.

(2) were also consistent with the presence of a tetrasaccharide unit at C-9.

Treatment of *O*-β-D-kijanosyl-(1→17)-kijanolide (14) with acetic anhydride in pyridine at 25 °C afforded the 9,32-di-*O*-acetyl derivative (16). The e.i. mass spectrum of the latter showed a molecular ion at *m/z* 867 (see Experimental section) (Figure 1) supporting the presence of two acetyl groups. A six proton singlet at δ_H 2.15 in the ^1H n.m.r. spectrum was also observed due to the acetyl groups. The ^{13}C n.m.r. spectrum of (16) (Table 1) confirmed the presence of two acetyl groups in the molecule and from the $\Delta\delta_C$ values observed in going from (14) to (16) it was apparent that these were located on the hydroxy groups at C-9 and C-32. Some long-range effects were also observed in neighbouring double bonds (C-11,C-12 and C-18,C-19) upon acetylation of (14). The methyl ether (15) on treatment with acetic anhydride in pyridine gave the 9,32-di-*O*-acetyl-26-*O*-methyl ether (17). The latter gave rise to a molecular ion at *m/z* 880 in the e.i. mass spectrum (see Experimental section and Figure 1) and exhibited a singlet at δ_H 4.17 in the ^1H n.m.r. spectrum (see Experimental section) due to the 26-*O*-methyl group and a six proton singlet at δ_H 2.15 due to the two acetyl groups. The ^{13}C n.m.r. for (17) was consistent with the structure (Table 1) and the $\Delta\delta_C$ values observed in going from (16) to (17) paralleled those observed in going from (1) to (2) and from (14) to (15). The $\Delta\delta_C$ values in going from (15) to (17) were also in good agreement with those observed in going from (14) to (16).

Although crystals of the methyl ether (15) were obtained from methanol–chloroform they were not acceptable for *X*-ray analysis. We therefore prepared a series of four heavy-atom derivatives as follows. *O*-β-D-Kijanosyl-(1→17)-kijanolide (14) on treatment with 4-iodobenzoyl chloride in pyridine under controlled conditions gave either the 32-*O*-(4-iodobenzoyl) derivatives (18), or the 9,32-di-*O*-(4-iodobenzoyl) derivative (20). Each on treatment with diazomethane gave the corresponding 26-*O*-methyl ether derivative (19) and (21) respectively. The chemical data for the derivatives (18)—(21) were consistent with the proposed structures. The ^{13}C n.m.r. data (Table 1) and the $\Delta\delta_C$ values confirmed the presence and location of the 4-iodobenzoyl groups in (18)—(21). The iodobenzoate (18) crystallized as fine needles from aqueous acetone, or from methanol, but the crystals were not acceptable for *X*-ray analysis. The di-iodobenzoate (20) crystallized from tetrahydrofuran–hexane as fine needles, which were also unsatisfactory for *X*-ray studies. The methyl ethers (19) and (21) could be obtained only as amorphous solids. Further degradations were therefore required to elucidate the structure of kijanimicin (1).

Vigorous methanolysis of *O*-β-D-kijanosyl-(1→17)-kijanolide (14) with 5M-methanolic hydrogen chloride at 65 °C for 3 h afforded methyl α-D-kijanoside (23) and the β-anomer (24), but caused extensive decomposition of the aglycone. The α-anomer (23) failed to give a molecular ion in the e.i. mass spectrum, while the β-anomer showed only a very weak $M - H^+$ ion at *m/z* 261 (Table 6). The e.i. mass spectra did however reveal a number of fragment ions (Table 6) which were diagnostic for the assigned structures (23) and (24) (Figure 3). The c.i. mass spectra of (23) and (24) each gave an MH^+ ion at *m/z* 263 (Table 6, footnotes *i* and *j*) thus confirming the composition of methyl D-kijanoside as $C_{10}H_{18}N_2O_6$. A satisfactory microanalysis (C, H, N, O) was obtained for the crystalline β-anomer (24), but not for the gummy α-anomer (23). The u.v. spectra of (23) and (24) exhibited a single maximum at $\lambda_{max.}$ 199 nm consistent with the presence of a methylcarbamoyl group in the molecule. The i.r. spectra of (23) and

Table 6. Mass spectra of the D-kijanose derivatives [*m*/*z* (%)]

Compd.	$M^{+\cdot}$	$(M + H)^+$	$(M - H)^+$	B_1	B_2	B_3	B_4	B_5
(23) [l]				231 (1.6) [b]	218 (0.7) [b]	218 (0.7) [b]	216 (0.5) [b]	204 (0.5) [b]
(24) [j]			261 (0.1)		218 (0.1) [b]	218 (0.1) [b]	216 (0.1) [b]	
(25)	232 (0.7) [a]	233 (0.5)		201 (1.5) [c]				
(27)	232 (2.5) [a]	233 (1.2)		201 (1.1) [c]				
(26)	274 (0.1)	275 (0.1)		243 (1.6) [d]				
(28)	274 (0.1) [a]	275 (0.3)	273 (0.1)	243 (0.6) [d]				
(29) [k]	276 (0.6)		275 (0.6)	245 (22.8) [e]			230 (3.2) [e]	
(29) [l]	276 (1.2)	277 (0.2)		245 (4.13) [e]			230 (3.4) [e]	
(56)		351 (0.01)	349 (0.01)	231 (14.9) [f]				
(57)				231 (0.7) [g]			216 (3.7) [g]	
(58)				231 (5.9) [h]				

Compd.	B_6	B_7	B_8	B_9	B_{10}	B_{11}	B_{12}
(23)	184 (23.7) [a,b]	184 (23.7) [a,b]	172 (34.8) [a,b]	156 (27.9) [a,b]	152 (5.1) [a,b]	140 (71.0) [a,b]	128 (86.8) [a,b]
(24)	184 (2.2) [a,b]	184 (2.2) [a,b]	172 (11.1) [a,b]	156 (15.2) [a,b]	152 (2.2) [a,b]	140 (36.7) [a,b]	128 (100) [a,b]
(25)	184 (5.6) [c]	184 (5.6) [c]					
(27)	184 (2.3) [c]	184 (2.3) [c]					
(26)	184 (10.8) [d]	184 (10.8) [d]					
(28)	184 (9.0) [d]	184 (9.0) [d]					
(29) [k]	198 (8.9) [e]		186 (100) [e]	170 (25.4) [e]			142 (98.4) [e]
(29) [l]	198 (25.1) [e]		186 (100) [e]	170 (22.2) [e]			142 (99.6) [e]
(56)	184 (98.7) [f]	184 (98.7) [f]		156 (32.5) [f]	152 (16.7) [f]		128 (100) [f]
(57)	184 (7.6) [g]	184 (7.6) [g]		156 (10.6) [g]	152 (4.2) [g]		128 (100) [g]
(58)	184 (41.5) [h]	184 (41.5) [h]		156 (7.1) [h]	152 (5.4) [h]		128 (33.5) [h]

Compd.	B_{13}	B_{14}	B_{15}	B_{16}	B_{17}	B_{18}	B_{19}
(23)	124 (8.5) [a]	96 (37.6) [a]	184 (23.7) [a,b]				
(24)	124 (4.5) [a]	96 (20.6) [a]	184 (2.2) [a,b]				
(25)			184 (5.6) [c]	169 (2.1) [c]	157 (9.4) [c]	140 (1.8) [c]	130 (2.8) [c]
(27)			184 (2.3) [c]	169 (4.5) [c]	157 (5.8) [c]	140 (2.0) [c]	130 (7.1) [c]
(26)			184 (10.8) [d]	211 (0.9) [d]	199 (4.9) [d]	140 (9.3) [d]	172 (4.4) [d]
(28)			184 (9.0) [d]	211 (0.5) [d]	199 (15.4) [d]	140 (30.8) [d]	172 (23.6) [d]
(29) [k]			198 (8.9) [e]			154 (90.3) [e]	
(29) [l]			198 (25.1) [e]			154 (81.5) [e]	
(56)	124 (12.3)	96 (26.0)	184 (98.7) [f]			140 (79.0) [f]	
(57)	124 (4.3)	96 (16.0)	184 (7.6) [g]			140 (47.7) [g]	
(58)	124 (4.0)	96 (8.1)	184 (41.5) [h]			140 (16.5) [h]	

Compd.	B_{20}	B_{21}	B_{22}	B_{23}	B_{24}	B_{25}	B_{26}
(23)							
(24)							
(25)	86 (30.3) [c]	118 (77.9) [c]	100 (100) [c]	131 (2.2)	130 (2.8)	86 (30.3)	
(27)	86 (35.8) [c]	118 (100) [c]	100 (99.7) [c]	131 (3.3)	130 (7.1)	86 (35.8)	
(26)	128 (11.3) [d]	160 (34.9) [d]	142 (38.3) [d]		130 (8.5)	86 (20.2)	115 (100)
(28)	128 (13.5) [d]	160 (20.0) [d]	142 (23.9) [d]		130 (41.6)	86 (21.9)	115 (100)
(29) [k]							
(29) [l]							
(56)							
(57)							
(58)							

[a] Composition confirmed by high resolution mass spectrometry. [b] $R^1 = CH_3$, $R^2 = NO_2$, $R^3 = H$. [c] $R^1 = CH_3$, $R^2 = NH_2$, $R^3 = H$. [d] $R^1 = CH_3$, $R^2 = NHCOCH_3$, $R^3 = H$. [e] $R^1 = CH_3$, $R^2 = NO_2$, $R^3 = CH_3$. [f] $R^1 = CH_3CHOH{-}CH{-}CH_2CH_2OH$, $R^2 = NO_2$, $R^3 = H$. [g] $R^1 = -CH_2CH_2CH_2OH$, $R_2 = NO_2$, $R^3 = H$. [h] $R^1 = -CH{=}CH_2$, $R^2 = NO_2$, $R^3 = H$. [i] The c.i.m.s. showed the following fragment ions: 263 (2.4), 231 (21.1), 216 (8.4), 184 (100), 172 (5.9), 156 (9.3), 140 (11.1), 128 (3.0). [j] The c.i.m.s. showed the following fragment ions: 263 (0.5), 231 (23.8), 216 (1.9), 184 (100), 172 (12.0), 156 (5.2), 140 (10.6), 128 (1.2). [k] Less polar product. [l] More polar product.

(24) revealed the presence of NH, nitro, and carbamoyl groups in kijanose. The ^1H n.m.r. data for (23) and (24) are given in Table 7. The α-anomer (23) revealed the anomeric proton as a doublet of doublets at δ_H 4.59 ($J_{1eq,2eq}$ 1 Hz, $J_{1eq,2ax}$ 4 Hz) and a methyl group at C-6 as a doublet at δ_H 1.19 ($J_{5ax,6}$ 6 Hz) indicating that kijanose was a 2,6-dideoxy sugar. The absence of any additional coupling between H_{2ax} or H_{2eq}, other than geminal coupling, or coupling with H_{1eq}, as well as the presence of a tertiary methyl group at δ_H 1.52, suggested that kijanose had a quaternary carbon at C-3 bearing a methyl group. The occurrence of 5_{ax}-H as a doublet of quartets at δ_H 4.22 ($J_{5ax,6}$ 6 Hz, $J_{5ax,4eq}$ 1 Hz) indicated that the substituent at C-4 was axially oriented. A doublet of doublets for 4eq-H at δ_H 4.40 ($J_{4eq,5ax}$ 1 Hz, $J_{4eq,NH}$ 10 Hz), and the presence of a doublet at δ_H 5.00 ($J_{4eq,NH}$ 10 Hz) and a singlet at δ_H 3.70 indicated that kijanose contained an axial methylcarbamoyl group at C-4. The nitro-group therefore was located on the quaternary carbon at C-3. The β-anomer (24) in addition to showing the expected larger couplings between 1ax-H and 2eq-H and 2ax-H, also showed long range W-coupling ($J_{2eq,4eq}$ 1 Hz) between 2eq-H and 4eq-H lending additional support to the presence of an axial substituent at C-4. The ^1H n.m.r. data did not reveal the relative stereochemistry at C-3 and has indeed in the past led to misassign-

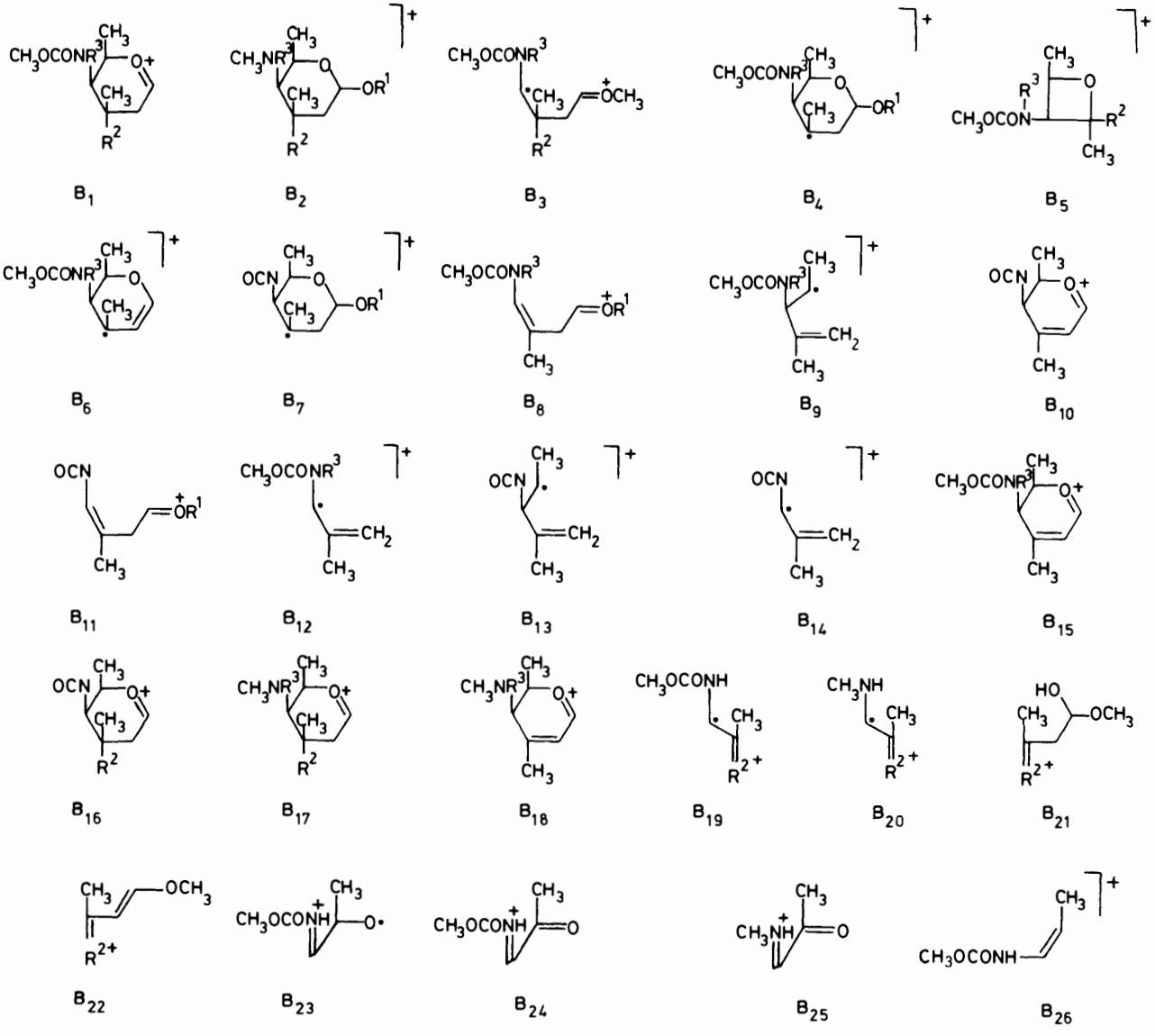
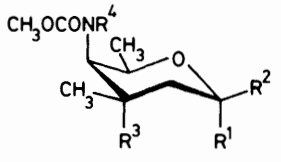


Figure 3. Mass spectral fragment ions for D-kijanose derivatives (Table 6)



(23) $R^1 = OCH_3, R^2 = R^4 = H, R^3 = NO_2$
(24) $R^1 = R^4 = H, R^2 = OCH_3, R^3 = NO_2$
(25) $R^1 = OCH_3, R^2 = R^4 = H, R^3 = NH_2$
(26) $R^1 = OCH_3, R^2 = R^4 = H, R^3 = NHAc$
(27) $R^1 = R^4 = H, R^2 = OCH_3, R^3 = NH_2$
(28) $R^1 = R^4 = H, R^2 = OCH_3, R^3 = NHAc$
(29) $R^1 = OCH_3, R^2 = H, R^3 = NO_2, R^4 = CH_3$

ment of the stereochemistry of the tertiary carbon in L-evernitrose.[18,19] The ^{13}C n.m.r. data for (23) and (24) are given in Table 8. The chemical shifts were consistent with the location of the various substituents as described above. From the shielding at C-4 it was apparent that the carbon was directly bound to the NH of the methylcarbamoyl group. The strong deshielding of C-3 was also indicative of the presence of a nitro-group on a quaternary carbon at C-3. From the chemical shifts of the 3-methyl group at δ_C 26.4 in (23) and at δ_C 25.4 in (24) it is now possible to conclude that the 3-methyl group is equatorially oriented [20] and that the nitro-group is therefore axial. However, at the time the structures of (23) and (24) were elucidated, the results of this ^{13}C n.m.r. study [20] were not available to us and we therefore used an alternative, unambigous proof, to deduce the relative stereochemistry at C-3, which was not dependent on having both epimers at C-3. This was done as follows.

A mixture of methyl α-L-mycaroside (30) and the β-anomer (31) [21] on treatment with toluene-*p*-sulphonyl chloride in pyridine at ambient temperature, afforded methyl 4-*O*-(*p*-tolylsulphonyl)-α-L-mycaroside (32) and the β-anomer (33) which were separated by chromatography. The α-anomer (32) and β-anomer (33) were each treated with sodium azide in hexamethylphosphoric triamide at 115 °C for 66 h to give

Table 7. ^{1}H N.m.r. data for D-kijanose derivatives [δ_H($CDCl_3$), multiplicity, J in Hz]

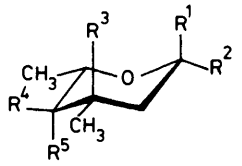
Compd.	1-H	2eq-H	2ax-H	4eq-H	5ax-H	6-CH_3	3-CH_3	1-OCH_3	4-N*H*COOCH$_3$	4-NHCOOC*H*$_3$	Other substituents		
(23)	4.59dd $J_{1eq,2eq}$ 1 $J_{1eq,2ax}$ 4 1eq-H	2.71dd $J_{1eq,2eq}$ 1 $J_{2eq,2ax}$ 15	1.78dd $J_{1eq,2ax}$ 4 $J_{2eq,2ax}$ 15	4.40dd $J_{4eq,NH}$ 10 $J_{4eq,5ax}$ 1	4.22dq $J_{4eq,5ax}$ 1 $J_{5ax,6}$ 6	1.19d $J_{5ax,6}$ 6	1.52s	3.19s	5.00d $J_{4eq,NH}$ 10	3.70s			
(24)	4.47dd $J_{1ax,2eq}$ 2.5 $J_{1ax,2ax}$ 10 1ax-H	2.69ddd $J_{1ax,2eq}$ 2.5 $J_{2eq,2ax}$ 15 $J_{2eq,4eq}$ 1	1.54dd $J_{1ax,2ax}$ 10 $J_{2eq,2ax}$ 15	4.44ddd $J_{2eq,4eq}$ 1 $J_{4eq,NH}$ 10 $J_{4eq,5ax}$ 1	3.60dq $J_{4eq,5ax}$ 1 $J_{5ax,6}$ 6	1.23d $J_{5ax,6}$ 6	1.59s	3.51s	5.14d $J_{4eq,NH}$ 10	3.75s			
(25)	4.69dd $J_{1eq,2eq}$ 2.5 $J_{1eq,2ax}$ 2.5 1eq-H			3.26ddd $J_{2eq,4eq}$ 1 $J_{4eq,NH}$ 10 $J_{4eq,5ax}$ 1	4.38dq $J_{4eq,5ax}$ 1 $J_{5ax,6}$ 6.5	1.16d $J_{5ax,6}$ 6.5	1.09s	3.32s	4.95d $J_{4eq,NH}$ 10	3.68s			
(27)	4.63dd $J_{1ax,2eq}$ 4 $J_{1ax,2ax}$ 8 1ax-H			3.17ddd $J_{2eq,4eq}$ 1 $J_{4eq,NH}$ 10.5 $J_{4eq,5ax}$ 2	4.31dq $J_{4eq,5ax}$ 2 $J_{5ax,6}$ 6.5	1.17d $J_{5ax,6}$ 6.5	1.18s	3.46s	5.07d $J_{4eq,NH}$ 10.5	3.66s			
(26)	4.68dd $J_{1eq,2eq}$ 3 $J_{1eq,2ax}$ 3 1eq-H			4 32ddd $J_{2eq,4eq}$ 1 $J_{4eq,NH}$ 10 $J_{4eq,5ax}$ 1	4.22dq $J_{4eq,5ax}$ 1 $J_{5ax,6}$ 6	1.14d $J_{5ax,6}$ 6	1.45s	3.37s	4.83d $J_{4eq,NH}$ 10	3.68s	1.93s 3-NHAc		
(28)	4.45dd $J_{1ax,2eq}$ 3 $J_{1ax,2ax}$ 10 1ax-H					1.18d $J_{5ax,6}$ 6	1.57s	3.47s	5.07d $J_{4eq,NH}$ 10	3.67s	1.97s 3-NHAc		
(29) [a,c]	4.87dd $J_{1eq,2eq}$ 3 $J_{1eq,2ax}$ 4 1eq-H	1.97dd $J_{1eq,2eq}$ 3 $J_{2eq,2ax}$ 14	2.34/2.82dd $J_{1eq,2ax}$ 4 $J_{2eq,2ax}$ 14		4.02m	1.25d $J_{5ax,6}$ 6	1.63/ 1.70/ 1.77s	3.38s		3.69/ 3.70/ 3.73s	2.87/ 2.88/ 3.03s 4-N-CH_3		
(29) [a,d]	4.76dd $J_{1eq,2eq}$ 1 $J_{1eq,2ax}$ 4	2.93ddd $J_{1eq,2eq}$ 1 $J_{2eq,2ax}$ 15 $J_{2eq,4eq}$ 1	1.98/2.02dd $J_{1eq,2ax}$ 4 $J_{2eq,2ax}$ 15	5.13/5.34dd $J_{2eq,4eq}$ 1 $J_{4eq,5ax}$ 3.5	4.34dq $J_{4eq,5ax}$ 3.5 $J_{5ax,6}$ 7	1.24d $J_{5ax,6}$ 7	1.47/ 1.48s	3.25s		3.97s	3.08/ 3.10s 4-N-CH_3		
(56) [b]	4.70/ 4.72dd $J_{1ax,2eq}$ 2 $J_{1ax,2ax}$ 10 1ax-H	2.71 broad ddd $J_{1ax,2eq}$ 2 $J_{2eq,2ax}$ 15 $J_{2eq,4eq}$ 1	1.60 broad dd $J_{1ax,2ax}$ 10 $J_{2eq,2ax}$ 15	4.38ddd $J_{2eq,4eq}$ 1 $J_{4eq,NH}$ 10 $J_{4eq,5ax}$ 1		1.20d $J_{5ax,6}$ 6	1.57s		5.19d $J_{4eq,NH}$ 10	3.73s	1.13/ 1.17d J_{5',CH_3} 6		
(57)	4.50dd $J_{1ax,2eq}$ 2 $J_{1ax,2ax}$ 10 1ax-H	2.63ddd $J_{1ax,2eq}$ 2 $J_{2eq,2ax}$ 15 $J_{2eq,4eq}$ 1	1.50dd $J_{1ax,2ax}$ 10 $J_{2eq,2ax}$ 15	4.36ddd $J_{2eq,4eq}$ 1 $J_{4eq,NH}$ 10 $J_{4eq,5ax}$ 1		1.20d $J_{5ax,6}$ 6	1.57s		5.12d $J_{4eq,NH}$ 10	3.70s	1.80tt $J_{1',2'}$ 6 $J_{2',3'}$ 6 2'-CH_2		
(58)	4.84dd $J_{1ax,2eq}$ 3 $J_{1ax,2ax}$ 10 1ax-H	2.72ddd $J_{1ax,2eq}$ 3 $J_{2eq,2ax}$ 14.5 $J_{2eq,4eq}$ 1	1.62dd $J_{1ax,2ax}$ 10 $J_{2eq,2ax}$ 14.5	4.37ddd $J_{2eq,4eq}$ 1 $J_{4eq,NH}$ 10 $J_{4eq,5ax}$ 1	3.60dq $J_{4eq,5ax}$ 1 $J_{5ax,6}$ 6	1.20d $J_{5ax,6}$ 6	1.59s		5.08d $J_{4eq,NH}$ 10	3.74s	4.20dd $J_{Ha,Hb}$ 2 $J_{Hb,Hc}$ 6 H_b	4.52dd $J_{Ha,Hb}$ 2 $J_{Ha,Hc}$ 14 H_a	6.38dd $J_{Ha,Hc}$ 14 $J_{Hb,Hc}$ 6 H_c

[a] Mixture of rotamers at ambient temperature. [b] Appears to be a mixture of diastereoisomers at C-4'. [c] Less polar product. [d] More polar product.

Table 8. ^{13}C N.m.r. data for D-kijanose derivatives [$\delta_C(CDCl_3)$, SFOR]

Carbon	(23)	(24)	$\Delta\delta_C(24) \rightarrow (23)$	(25)	(27)	$\Delta\delta_C(27) \rightarrow (25)$	(26)	(29) [b,d]	(29) [b,e]	(56) [c]	(57)	(58)
C-1	96.9 (d)	99.9	−3.0	99.1	100.4	−1.3	98.5	97.3/97.4	96.6	98.9/99.7 (d)	98.8 (d)	97.5 (d)
C-2	34.6 (t)	35.8	−1.2	37.4	40.2	−2.8	36.7	31.5/31.6	31.7/32.1	35.8/35.9 (t)	35.9 (t)	35.1 (t)
C-3	86.0 (s)	90.7	−4.7	59.3	57.8	+1.5	52.4	*a*	86.3/86.4	90.7 (s)	90.7 (s)	90.6 (s)
C-4	52.8 (d)	53.9	−1.1	50.7	53.0	−2.3	54.5	53.2/53.3	53.3	53.6 (d)	53.8 (d)	53.7 (d)
C-5	62.3 (d)	68.9	−6.6	61.4	68.2	−6.8	62.6	62.7/62.0/61.7	62.7/63.0	69.2 (d)	68.9 (d)	69.3 (d)
C-6	16.9 (q)	16.9		17.4	17.2	+0.2	17.3	18.8	16.9/17.0	16.9 (q)	17.0 (q)	16.9 (q)
1-OCH_3	55.0 (q)	56.6	−1.6	55.2	56.4	−1.2	55.3	55.0/55.2	54.7			
3-CH_3	26.4 (q)	25.4	+1.0	23.1	30.7	−7.6	24.6	21.6/22.3	25.6/25.8	25.3 (q)	25.4 (q)	25.3 (q)
3-$NHCOCH_3$							*a*					
3-$NHCOCH_3$							22.9					
4-$NHCOOCH_3$	157.4 (s)	157.4		157.6	157.6		*a*	*a*	*a*	157.5 (s)	157.4 (s)	157.4 (s)
4-$NHCOOCH_3$	52.8 (q)	52.7	+0.1	52.3	52.3		52.4	52.8/53.1	53.7/53.9	52.8 (q)	52.8 (q)	52.8 (q)
N-CH_3								39.8	36.5/36.6			
C-1′										59.0 (t)	60.3 (t)	148.9 (d)
C-2′										32.2/33.7 (t)	32.3 (t)	92.5 (t)
C-3′										80.8/81.3 (d)	67.3 (t)	
C-4′										69.2/69.9 (d)		
C-5′										18.0/19.2 (q)		

[a] Not measured under conditions that the spectrum was run. [b] Mixture of rotamers at ambient temperature. [c] Mixture of diastereoisomers at C-4′. [d] Less polar product. [e] More polar product.



(30) $R^1 = OCH_3, R^2 = R^5 = H, R^3 = R^4 = OH$
(31) $R^1 = R^5 = H, R^2 = OCH_3, R^3 = R^4 = OH$
(32) $R^1 = OCH_3, R^2 = R^5 = H, R^3 = OH, R^4 = 4\text{-}CH_3C_6H_4SO_2O$
(33) $R^1 = R^5 = H, R^2 = OCH_3, R^3 = OH, R^4 = 4\text{-}CH_3C_6H_4SO_2O$
(34) $R^1 = OCH_3, R^2 = R^4 = H, R^3 = OH, R^5 = N_3$
(35) $R^1 = OCH_3, R^2 = R^4 = H, R^3 = OH, R^5 = NH_2$
(36) $R^1 = R^4 = H, R^2 = OCH_3, R^3 = OH, R^5 = N_3$
(37) $R^1 = R^4 = H, R^2 = OCH_3, R^3 = OH, R^5 = NH_2$
(38) $R^1 = R^4 = H, R^2 = OCH_3, R^3 = OH, R^5 = NHCOOCH_3$
(39) $R^1 = R^5 = H, R^2 = OCH_3, R^3 = OH, R^4 = OCOCH_2CH_3$
(40) $R^1 = R^5 = H, R^2 = OCH_3, R^3 = OAc, R^4 = OCOCH_2CH_3$
(41) $R^1 = R^5 = H, R^2 = OCH_3, R^3 = OH, R^4 = OAc$

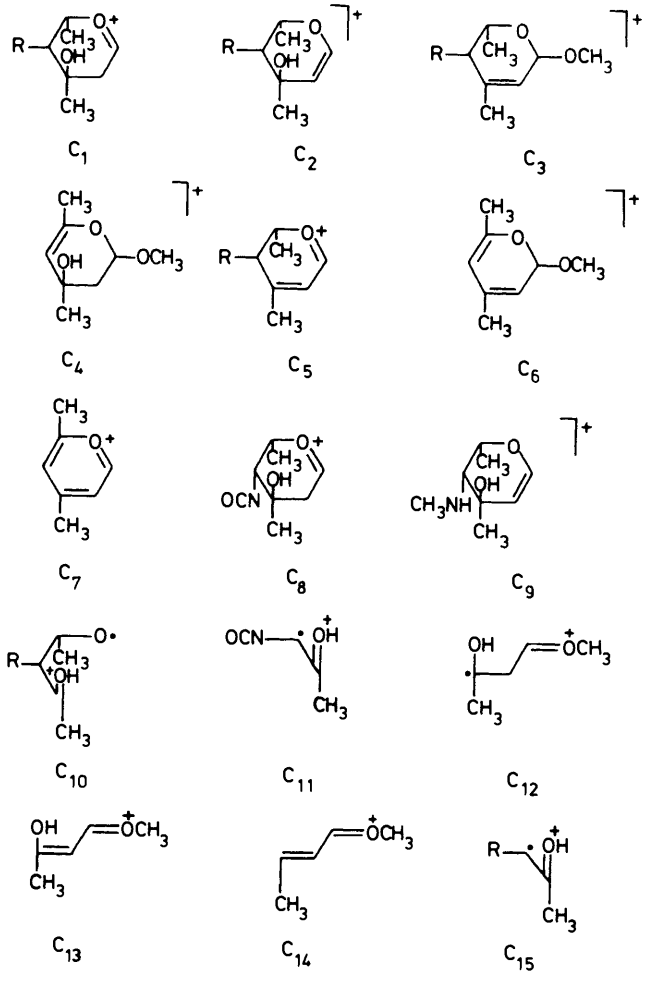


Figure 4. Mass spectral fragment ions for L-mycarose derivatives (Table 9)

Table 9. Mass spectra of the L-mycarose derivatives [*m*/*z* (%)]

Compd.	$M^{+\cdot}$	$(M + H)^+$	$(M - H)^+$	C_1	C_2	C_3
(32) [c]		331 (0.1)	329 (0.1)	299 (2.1) [b]	298 (5.8) [b]	
(33) [c]			329 (0.5)	299 (0.8) [b]	298 (0.3) [b]	
(34)				170 (0.8) [a,d]	169 (0.2) [d]	
(36)	201 (0.1)		200 (0.3)	170 (0.1) [d]		
(35)	175 (0.5)	176 (0.1)	174 (0.1)	144 (1.8) [e]	143 (1.6) [e]	157 (0.9) [e]
(37)		176 (0.2)	174 (0.3)	144 (1.8) [e]	143 (1.2) [e]	157 (0.4) [e]
(38)	233 (0.2)		232 (0.2)	202 (0.2) [f]	201 (0.4) [f]	

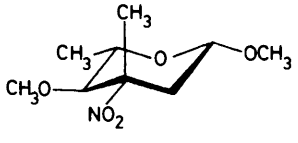
Compd.	C_4	C_5	C_6	C_7	C_8	C_9
(32)		281 (5.3) [b]	140 (6.3)	109 (16.6)		
(33)		281 (2.3) [b]	140 (21.8)	109 (9.6)		
(34)	158 (0.1) [a]		140 (0.2)	109 (0.3) [a,d]		
(36)			140 (0.1)	109 (0.5) [d]		
(35)	158 (0.5)	126 (3.5) [a,e]		109 (4.5) [a,e]		
(37)	158 (0.2)	126 (2.7) [e]		109 (0.4) [e]		
(38)	158 (0.2)	184 (2.2) [a,f]		109 (1.0) [a,f]	170 (0.7)	157 (1.1)

Compd.	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}
(32)			102 (3.2)	101 (37.8)	85 (15.8)	228 (8.0) [b]
(33)			102 (3.5)	101 (30.1)	85 (14.0)	228 (94.5) [b]
(34)			102 (5.2) [a]	101 (22.5) [a]	85 (4.3)	99 (2.5) [a,d]
(36)			102 (30,8)	101 (15.4)	85 (5.8)	99 (5.5) [d]
(35)	117 (1.2) [a,e]		102 (1.6)	101 (4.4) [a]	85 (10.7)	73 (30.9) [e]
(37)	117 (1.8) [e]		102 (1.1)	101 (3.5)	85 (16.5)	73 (100) [e]
(38)	175 (0.4) [a,f]	99 (12.2) [a]	102 (3.2)	101 (3.5) [a]	85 (3.8) [a]	131 (100) [a,f]

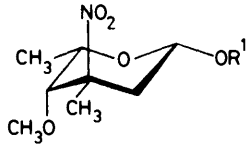
[a] Composition confirmed by high resolution mass spectrometry. [b] R = 4-$CH_3C_6H_4SO_2O$. [c] Also contained fragment ions at: *m*/*z* 155 (100) $CH_3C_6H_4SO_2^+$ and *m*/*z* 91 (86.2) $CH_3C_6H_4^+$ for (32) and *m*/*z* 91 (91.0) $CH_3C_6H_4^+$ for (33). [d] R = N_3. [e] R = NH_2. [f] R = $NHCOOCH_3$.

the corresponding azido-derivatives (34) and (36) respectively. The latter were each subjected to catalytic hydrogenation over 10% palladium on carbon to give the 4-amino sugars (35) and (37) respectively. The β-anomer (37) on treatment with methyl chloroformate in the presence of sodium carbonate afforded methyl 2,4,6-trideoxy-4-(methoxycarbonylamino)-3-*C*-methyl-β-L-*xylo*-hexopyranoside (38). The e.i. mass spectral data for (32)—(38) are given in Table 9 and the fragment ions are given in Figure 4. The ^{1}H n.m.r. data for (32)—(38) are given in Table 10 and the ^{13}C n.m.r. data are given in Table 11. Comparison of the ^{1}H n.m.r. data for the model compound (38) with that of methyl β-D-kijanoside (24) (Table 12) revealed marked deshielding of the vicinal *cis* protons 2eq-H and 4eq-H in going from the 3-axial hydroxy compound (38) to the 3-axial nitro compound (24), while little effect was observed on the vicinal *trans* proton 2ax-H. This was in excellent agreement with the results obtained in going from methyl 4-*O*-propionyl-β-L-mycaroside (39) [21] to methyl 3-*O*-acetyl-4-*O*-propionyl-β-L-mycaroside (40) [21] (Table 12) and are what one would have predicted *a priori*. Comparison of the ^{13}C n.m.r. data for (38) with that of methyl β-D-kijanoside (24) (Table 13) revealed pronounced shielding of C-2 and C-4, both of which bear vicinal *cis* protons to the axial nitro group.[15] These data were in excellent agreement with the observed shielding at C-2, which bears a vicinal *cis* proton, and the complete absence of any shielding at C-4, which does not bear a vicinal *cis* proton to the 3-*O*-acetyl group, in going from methyl 4-*O*-acetyl-β-L-mycaroside (41) [15] to methyl 3-*O*-acetyl-4-*O*-propionyl-β-L-mycaroside (40) [15] (Table 13). The above results unambiguously establish the fact that kijanose has the *xylo*-configuration.

It remained therefore to establish the absolute stereochemistry of kijanose and this was done as follows: The molecular rotations of methyl α-D-kijanoside (23) and the β-anomer (24) are given in Table 5 and the application of Hudson's Rules of Isorotation [16] to these gave a 2A value of

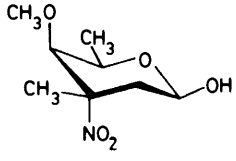


(42)



(43) R^1 = H

(44) R^1 = Ac



(45)

+252° which was in excellent agreement with what would be expected for a D-sugar. By way of comparison methyl α-L-mycaroside (30) [21] and the β-anomer (31) [21] (Table 5) give a 2A value of −280°. It therefore follows that the structure of D-kijanose is 2,3,4,6-tetradeoxy-4-methoxycarbonylamino-3-*C*-methyl-3-nitro-D-*xylo*-hexopyranose. D-Kijanose therefore represents the third nitro sugar to have been isolated from an antibiotic, the others being L-evernitrose [18,19] [methyl β-L-evernitroside (42)] and L-rubranitrose (43) [22] the latter being isolated from the antibiotic rubradirin.[23] Rubranitrose which has the same relative stereochemistry as D-kijanose was claimed to have the L-configuration by comparison of the c.d. spectra of the β-1-acetate (44) with that of methyl β-L-evernitroside (42).[22] The 1-*O*-acetyl-β-rubranitrose (44) was found to have a positive extremum at $[\theta]_{285}$ + 2 500, whereas methyl β-L-evernitroside (42) exhibited a negative extremum at $[\theta]_{285}$ − 1 200. It was therefore concluded [22] that the absolute stereochemistry at C-3 was opposite in these two sugars. Since the relative stereochemistry of rubranitrose was known from a single crystal *X*-ray analysis [22] it was concluded that the

Table 10. ^{1}H N.m.r. data for L-mycarose derivatives [$\delta_H(CDCl_3)$, multiplicity, J in Hz]

Compd.	1-H	2eq-H	2ax-H	4-H	5ax-H	6-CH_3	3-CH_3	1-OCH_3	Other substituents		
(32)	4.72dd $J_{1eq,2eq}$ 2 $J_{1eq,2ax}$ 3 1eq-H	2.04dd $J_{1eq,2eq}$ 2 $J_{2eq,2ax}$ 14.5	1.80dd $J_{1eq,2ax}$ 3 $J_{2eq,2ax}$ 14.5	4.31d $J_{4ax,5ax}$ 10 4ax-H	4.00dq $J_{4ax,5ax}$ 10 $J_{5ax,6}$ 6	1.23d $J_{5ax,6}$ 6	1.12s	3.33s	2.42s $-C_6H_4CH_3$	7.29d $J_{2',3'}$ 8 $J_{5',6'}$ 8 2′-H, 6′-H-Ar	7.78d $J_{2',3'}$ 8 $J_{5',6'}$ 8 3′-H, 5′-H-Ar
(33)	4.64dd $J_{1ax,2eq}$ 2.5 $J_{1ax,2ax}$ 9 1ax-H	2.02dd $J_{1ax,2eq}$ 2.5 $J_{2eq,2ax}$ 13.5	1.58ddd $J_{1ax,2ax}$ 9 $J_{2eq,2ax}$ 13.5 $J_{2ax,?}$ 2	4.27d $J_{4ax,5ax}$ 10 4ax-H	3.83dq $J_{4ax,5ax}$ 10 $J_{5ax,6}$ 6	1.06d $J_{5ax,6}$ 6	1.22s	3.42s	2.42s $-C_6H_4CH_3$	7.30d $J_{2',3'}$ 8 $J_{5',6'}$ 8 2′-H, 6′-H-Ar	7.78d $J_{2',3'}$ 8 $J_{5',6'}$ 8 3′-H, 5′-H-Ar
(34)	4.76dd $J_{1eq,2eq}$ 1.5 $J_{1eq,2ax}$ 4 1eq-H	1.61ddd $J_{1eq,2eq}$ 1.5 $J_{2eq,2ax}$ 14 $J_{2eq,4eq}$ 1.5	1.84dd $J_{1eq,2ax}$ 4 $J_{2eq,2ax}$ 14	2.97dd $J_{2eq,4eq}$ 1.5 $J_{4eq,5ax}$ 2 4eq-H	4.30dq $J_{4eq,5ax}$ 2 $J_{5ax,6}$ 6.5	1.28d $J_{5ax,6}$ 6.5	1.24s	3.32s	4.28bs 3-OH		
(36)	4.60dd $J_{1ax,2eq}$ 4 $J_{1ax,2ax}$ 7 1ax-H	1.72ddd $J_{1ax,2eq}$ 4 $J_{2eq,2ax}$ 14 $J_{2eq,4eq}$ 1	1.54dd $J_{1ax,2ax}$ 7 $J_{2eq,2ax}$ 14	2.87dd $J_{2eq,4eq}$ 1 $J_{4eq,5ax}$ 1 4eq-H	4.17dq $J_{4eq,5ax}$ 1 $J_{5ax,6}$ 7	1.37d $J_{5ax,6}$ 7	1.42s	3.48s			
(35)	4.74dd $J_{1eq,2eq}$ 1 $J_{1eq,2ax}$ 4 1eq-H	1.58ddd $J_{1eq,2eq}$ 1 $J_{2eq,2ax}$ 14 $J_{2eq,2eq}$ 1	1.89dd $J_{1eq,2eq}$ 4 $J_{2eq,2ax}$ 14	2.35dd $J_{2eq,4eq}$ 1 $J_{4eq,5ax}$ 2 4eq-H	4.32dq $J_{4eq,5ax}$ 2 $J_{5ax,6}$ 6	1.23d $J_{5ax,6}$ 6	1.27s	3.39s			
(37)	4.66dd $J_{1ax,2eq}$ 4 $J_{1ax,2ax}$ 8 1ax-H	1.66ddd $J_{1ax,2eq}$ 4 $J_{2eq,2ax}$ 14 $J_{2eq,4eq}$ 1	1.50dd $J_{1ax,2ax}$ 8 $J_{2eq,2ax}$ 14	2.23dd $J_{2eq,4eq}$ 1 $J_{4eq,5ax}$ 2 4eq-H	4.21dq $J_{4eq,5ax}$ 2 $J_{5ax,6}$ 7	1.24d $J_{5ax,6}$ 7	1.33s	3.50s			
(38)	4.68dd $J_{1ax,2eq}$ 3 $J_{1ax,2ax}$ 10 1ax-H	1.76ddd $J_{1ax,2eq}$ 3 $J_{2eq,2ax}$ 14 $J_{2eq,4eq}$ 1	1.38dd $J_{1ax,2ax}$ 10 $J_{2eq,2ax}$ 14	3.45ddd $J_{2eq,4eq}$ 1 $J_{4eq,NH}$ 10 $J_{4eq,5ax}$ 2 4eq-H	4.27dq $J_{4eq,5ax}$ 2 $J_{5ax,6}$ 6	1.18d $J_{5ax,6}$ 6	1.29s	3.52s	2.42bs 3-OH	3.70s 4-NHCOOCH_3	5.25d $J_{4eq,NH}$ 10 4-NHCOOCH$_3$

Table 11. ^{13}C N.m.r. data for L-mycarose derivatives [$\delta_C(CDCl_3)$]

Carbon	(34)	(36)	$\Delta\delta_C(36) \longrightarrow (34)$	(35)	(37)	$\Delta\delta_C(37) \longrightarrow (35)$	(38)
C-1	99.0	99.9	−0.9	99.2	100.5	−1.3	100.5
C-2	35.9	39.5	−3.6	35.4	39.0	−3.6	39.7
C-3	70.8	72.9	−2.1	74.1	73.3	+0.8	72.6
C-4	69.4	68.7	+0.7	58.3	58.4	−0.1	57.6
C-5	62.5	68.8	−6.3	62.5	68.8	−6.3	68.1
C-6	17.7	17.7		17.3	17.2	+0.1	17.0
1-OCH_3	55.2	56.3	−1.1	55.0	56.4	−1.4	55.5
3-CH_3	27.0	28.6	−1.6	26.8	28.5	−1.7	27.5
4-$NHCOOCH_3$							157.7
4-$NHCOOCH_3$							52.4

Table 12. $\Delta\delta_H$ Values for H_{cis} and H_{trans} at C-2 and C-4 in 3-axially substituted sugars

3-OH ⟶ 3-NO_2					3-OH ⟶ 3-OAc				
H	$\delta_H(38)$ [21]	$\delta_H(24)$	Stereochemistry [a]	$\Delta\delta_H(38) \longrightarrow (24)$	H	$\delta_H(39)$ [21]	$\delta_H(40)$	Stereochemistry [a]	$\Delta\delta_H(39) \longrightarrow (40)$
2eq-H	1.76	2.69	*cis*	+0.93	2eq-H	2.05	3.02	*cis*	+0.97
2ax-H	1.38	1.54	*trans*	+0.16	2ax-H	1.59	1.56	*trans*	−0.03
4eq-H	3.45	4.44	*cis*	+0.99	4ax-H	4.64	4.60	*trans*	−0.04

[a] Stereochemistry of the protons at C-2 and C-4 relative to the hetero substituent at C-3.

Table 13. $\Delta\delta_C$ Values for C-2 and C-4 in 3-axially substituted sugars

3-OH ⟶ 3-NO_2					3-OH ⟶ 3-OAc				
Carbon	$\delta_C(38)$	$\delta_C(24)$	Stereochem. of proton	$\Delta\delta_C(38) \longrightarrow (24)$	Carbon	$\delta_C(41)$ [15]	$\delta_C(40)$ [15]	Stereochem. of proton	$\Delta\delta_C(41) \longrightarrow (40)$
C-2	39.7	35.8	2eq-H *cis*	−3.9	C-2	43.3	39.4	2eq-H *cis*	−3.9
C-4	57.6	53.9	4eq-H *cis*	−3.7	C-4	77.8	77.4	4ax-H *trans*	−0.4

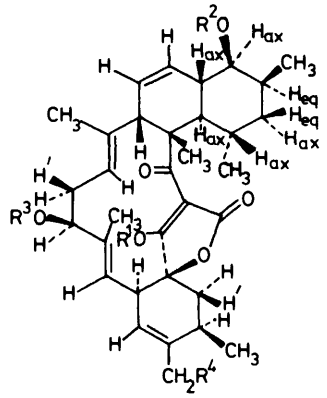
absolute stereochemistry of rubranitrose was therefore L. However, the published rotations of rubranitrose (43) {$[\alpha]_D^{26}$ 127° ⟶ +86° (*c* 1, ethanol)} [22] and the β-1-acetate (44) {$[\alpha]_D^{26}$ +36° (*c* 0.4, ethanol)} [22] did not agree with an L-configuration but were clearly in agreement with a D-configuration. Comparison of the c.d. data for methyl β-D-kijanoside (24), which shows a positive extremum at $[\theta]_{282}$ +2 308, with that of (44), clearly indicates that both compounds have the same absolute stereochemistry. We therefore propose that rubranitrose is in fact a D-sugar and that the correct structure is (45). This is the second example where a c.d. proof of the absolute stereochemistry of a sugar has failed to give the correct result [15,21] and great care should be exercised unless adequate model studies are carried out to first validate the method. The c.d. spectrum of methyl β-D-kijanoside (24) also showed a negative extremum at $[\theta]_{234}$ −962. The α-anomer (23) exhibited a negative extremum at $[\theta]_{234}$ −326 and a positive extremum at $[\theta]_{280}$ +340.

Methyl α-D-kijanoside (23) and the β-anomer (24) on hydrogenation in the presence of Raney nickel afforded the corresponding 3-amino sugars (25) and (27) respectively. Acetylation of each using acetic anhydride in methanol, afforded the corresponding 3-*N*-acetyl derivatives (26) and (28). The physical data for (25)—(28) are given in Tables 6—8 and the mass spectral fragment ions are described in Figure 3. The application of Hudson's Rules of Isorotation [16] to the molecular rotations (Table 5) gave a 2A value of +293°. This was in agreement with a D-configuration and serves to rule out the possibility of any anomalous rotational effects arising from the 3-nitro substituents when Hudson's Rule is used.

Methylation of methyl α-D-kijanoside (23) with sodium hydride and methyl iodide afforded two products. Both gave molecular ions at *m/z* 276 (Table 6) (Figure 3) in the e.i. mass spectra, consistent with a monomethylated derivative. Both were α-glycosides from the specific rotations and ^1H n.m.r. data (Table 7). Both also existed as complex mixtures of rotamers at ambient temperature. The ^{13}C n.m.r. data are given in Table 8. It is uncertain which product corresponds to the expected structure (29).

In view of the extensive degradation of the aglycone that occurred during the methanolysis to give the methyl kijanosides, milder hydrolytic conditions were therefore tried. Thus *O*-β-D-kijanosyl-(1→17)-kijanolide (14) on treatment with 5M-hydrogen chloride in methanol at 25 °C for 16 h afforded traces of kijanolide (46) (see Table 1 for the ^{13}C n.m.r. data) which was only partially characterized due to the low yield. The major aglycone derivative isolated from the above reaction was shown to be 32-chloro-32-deoxykijanolide (47). The ^1H n.m.r. spectrum of (47) (see Experimental section) showed two doublets at δ_H 4.13 and 4.27 with $J_{32,32'}$ 11.7 Hz, due to the allylic chloromethylene group. The ^{13}C n.m.r. data were also consistent with the eventual structure (Table 1), although, due to the limited solubility in deuteriochloroform, deuteriomethanol had to be used, which obscured the signal due to C-32. Numerous minor degradation products were also formed during the methanolysis and were not isolated. Methyl α-D-kijanoside (23) and the β-anomer (24) were again isolated from this reaction. It was obvious that an alternative method was needed to generate reasonable quantities of the aglycone for further structural studies.

Hence, kijanimicin (1) was exhaustively permethylated using sodium hydride and methyl iodide in dry dimethylformamide to give a per-*N*,*O*-methylated derivative. The latter was subjected to methanolysis using 0.5M-hydrogen chloride in methanol at 25 °C for 20 h to give methyl 2,6-di-



(46) $R^1 = R^2 = R^3 = H$, $R^4 = OH$

(47) $R^1 = R^2 = R^3 = H$, $R^4 = Cl$

(48) $R^1 = R^2 = R^3 = H$, $R^4 = OCH_3$

(49) $R^1 = CH_3$, $R^2 = R^3 = H$, $R^4 = OCH_3$

(50) $R^1 = H$, $R^2 = R^3 = CH_3$, $R^4 = OCH_3$

(51) $R^1 = H$, $R^2 = R^3 = Ac$, $R^4 = OCH_3$

(52) $R^1 = CH_3$, $R^2 = R^3 = Ac$, $R^4 = OCH_3$

deoxy-3,4-di-*O*-methyl-α-L-*ribo*-hexopyranoside (9), the β-anomer (10), methyl 2,6-dideoxy-4-*O*-methyl-α-L-*ribo*-hexopyranoside (4), the β-anomer (5), methyl 2,6-dideoxy-α-L-*ribo*-hexopyranoside (6), the β-anomer (7), methyl 2,6-dideoxy-α-L-*ribo*-hexofuranoside (11) and the β-anomer (12). The chemical data for the above sugars were in agreement with the data presented earlier in the text. Two fragments containing the aglycone were also isolated from this hydrolysis. These were *O*-(4^E-*N*-methyl-β-D-kijanosyl)-(1→17)-32-*O*-methylkijanolide (22), which existed as a mixture of rotamers at ambient temperature, and 32-*O*-methylkijanolide (48), the latter being the minor product. In order to improve the yield of (48) the reaction was repeated using 8.5M-hydrogen chloride in methanol at 25 °C for 43 h and this afforded sizeable quantities of pure (48) which was obtained as an amorphous solid. The e.i. mass spectrum of (48) showed a molecular ion at *m/z* 566 and a high resolution mass measurement was in agreement with a composition of $C_{34}H_{46}O_7$. The mass spectral fragment ions (see Experimental section) are given in Figure 1. The i.r. spectrum of (48) revealed the presence of hydroxy, lactone, carbonyl and ether functions in the aglycone. The u.v. spectrum of (48) showed maxima at 245 and 262 nm due to the 1,3,3′-diketolactone system. Prior to carrying out extensive ^1H n.m.r. and ^{13}C n.m.r. studies on (48), four derivatives were first prepared.

32-*O*-Methylkijanolide (48) on treatment with diazomethane afforded 26,32-di-*O*-methylkijanolide (49). Methylation of (48) using sodium hydride and methyl iodide on the other hand gave 9,17,32-tri-*O*-methylkijanolide (50). Acetylation of (48) with acetic anhydride in pyridine at 25 °C gave 9,17-di-*O*-acetyl-32-*O*-methylkijanolide (51). Acetylation of 26,32-di-*O*-methylkijanolide (49) with acetic anhydride in pyridine gave 9,17-di-*O*-acetyl-26,32-di-*O*-methylkijanolide (52). The e.i. mass spectral fragment ions (see Experimental section) for (48)—(52) are given in Figure 1. The ^{13}C n.m.r. data for (48)—(52) are given in Table 1 and it is evident from the data for (48), that no rearrangements had occurred within the aglycone during the course of the methanolysis. The deshielding (+10.3) observed at C-32 in (48) relative to kijanimicin (1) clearly showed that the hydroxy group that was free in the

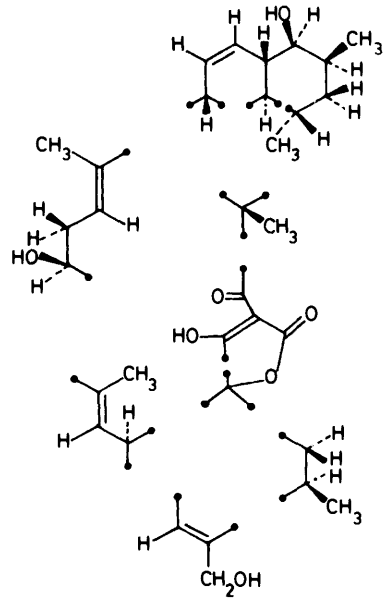


Figure 5. Partial structure of kijanolide

kijanimicin aglycone and which had undergone methylation, was the primary allylic hydroxy group at C-32. The $\Delta\delta_C$ values observed in going from (48) to kijanimicin (1) show deshielding at C-9 (+8.4) and shielding at C-8 (−0.7) due to the presence of the tetrasaccharide unit in (1). Deshielding was also observed at C-17 (+5.5), while C-16 (−1.0), C-18 (−4.2) and C-19 (−2.4) were all shielded in (1) due to the attachment of the D-kijanose unit at C-17. The $\Delta\delta_C$ values in going from (48) to (14) showed similar values for C(16)—C(19) to those just discussed, indicating that the kijanose unit was glycosidically linked at C-17. The $\Delta\delta_C$ values observed in going from (48) to the 26-*O*-methyl ether (49) were consistent with what had previously been found with (2) and (15). The $\Delta\delta_C$ values observed in going from (48) to (50) indicated that the three methyl ether groups were at C-9, C-17, and C-32, and that no methylation of the acidic enol group at C-26 had occurred. In (50), C-9 was deshielded (+8.9), while C-8 (−0.9) and C-10 (−4.6) both experienced shielding due to the 9-*O*-methyl group. Deshielding was also observed at C-17 (+9.2), while both C-16 (−2.4) and C-18 (−4.0) were shielded in (50) relative to (48). The ^{13}C n.m.r. data for the diacetates (51) and (52) were consistent with the presence of two acetyl groups in each molecule and from the $\Delta\delta_C$ values observed in going from (48) to (51), and from (49) to (52), they were located at C-9 and C-17.

The ^1H n.m.r. spectra of the kijanolide derivatives (48)—(52) were recorded at 600 MHz and the data are given in Table 17. Extensive off-resonance decoupling experiments were performed on (50) and (52) (see Table 17) and this led to the unambigous assignment of all but two protons in kijanolide. The signals due to 7ax-H and 6ax-H in (50) could not be located and hence the coupling constants for these protons could not be determined. It was therefore not possible to confirm the existence of the C(5)–C(6) bond in kijanolide. It was evident from the data in Table 17 that the *O*-methyl groups in (50) were located at C-9 and C-17 in addition to the group at C-32, from the observed deshielding of 9-H and 17-H. Acetylation of the hydroxy groups at C-9 and C-17 in (51) and (52) resulted in the expected deshielding of 9-H and 17-H. The data in Table 17 together with the ^{13}C n.m.r. data in Table 1 enabled us to derive the various component structural units of kijanolide shown in Figure 5. The data, however,

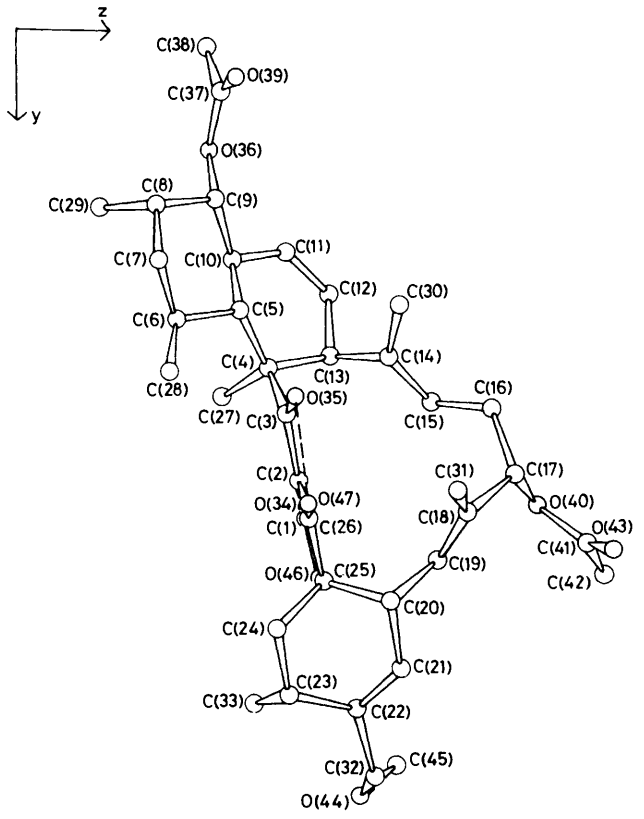


Figure 6. Structure and solid state conformation of (51), with atom numbering scheme. The broken line denotes an intramolecular O–H · · · O hydrogen bond

did not enable us to assign an unequivocal structure to kijanolide. Fortunately at this point both of the acetyl derivatives (51) and (52) crystallized from methanol–chloroform and of these only the crystals of (51) were suitable for an *X*-ray determination.

A single-crystal *X*-ray analysis defined the complete structure and relative stereochemistry of (51). A view of the solid-state conformation is shown in Figure 6, which also provides the atom numbering scheme used in the *X*-ray analysis. Final atomic co-ordinates are given in Tables 14 and 15. Interatomic distances and angles involving the non-hydrogen atoms are given in Table 16. Crystals of (51) contain discrete molecules separated by normal van der Waals' distances. Torsion angles defining the molecular conformation are given in the Supplementary publication.*

The cyclohexane ring, *trans*-fused to one of the cyclohexene rings, adopts a chair form with equatorial 16-methyl and 9-acetoxy substituents and an axial methyl group at C-8. Although the substitution pattern forces this ring (mean dihedral angle 59.0°) to be overall more puckered than cyclohexane (mean dihedral angle 56° [24]), it is flattened around C(8) in order to minimize non-bonded repulsive interactions involving the axial methyl substituent. Both cyclohexene rings approximate more closely to half-chair (C_2) than to envelope (C_s) forms.† The macrocyclic ring may be viewed either as a 13-membered carbocyclic ring, or as a 14-membered lactone ring. In addition to providing an appropriate geometry for the intramolecular O(26)–H(26) · · · O(35) hydrogen bond (O · · · O 2.52 Å), the approximate coplanarity of the carbonyl group [C(26)–C(2)–C(3)–O(35) 10.0°] and the spiro α,β-unsaturated γ-lactone ring atoms (mean endocyclic dihedral angle 2.3°) maximizes π-interactions between these systems. The presence of two additional planar fragments, in the form of trisubstituted double bonds, imposes considerable conformational constraints on the macrocyclic ring system, which consequently approximates to a triangular shape, in which the C(14) and C(18) methyl substituents are *syn*-oriented with respect to

Table 14. Non-hydrogen atom fractional co-ordinates (× 10^4) with estimated standard deviations in parentheses

Atom	*x*	*y*	*z*
C(1)	−898(7)	1 576(3)	676(7)
C(2)	105(6)	1 289(3)	484(7)
C(3)	316(6)	789(2)	214(7)
C(4)	−571(6)	437(3)	−181(8)
C(5)	−34(7)	−7(2)	−811(8)
C(6)	525(7)	68(3)	−2 165(7)
C(7)	1 108(8)	−388(3)	−2 571(9)
C(8)	309(8)	−813(3)	−2 648(9)
C(9)	−323(7)	−855(3)	−1 371(9)
C(10)	−938(6)	−396(3)	−986(8)
C(11)	−1 674(8)	−459(3)	151(9)
C(12)	−1 817(8)	−137(3)	1 065(10)
C(13)	−1 215(7)	335(3)	1 129(9)
C(14)	−475(8)	334(3)	2 377(9)
C(15)	−637(8)	675(3)	3 246(8)
C(16)	−49(10)	715(3)	4 538(9)
C(17)	261(8)	1 207(3)	5 019(8)
C(18)	888(7)	1 505(3)	4 034(9)
C(19)	402(7)	1 867(3)	3 423(8)
C(20)	932(7)	2 190(3)	2 446(9)
C(21)	623(7)	2 700(3)	2 690(9)
C(22)	468(8)	3 017(3)	1 774(11)
C(23)	585(7)	2 925(3)	349(9)
C(24)	1 072(7)	2 428(3)	82(10)
C(25)	638(6)	2 059(2)	1 052(8)
C(26)	991(6)	1 579(3)	718(8)
C(27)	−1 433(6)	662(3)	−1 161(9)
C(28)	1 388(8)	473(3)	−2 298(9)
C(29)	−461(9)	−778(3)	−3 851(9)
C(30)	377(9)	−63(3)	2 569(10)
C(31)	2 082(9)	1 343(4)	3 839(13)
C(32)	201(10)	3 526(3)	2 174(12)
C(33)	−514(8)	2 997(3)	−390(10)
O(34)	−1 882(4)	1 479(2)	665(7)
O(35)	1 292(5)	651(2)	401(6)
O(36)	−1 183(5)	−1 216(2)	−1 505(6)
C(37)	−861(8)	−1 665(3)	−1 277(9)
C(38)	−1 835(9)	−1 994(3)	−1 576(11)
O(39)	55(6)	−1 773(2)	−954(8)
O(40)	−752(5)	1 436(2)	5 471(5)
C(41)	−626(10)	1 714(4)	6 512(10)
C(42)	−1 703(11)	1 948(6)	6 891(13)
O(43)	251(7)	1 764(3)	7 063(7)
O(44)	−892(8)	3 674(3)	1 861(9)
C(45)	−1 733(13)	3 434(6)	2 622(17)
O(46)	−572(4)	2 023(2)	956(5)
O(47)	2 066(4)	1 467(2)	720(6)

* For details of the Supplementary Publications scheme see Instructions for Authors (1983), *J. Chem. Soc., Perkin Trans. 1*, 1983, Issue 1.

† For the cyclohexene ring defined by atoms C(4), C(5), C(10)—C(13), $\Delta(C_2\text{-half-chair}) = |\omega_{11,12}| + |\omega_{10,11} - \omega_{12,13}| + |\omega_{5,10} - \omega_{4,13}| = 19.4°$, $\Delta(C_s\text{-envelope}) = |\omega_{11,12}| + |\omega_{12,13}| + |\omega_{10,11} + \omega_{4,13}| + |\omega_{5,10} + \omega_{4,5}| = 40.6°$, where ω_{ij} is the endocyclic torsion angle about the C(*i*)–C(*j*) bond.

For the cyclohexene ring defined by atoms C(20)—C(25), $\Delta(C_2\text{-half-chair}) = |\omega_{21,22}| + |\omega_{20,21} - \omega_{22,23}| + |\omega_{20,25} - \omega_{23,24}| = 23.8°$, $\Delta(C_s\text{-envelope}) = |\omega_{21,22}| + |\omega_{22,23}| + |\omega_{20,21} + \omega_{23,24}| + |\omega_{20,25} + \omega_{24,25}| = 36.8°$.

Table 15. Calculated fractional co-ordinates (× 10^3) for hydrogen atoms [a]

Atom	x	y	z
H(5)	59	−12	−15
H(6)	−13	14	−282
H(7A)	150	−34	−344
H(7B)	172	−47	−183
H(8)	80	−112	−276
H(9)	26	−94	−62
H(10)	−144	−30	−175
H(11)	−214	−78	17
H(12)	−240	−20	183
H(13)	−180	60	126
H(15)	−121	95	303
H(16A)	−57	56	525
H(16B)	71	52	448
H(17)	79	117	583
H(19)	−45	195	363
H(20)	181	216	255
H(21)	504	279	368
H(23)	115	318	0
H(24A)	194	244	14
H(24B)	83	232	−87
H(27A)	−103	74	−197
H(27B)	−209	42	−126
H(27C)	−175	97	−66
H(28A)	98	79	−206
H(28B)	206	38	−171
H(28C)	164	46	−329
H(29A)	−101	−107	−391
H(29B)	−89	−46	−373
H(29C)	8	−75	−464
H(33A)	−84	333	−22
H(33B)	−32	293	−137
H(33C)	−104	272	−6
H(32A)	34	355	320
H(32B)	82	375	173
H(47)	229	117	53

[a] Hydrogen atoms bear the same labels as the atoms to which they are bonded; all were assigned an isotropic temperature factor, $U = 0.051$ Å^2.

the mean ring plane. The conformation found in the present study is very similar to that encountered in tetronolide,[12] where the basic ring system differs only in the location of the double bond in the cyclohexene ring bearing the spiro γ-lactone ring.

It remained at this point to complete the structure of the tetrasaccharide moiety and to determine the absolute stereochemistry of kijanolide. The isolation of the four digitoxose derivatives (6), (7), (11), and (12) from the methanolysis of per-*N*,*O*-methylkijanimicin described earlier, clearly indicated that the tetrasaccharide moiety had a branched chain. From the ^{13}C n.m.r. data (Table 1) it was evident that one of the terminal sugar units of the tetrasaccharide was a 2,6-dideoxy-4-*O*-methyl-β-L-*ribo*-hexopyranosyl unit. It followed therefore that the other terminal sugar was a 2,6-dideoxy-α-L-*ribo*-hexopyranosyl unit. At this point four possible structures could be written for the tetrasaccharide moiety, that would satisfy the hydrolytic data and in order to reduce this number, the following reactions were carried out. Kijanimicin (1) on treatment with sodium metaperiodate followed by a sulphonic acid resin, afforded 3^B-*O*-dedigitoxosylkijanimicin (53). The ^{1}H n.m.r. spectrum of (53) at 220 MHz contained a doublet of doublets at δ_H 4.46 ($J_{1^E_{ax},2^E_{ax}}$ 10 Hz, $J_{1^E_{ax},2^E_{eq}}$ 2 Hz) due to 1^E_{ax}-H of the kijanosyl unit. The terminal 2,6-dideoxy-4-*O*-methyl-β-L-*ribo*-hexopyranosyl unit gave rise to a doublet of doublets at δ_H 4.92 ($J_{1^D_{ax},2^D_{ax}}$ 9.5 Hz, $J_{1^D_{ax},2^D_{eq}}$ 1.5 Hz) due to

Table 16. Interatomic distances (Å) and angles (°), with estimated standard deviations in parentheses

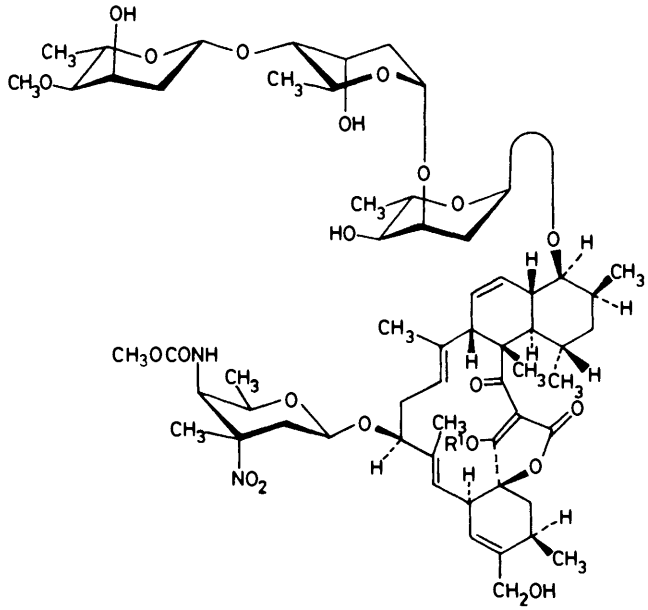
(a) Bond lengths

C(1)–C(2)	1.460(10)	C(16)–C(17)	1.529(12)
C(1)–O(34)	1.202(10)	C(17)–C(18)	1.515(12)
C(1)–O(46)	1.359(9)	C(17)–O(40)	1.446(11)
C(2)–C(3)	1.469(10)	C(18)–C(19)	1.338(11)
C(2)–C(26)	1.360(10)	C(18)–C(31)	1.506(13)
C(3)–C(4)	1.510(10)	C(19)–C(20)	1.500(11)
C(3)–O(35)	1.241(9)	C(20)–C(21)	1.517(11)
C(4)–C(5)	1.556(10)	C(20)–C(25)	1.520(12)
C(4)–C(13)	1.576(12)	C(21)–C(22)	1.315(13)
C(4)–C(27)	1.575(12)	C(22)–C(23)	1.494(14)
C(5)–C(6)	1.558(11)	C(22)–C(32)	1.537(13)
C(5)–C(10)	1.552(10)	C(23)–C(24)	1.553(11)
C(6)–C(7)	1.529(11)	C(23)–C(33)	1.526(13)
C(6)–C(28)	1.548(12)	C(24)–C(25)	1.536(12)
C(7)–C(8)	1.538(12)	C(25)–C(26)	1.470(10)
C(8)–C(9)	1.518(12)	C(25)–O(46)	1.447(9)
C(8)–C(29)	1.543(13)	C(26)–O(47)	1.317(9)
C(9)–C(10)	1.546(11)	C(32)–O(44)	1.404(15)
C(9)–O(36)	1.456(10)	O(36)–C(37)	1.352(10)
C(10)–C(11)	1.472(12)	C(37)–C(38)	1.520(13)
C(11)–C(12)	1.324(13)	C(37)–O(39)	1.180(11)
C(12)–C(13)	1.521(12)	O(40)–C(41)	1.338(11)
C(13)–C(14)	1.556(13)	C(41)–C(42)	1.495(18)
C(14)–C(15)	1.332(12)	C(41)–O(43)	1.196(14)
C(14)–C(30)	1.530(13)	O(44)–C(45)	1.443(19)
C(15)–C(16)	1.505(13)		

(b) Bond angles

C(2)–C(1)–O(34)	131.7(7)	C(15)–C(16)–C(17)	117.9(7)
C(2)–C(1)–O(46)	108.6(6)	C(16)–C(17)–C(18)	114.4(7)
O(34)–C(1)–O(46)	119.6(7)	C(16)–C(17)–O(40)	108.3(8)
C(1)–C(2)–C(3)	135.0(6)	C(18)–C(17)–O(40)	112.0(7)
C(1)–C(2)–C(26)	105.7(6)	C(17)–C(18)–C(19)	122.0(7)
C(3)–C(2)–C(26)	119.2(6)	C(17)–C(18)–C(31)	112.5(8)
C(2)–C(3)–C(4)	124.9(6)	C(19)–C(18)–C(31)	125.4(8)
C(2)–C(3)–O(35)	115.9(6)	C(18)–C(19)–C(20)	127.2(7)
C(4)–C(3)–O(35)	119.0(6)	C(19)–C(20)–C(21)	111.9(7)
C(3)–C(4)–C(5)	111.3(6)	C(19)–C(20)–C(25)	112.7(6)
C(3)–C(4)–C(13)	103.4(6)	C(21)–C(20)–C(25)	109.5(7)
C(3)–C(4)–C(27)	110.9(6)	C(20)–C(21)–C(22)	124.7(9)
C(5)–C(4)–C(13)	114.0(6)	C(21)–C(22)–C(23)	124.8(8)
C(5)–C(4)–C(27)	109.3(6)	C(21)–C(22)–C(32)	118.7(9)
C(13)–C(4)–C(27)	107.8(6)	C(23)–C(22)–C(32)	116.4(8)
C(4)–C(5)–C(6)	115.9(6)	C(22)–C(23)–C(24)	111.5(7)
C(4)–C(5)–C(10)	110.0(6)	C(22)–C(23)–C(33)	112.7(8)
C(6)–C(5)–C(10)	106.8(6)	C(24)–C(23)–C(33)	110.7(7)
C(5)–C(6)–C(7)	108.8(6)	C(23)–C(24)–C(25)	112.4(7)
C(5)–C(6)–C(28)	117.6(6)	C(20)–C(25)–C(24)	111.6(6)
C(7)–C(6)–C(28)	107.8(7)	C(20)–C(25)–C(26)	112.4(6)
C(6)–C(7)–C(8)	113.5(7)	C(20)–C(25)–O(46)	108.1(6)
C(7)–C(8)–C(9)	108.8(7)	C(24)–C(25)–C(26)	112.7(7)
C(7)–C(8)–C(29)	110.9(7)	C(24)–C(25)–O(46)	109.8(6)
C(9)–C(8)–C(29)	113.9(7)	C(26)–C(25)–O(46)	101.7(5)
C(8)–C(9)–C(10)	113.0(7)	C(2)–C(26)–C(25)	112.5(6)
C(8)–C(9)–O(36)	108.7(7)	C(2)–C(26)–O(47)	127.3(7)
C(10)–C(9)–O(36)	106.6(6)	C(25)–C(26)–O(47)	120.1(6)
C(5)–C(10)–C(9)	107.6(6)	C(22)–C(32)–O(44)	114.4(8)
C(5)–C(10)–C(11)	114.0(7)	C(9)–O(36)–C(37)	116.8(6)
C(9)–C(10)–C(11)	112.5(7)	O(36)–C(37)–C(38)	109.3(7)
C(10)–C(11)–C(12)	123.8(8)	O(36)–C(37)–O(39)	123.7(8)
C(11)–C(12)–C(13)	125.5(9)	C(38)–C(37)–O(39)	127.0(8)
C(4)–C(13)–C(12)	110.7(7)	C(17)–O(40)–C(41)	115.4(7)
C(4)–C(13)–C(14)	115.5(7)	O(40)–C(41)–C(42)	112.1(9)
C(12)–C(13)–C(14)	107.5(7)	O(40)–C(41)–O(43)	123.1(10)
C(13)–C(14)–C(15)	118.1(8)	C(42)–C(41)–O(43)	124.8(10)
C(13)–C(14)–C(30)	118.8(7)	C(32)–O(44)–C(45)	112.0(9)
C(15)–C(14)–C(30)	123.1(8)	C(1)–O(46)–C(25)	111.4(5)
C(14)–C(15)–C(16)	125.5(8)		

1^{D}_{ax}-H. The remaining 2,6-dideoxy-α-L-*ribo*-hexopyransoyl units each gave rise to doublet of doublet signals at δ_H 4.78 ($J_{1eq,2ax}$ 5 Hz, $J_{1eq,2eq}$ < 1 Hz), and δ_H 5.13 ($J_{1eq,2ax}$ 4 Hz, $J_{1eq,2eq}$ < 1 Hz) due to the equatorial anomeric protons in these sugars. The ^{13}C n.m.r. data for (53) are given in Table 1. Methylation of (53) with diazomethane afforded the 26-*O*-methyl ether (54). The ^1H n.m.r. spectrum of (54) run at 600 MHz showed a doublet of doublets at δ_H 4.45 ($J_{1^E_{ax},2^E_{ax}}$ 10 Hz, $J_{1^E_{ax},2^E_{eq}}$ 1.9 Hz) due to 1^{E}_{ax}-H of the kijanosyl unit. The terminal 2,6-dideoxy-4-*O*-methyl-β-L-*ribo*-hexopyranosyl unit gave rise to a doublet of doublets at δ_H 4.92 ($J_{1^D_{ax},2^D_{ax}}$ 9.5 Hz, $J_{1^D_{ax},2^D_{eq}}$ 1.4 Hz) due to 1^{D}_{ax}-H. The remaining 2,3-dideoxy-α-L-*ribo*-hexopyranosyl units gave rise to a pair of doublet of doublet signals at δ_H 4.77 ($J_{1eq,2ax}$ 4.8 Hz, $J_{1eq,2eq}$ < 1 Hz) and δ_H 5.14 ($J_{1eq,2ax}$ 3.8 Hz, $J_{1eq,2eq}$ < 1 Hz) due to 1_{eq}-H in these sugar units. The ^{13}C n.m.r. data for (54) are given in Table 1 and the $\Delta\delta_C$ values in going from (53) to (54) were in agreement with those observed previously for the other 26-*O*-methyl derivatives.

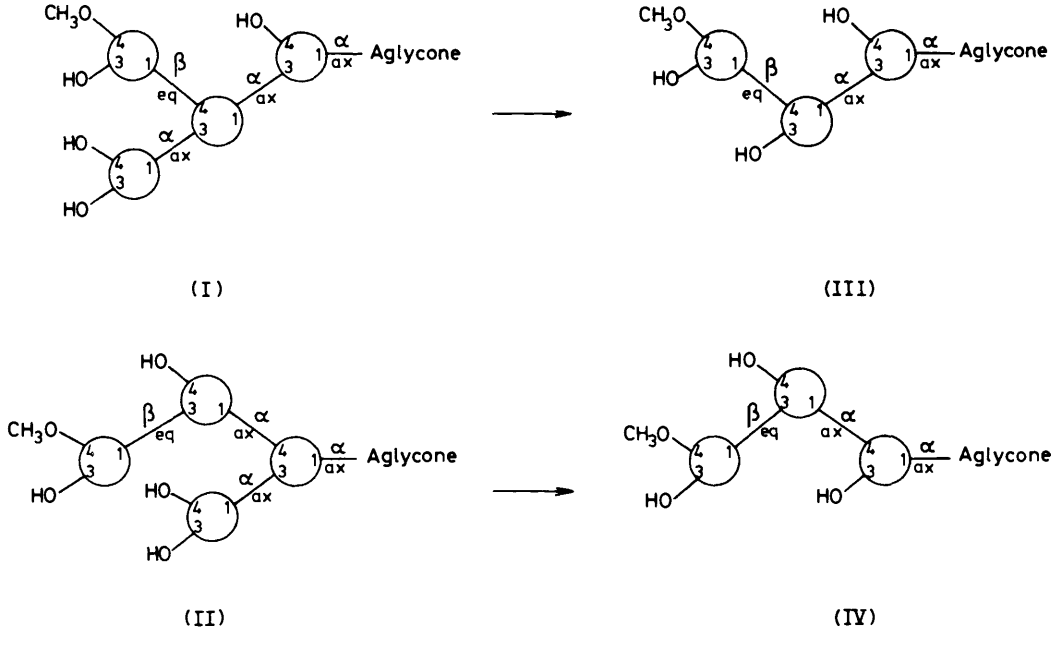


(53) R^1 = H

(54) R^1 = CH_3

The 3^B-*O*-dedigitoxosylkijanimicin (53) was per-*N*,*O*-methylated using sodium hydride and methyl iodide, and the product was subjected to methanolysis using 0.5M-hydrogen chloride in methanol at 25 °C. Isolation of all the monosaccharide components of the hydrolysis afforded methyl 2,6-dideoxy-3,4-di-*O*-methyl-α-L-*ribo*-hexopyranoside (9), the β-anomer (10), methyl 2,6-dideoxy-4-*O*-methyl-α-L-*ribo*-hexopyranoside (4), the β-anomer (5) and methyl 2,6-dideoxy-3-*O*-methyl-β-L-*ribo*-hexofuranoside (13). The physical data for (9), (10), (4), and (5) were identical with those described earlier, thus confirming their identities. The furanoside (13) did not give a molecular ion in the e.i. mass spectrum, but did show an $(M - H)^+$ peak at *m/z* 175 (Table 2). The typical fragment ions A_8—A_{15} for a furanoside were observed (Figure 2) and the ions A_9, A_{14}, and A_{15} supported the location of the methyl ether at C-3 in the molecule. The rotation was consistent with that of a β-furanoside. The ^1H n.m.r. spectrum of (13) (Table 3) revealed few coupling constants, but did reveal the 3-*O*-methyl group as a singlet at δ_H 3.33 and showed shielding of 3-H relative to what was observed in (12). The ^{13}C n.m.r. spectrum of the furanoside (13) (Table 4) was consistent with the proposed structure. The isolation of the above monosaccharides could only be accommodated by two possible trisaccharide structures namely structures III and IV, which, in turn, were derived from the tetrasaccharide structures I and II respectively (Figure 7). The other two tetrasaccharide structures that were possible structures were ruled out at this point, as they would have given the same trisaccharide which on permethylation and acidic hydrolysis would have produced only sugars (9), (10), (4), and (5) and no 3-*O*-methylfuranoside (13).

In order to determine which of the two possible structures I, or II correctly represents the tetrasaccharide portion of kijanimicin (1), it was necessary to consider the preferred



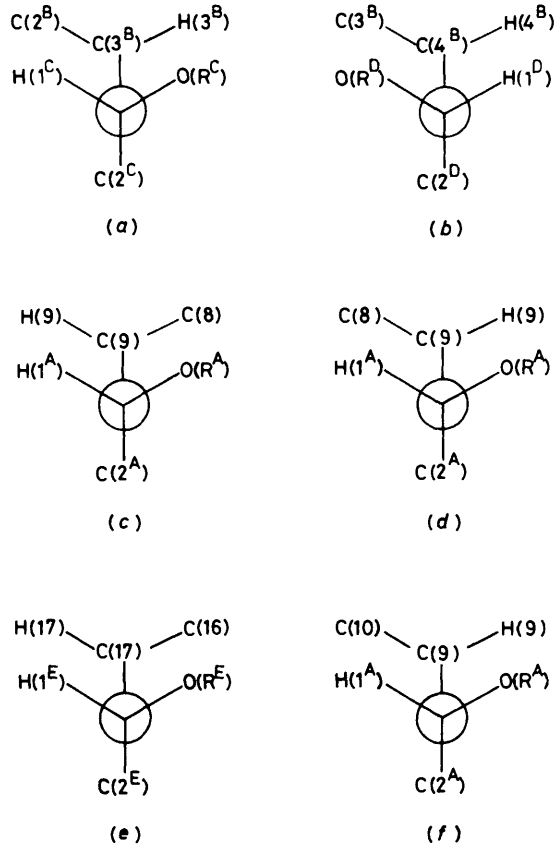
(I) (III)

(II) (IV)

Figure 7. Possible tetrasaccharide andtrisaccharide structures that accommodate the methylation/hydrolytic data

rotamers about the glycosidic bonds. The solution conformations about the glycosidic bonds have been shown to be extremely sensitive to the stereochemical environment of the glycosidic bonds,[25-27] as well as the absolute stereochemistry about that bond.[28,29] Knowing the absolute stereochemistry of each of the component sugars of the tetrasaccharide, as well as the conformation ($^1C_4$) and the anomeric configuration, it was now possible to evaluate the ^{13}C n.m.r. data for the tetrasaccharide unit (Table 1) taking into account the stereochemical environment of each glycosidic bond. If we first consider structure I it is possible to readily assign the carbons of the terminal sugar D. The other terminal sugar C showed the expected chemical shifts for C(2)—C(6). The anomeric carbon, however, was strongly shielded (δ_C 92.2). It was also apparent when considering the $\Delta\delta_C$ values in going from 3^B-*O*-dedigitoxosylkijanimicin (53) to kijanimicin (1) that almost no change was observed at C-3 of sugar B (+0.5) in spite of the fact that sugar C had been glycosidically attached at C-3. It was also evident from the $\Delta\delta_C$ values in going from (53) to (1), that introduction of sugar C produced marked shielding at $C\text{-}2^B$ (−4.7). These results are in excellent agreement with what one would expect considering the stereochemical environment at $C\text{-}3^B$, from previous ^{13}C n.m.r. studies carried out on 1-, 3-, and 5-*epi*-aminoglycoside antibiotics.[25,26] It is evident in (1), that the deshielding at $C\text{-}3^B$ of sugar B resulting from the glycosidic linkage of sugar C is being cancelled out by the shielding by sugar C due to its configuration about the glycosidic linkage. Assuming that the *exo*-anomeric effect is operational [28] it may be reasoned that sugar C adopts rotamer *a* about the glycosidic bond.[25] The interactions between $1^C\text{-}H$ and $2^B_{eq}\text{-}H$ would be expected to shield $C\text{-}1^C$ and $C\text{-}2^B$,[25,30] while the interaction between the $C(3^B)\text{–}H(3^B)$ and $C(1^C)\text{–}O(R^C)$ bonds would be expected to produce marked shielding at $C\text{-}3^C$.[25,26] It is also evident that similar shieldings are present at $C\text{-}1^B$ in sugar B (δ_C 90.8) as well as at $C\text{-}3^A$ (δ_C 66.8) and $C\text{-}2^A$ (δ_C 29.9) in sugar A in (1). Similar effects are observed in (53). It is apparent from these data that sugar B also adopts rotamer *a* about the glycosidic bond with sugar A. The glycosidic attachment of sugar D to $C\text{-}4^B$ of sugar B would be expected to deshield $C\text{-}4^B$ (δ_C 79.6) which was the case. Sugar D would be expected to adopt rotamer *b* about the glycosidic bond [25,26] and this would result in moderate shielding at $C\text{-}3^B$ (δ_C 62.6) of sugar B in (1) relative to $C\text{-}3^A$ (δ_C 66.8) of sugar A, as was indeed observed.[25] Similar effects were apparent in (53). Consideration of the ^{13}C n.m.r. data for the methyl ethers (2) and (54) (Table 1) led to similar conclusions. Thus the ^{13}C n.m.r. data are readily accommodated by structures I and III. When similar conformational arguments are applied to the ^{13}C n.m.r. data in an attempt to fit the data to structures II and IV (Figure 7), no correlation between the data and the structures is possible. We therefore conclude that structures I and III correctly represent the tetrasaccharide portion of (1) and the trisaccharide portion of (53) respectively. The ^{13}C n.m.r. data also correlate well with the assigned anomeric linkages as determined from the ^1H n.m.r. data. The reason for the anomalously low $J_{^{13}C\text{-}^1H}$ value for $1^D\text{-}H$ (160 Hz) is not apparent. The ^{13}C n.m.r. data also suggest that some rotation occurs about the $O\text{–}C\text{-}4^D$ bond when the sugar C is removed in going from (1) to (53), resulting in increased shielding at $C\text{-}1^D$ and $C\text{-}4^B$ in (53) relative to (1). The application of Klyne's rule to the molecular rotations of (1), (53), (14), and (48) (Table 5) also lends further support to the assigned glycosidic linkages.

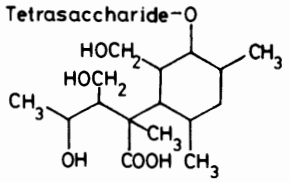
It remained to determine the absolute stereochemistry of kijanolide and this was done as follows. As a consequence of knowing the absolute stereochemistry of sugar A (L, $^1C_4$ conformation) and the nature of the glycosidic linkage to the aglycone (α), as well as the point of attachment to kijanolide



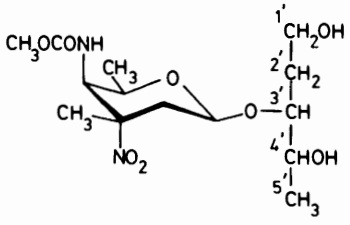
(C-9) and the relative stereochemical environment at C-8, C-9, and C-10, in a rigid cyclohexane ring whose precise geometry is known, it was possible to deduce the absolute stereochemistry at C-9 from the observed glycosylation shifts in the ^{13}C n.m.r. spectra in going from (14) to (1).[25,27,28] Thus the observed deshielding at C-9 (+8.4) coupled with the shielding at C-8 (−0.7) and absence of any shielding at C-10, were in excellent agreement with the predicted glycosylation shifts for an α-L-2-deoxyhexopyranoside glycosidically attached to an aglycone having the stereochemical features present at C-8, C-9, and C-10 in (1).[25,27] It therefore follows that C-9 has the *S*-configuration as shown in (1) and that the rotamer about the O–C-9 bond is *c*.[25] Had the configuration at C-9 been *R*, then marked shielding would have been predicted to occur at C-8, C-9 and $C\text{-}1^A$ [25,26] due to adoption of rotamer *d*, which was not the case. The chemical shift of $C\text{-}1^A$ (δ_C 98.2) is normal for that of an α-L-digitoxoside and from its relaxation properties it was clearly the anomeric carbon of sugar A.

The ^{13}C n.m.r. data also afforded useful information about the conformation of the D-kijanosyl unit about the *O*–C-17 glycosidic bond. It is obvious that in kijanimicin (1) and its various kijanosyl containing derivatives, that $C\text{-}1^E$ is shielded (δ_C 97.1) relative to C-1 in methyl β-D-kijanoside (24) (δ_C 99.9). It is also apparent that C-17 is experiencing some shielding as the net deshielding observed at C-17 upon glycosylation is only +5.5.[25] Both C-16 (−1.0) and C-18 (−4.2) are shielded in going from (48) to (1). It is evident that reduced steric interaction with the C-18 substituent due to the conformational properties of the macrocyclic ring, enables the kijanosyl unit to undergo partial counter-clockwise rotation about the O–C-17 bond relative to rotamer *e*, which would satisfactorily explain the observed $\Delta\delta_C$ values.[25,26]

Finally, chemical proof for the linkage of the tetrasaccharide



(55)



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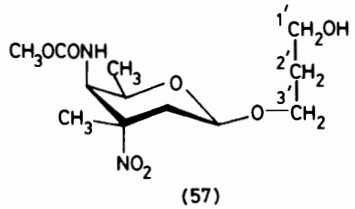

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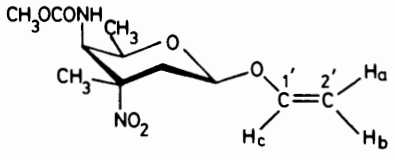

(58)

moiety at C-9 and of the kijanose unit at C-17 was obtained by subjecting kijanimicin (1) to ozonolysis followed by sodium borohydride reduction. The reaction gave an extremely complex mixture of products. The tetrasaccharide acid (55), which retained sixteen carbon atoms of the aglycone, was obtained in low yield. The ^{13}C n.m.r. data (Table 1) showed that epimerization had occurred at C-10 during the course of the reaction as evidenced by the pronounced shielding observed at C-1^A (δ_C 92.9), C-9 (δ_C 74.8), and C-10 (δ_C 25.9) relative to (1). These data are consistent with the existence of rotamer *f* about the *O*–C-9 glycosidic bond as expected [25,26] for a cyclohexane derivative having an axial C-10 substituent. The isolation of (55) clearly indicated that the tetrasaccharide unit was glycosidically linked at C-9. The principal product of the ozonolysis of (1) was *O*-β-D-kijanosyl-(1→3)-3,4-dihydroxypentanol (56). The mass spectral data (Table 6, Figure 3), ^1H n.m.r. data (Table 7) and ^{13}C n.m.r. data (Table 8) were consistent with the assigned structure. The isolation of (56) was consistent with the glycosidic linkage of the kijanose at C-17 in (1). Two other minor kijanose containing artefacts (57) and (58) were also isolated in low yield.

The ^1H n.m.r. spectrum of 26-*O*-methylkijanimicin (2) at 600 MHz contained too many complex overlapping multiplets to be decoupled successfully by conventional techniques. We therefore resorted to proton 2D J spectroscopy and n.O.e. difference spectroscopy [31] in order unambiguously to assign as many of the protons as possible in (2). The results of this study are summarized in Table 18. The chemical shifts of all of the protons in (2), with the exception of the 32-hydroxymethylene protons, were unambiguously assigned from the SECSY and difference spectra. All of the coupling constants for the protons of the five sugar moieties in (2) were also obtained, as well as many of the coupling constants of the various protons in the kijanolide unit. Due to the complexity of the spectrum the remaining coupling constants for the kijanolide unit could not be ascertained in this experiment. Most of these latter coupling constants had already been determined for the kijanolide derivatives (48)—(52) (Table 17).

The chemical shifts and coupling constants for the protons of the tetrasaccharide unit were of particular interest. The chemical shifts of the protons of the tetrasaccharide unit in (2) were in good agreement with the structure already deduced for (2). Pronounced deshielding was observed for 1-H in sugars B and C consistent with the rotamers adopted by these units. Shielding was also evident for 2_{ax}-H in sugars A and B which was consistent with 3-substituents in these units. The small couplings observed for 1-H of sugars A, B, and C were consistent with α-glycosidic linkages for these units, while the large axial-axial couplings observed for 1-H of sugars D and E clearly indicated that these were β-glycosides. The coupling constants also clearly support $^1C_4$ conformations for sugars A, B, C, and D in spite of the bulky 1,3-diaxial substituents present in sugars A and B. The n.O.e. difference spectrum obtained when 4^B_{ax}-H was irradiated revealed an n.O.e. effect at 1^D_{ax}-H, confirming the spacial proximity of these two protons due to the rotamer adopted by sugar D.

It is therefore concluded that the total structure and absolute stereochemistry of kijanimicin may be represented by (1). It is evident from the ^1H n.m.r. data (Table 17) of the kijanolide derivatives, that the aglycone adopts a similar solution conformation about the macrocyclic ring, to that observed in the solid state by *X*-ray studies.

Kijanimicin (1) exhibits antitumour activity, antimalarial activity, and is active against anaerobic bacteria, in particular *Propionibacterium acnes*.[3] It is a member of a new class of tetronic acid containing antibiotics of which the tetrocarcins [9-12] and antlermicins [13,14] are the only other known members. The latter differ in the structure of the aglycone as well as in the structures of some of the glycosidic components and full structures for these antibiotics have not yet been published. It is also interesting to note the similarity to chlorothricin, which has an extra oxygen atom in the macrocyclic ring.[32]

Experimental

Unless otherwise stated optical rotations were recorded at *c* 0.3%. I.r. spectra were recorded on a Perkin-Elmer Infracord 137, or 221 spectrometer. U.v. spectra were run on a Cary 118 spectrometer and c.d. spectra were run on a Cary 61 spectrometer. Low-resolution e.i. mass spectra were recorded on a Varian MAT CH5 spectrometer. C.i. mass spectra were recorded on a Varian MAT 312 spectrometer. ^1H N.m.r. spectra were recorded at 79.5 MHz on a Varian CFT-20 instrument and at 100 MHz on a Varian XL-100-15 spectrometer. The 600 MHz ^1H n.m.r. spectra were run on a non-commercially available spectrometer at Carnegie-Mellon University, Pittsburgh, Pennsylvania. ^{13}C N.m.r. spectra were obtained on a Varian XL-100-15 spectrometer in the Fourier-transform mode using a Varian 620L-100 16K computer equipped with a 2.5 Megabyte disc system, or on a Varian CFT-20 spectrometer. The $J_{^{13}C-^1H}$ values were measured on a Varian XL-100-15 spectrometer. All chemical shift values were reported in p.p.m. downfield from tetramethylsilane. Kijanimicin (1) and its derivatives showed a marked tendency

Table 17. ^{1}H N.m.r. data for kijanolide derivatives in $CDCl_3$ at 600 MHz (J in Hz) [a–c]

Assignment	Data key	Compound (48)	(49)	(50)	(51)	(52)
5ax-H	δ_H (dd)	2.01	2.06	2.00	2.11	2.16
	$J_{5ax,6ax}$	9.1	NA	9.5	10.8	9.9
	$J_{5ax,10ax}$	9.5	NA	10.2 [c]	10.8	10.2 [c]
6ax-H	δ_H (m)	NA	NA	NA	NA	NA
	$J_{5ax,6ax}$	NA	NA	NA	NA	NA
	$J_{6ax,6\text{-}CH_3}$	NA	NA	NA	NA	NA
	$J_{6ax,7eq}$	NA	NA	NA	NA	NA
	$J_{6ax,7ax}$	NA	NA	NA	NA	NA
7eq-H [d]	δ_H (m)	NA	NA	NA	NA	NA
	$J_{6ax,7eq}$	NA	NA	NA	NA	NA
	$J_{7eq,7ax}$	NA	NA	NA	NA	NA
	$J_{7eq,8eq}$	NA	NA	NA	NA	NA
7a-H [d]	δ_H (m)	1.55	1.49	1.56	1.53	1.49
	$J_{6ax,7ax}$	NA	NA	NA [c]	NA	NA
	$J_{7eq,7ax}$	NA	NA	NA	NA	NA
	$J_{7ax,8eq}$	NA	NA	3.7 [c]	NA	NA
8eq-H	δ_H (m)	2.26	2.24	2.42	2.43	2.45
	$J_{7eq,8eq}$	NA	NA	NA	NA	NA
	$J_{7ax,8eq}$	NA	NA	3.7 [c]	NA	NA
	$J_{8eq,8\text{-}CH_3}$	7.0	7.0	7.0 [c]	7.0	7.0 [c]
	$J_{8eq,9ax}$	5.4	5.4	4.9 [c]	5.4	5.8 [c]
9ax-H	δ_H (dd)	3.66	3.67	3.11	4.81	4.81
	$J_{8eq,9ax}$	5.4	5.4	4.9 [c]	5.4	5.8 [c]
	$J_{9ax,10ax}$	10.4	9.5	10.5 [c]	11.2	11.1 [c]
10ax-H	δ_H (ddddd)	2.07	2.06	2.10	2.29	2.27
	$J_{9ax,10ax}$	10.4	9.5	10.5 [c]	11.2	11.1 [c]
	$J_{5ax,10ax}$	9.5	NA	10.2 [c]	10.8	10.2 [c]
	$J_{10ax,11}$	NA	NA	2.0	NA	2.0
	$J_{10ax,12}$	2.1	NA	2.5 [c]	2.5	2.5 [c]
	$J_{10ax,13}$	NA	NA	<1.0 [c]	NA	NA
11-H	δ_H (ddd)	6.04	6.01	6.02	5.70	5.68
	$J_{10ax,11}$	NA	NA	2.0	NA	2.0
	$J_{11,12}$	10.4	9.9	10.4 [c]	10.4	9.9 [c]
	$J_{11,13}$	NA	NA	2.0 [c]	NA	NA
12-H	δ_H (ddd)	5.44	5.47	5.39	5.40	5.44
	$J_{10ax,12}$	2.1	NA	2.5 [c]	2.5	2.5 [c]
	$J_{11,12}$	10.4	9.9	10.4 [c]	10.4	9.9 [c]
	$J_{12,13}$	5.0	5.0	5.0	5.0	5.0 [c]
13-H	δ_H (ddd)	3.53	4.03	3.47	3.53	4.02
	$J_{10,13}$	NA	NA	<1.0 [c]	NA	NA
	$J_{11,13}$	NA	NA	2.0 [c]	NA	NA
	$J_{12,13}$	5.0	5.0	5.0	5.0	5.0 [c]
15-H	δ_H (ddq)	5.22	5.12	5.18	5.18	5.13
	$J_{15,14\text{-}CH_3}$	<1.0	<1.0	<1.0 [c]	<1.0	<1.0
	$J_{15,16}$	10.4	9.5	9.1 [c]	10.2	9.3 [c]
	$J_{15,16'}$	NA	NA	2.0 [c]	NA	NA
16-H	δ_H (ddd)	2.40	2.42	2.22	2.41	2.41
	$J_{15,16}$	10.4	9.5	9.1 [c]	10.2	9.3 [c]
	$J_{16,16'}$	16.6	16.6	16.6	17.0	17.0 [c]
	$J_{16,17}$	2.3	2.9	2.3	2.3	3.3 [c]
16′-H	δ_H (ddd)	2.23	2.23	2.31	2.21	2.19
	$J_{15,16'}$	NA	NA	2.0 [c]	NA	NA
	$J_{16,16'}$	16.6	16.6	16.6	17.0	17.0 [c]
	$J_{16',17}$	2.0	NA	2.1	2.0	3.3 [c]
17-H	δ_H (ddd)	4.20	4.17	3.61	5.21	5.23
	$J_{16,17}$	2.3	2.9	2.3	2.3	3.3 [c]
	$J_{16',17}$	2.0	NA	2.1	2.0	3.3 [c]
	$J_{17,19}$	NA	NA	2.1 [c]	NA	NA
19-H	δ_H (ddq)	5.28	5.17	5.28	5.11	4.99
	$J_{17,19}$	NA	NA	2.1 [c]	NA	NA
	$J_{18\text{-}CH_3,19}$	<1.0	<1.0	<1.0 [c]	<1.0	<1.0
	$J_{19,20}$	10.6	9.7	10.8	10.4	9.9 [c]
20-H	δ_H (dd)	3.65	3.42	3.65	3.62	3.40
	$J_{19,20}$	10.6	9.7	10.8	10.4	9.9 [c]
	$J_{20,?}$ [f]	1.5	NA	1.2	1.5	1.7
21-H	δ_H (s)	5.46	5.47	5.50	5.41	5.41
23-H	δ_H (dq)	2.65	2.61	2.66	2.66	2.61
	$J_{23,24}$	0	0	0	0	0
	$J_{23,24'}$	7.5	7.0	7.5	7.5	7.0 [c]
	$J_{23,33\text{-}CH_3}$	7.5	7.5	7.5	7.5	7.0 [c]

Table 17 (*continued*)

Assignment	Data key	Compound (48)	(49)	(50)	(51)	(52)
24-H	δ_H (d)	1.84	1.76	1.83	1.84	1.76
	$J_{23,24}$	0	0	0	0	0
	$J_{24,24'}$	14.3	14.1	14.1	14.1	14.1 [c]
24'-H	δ_H (dd)	2.37	2.33	2.37	2.38	2.34
	$J_{23,24'}$	7.5	7.0	7.5	7.5	7.0 [c]
	$J_{24,24'}$	14.3	14.1	14.1	14.1	14.1 [c]
32-H	δ_H (d)	4.11	4.10	4.12	4.12	4.11
	$J_{32,32'}$	12.0	12.0	11.8	12.0	11.6 [c]
32'-H	δ_H (d)	3.79	3.77	3.79	3.78	3.77
	$J_{32,32'}$	12.0	12.0	11.8	12.0	11.6 [c]
4-CH_3	δ_H (s)	1.62	1.54	1.62	1.64	1.56
6-CH_3	δ_H (d)	0.66	0.64	0.65	0.67	0.65
	$J_{6ax,6\text{-}CH_3}$	5.8	5.8	6.6 [c]	6.2	5.8
8-CH_3	δ_H (d)	1.05	1.04	0.97	1.01	0.99
	$J_{8eq,8\text{-}CH_3}$	7.0	7.0	7.0 [c]	7.0	7.0 [c]
14-CH_3	δ_H (d)	1.39	1.41	1.38	1.38	1.40
	$J_{15,14\text{-}CH_3}$	<1.0	<1.0	<1.0 [c]	<1.0	<1.0
18-CH_3	δ_H (d)	1.40	1.31	1.41	1.44	1.35
	$J_{19,18\text{-}CH_3}$	<1.0	<1.0	<1.0 [c]	<1.0	<1.0
23-CH_3	δ_H (d)	1.30	1.28	1.30	1.30	1.29
	$J_{23,23\text{-}CH_3}$	7.5	7.5	7.5	7.5	7.0 [c]
9-OCH_3	δ_H (s)			3.29 [e]		
17-OCH_3	δ_H (s)			3.36 [e]		
26-OCH_3	δ_H (s)		4.13			4.13
32-OCH_3	δ_H (s)	3.33	3.32	3.34 [e]	3.33	3.32
9-$OCOCH_3$	δ_H (s)				2.12	2.12
17-$OCOCH_3$	δ_H (s)				2.12	2.12

[a] NA = Could not be ascertained. [b] Run at Carnegie-Mellon University; NIH grant RR00292. [c] Confirmed by off-resonance decoupling. [d,e] May be interchanged. [f] Probably coupled to 21-H.

to form solvates particularly with chloroform and this property, coupled with the tendency of the free enols to form chelates with metal ions, made it extremely difficult to get accurate microanalyses on these compounds.

Isolation of Kijanimicin (1).—(i) Crude kijanimicin complex (30.1 g) was chromatographed on a silica gel column (4 kg) using 2.5% methanol in chloroform as the eluant to give kijanimicin (1) (14.8 g) as an off-white amorphous solid.*

(ii) Crude kijanimicin complex (40 g) was introduced onto a Waters Prep 500 h.p.l.c. instrument equipped with four silica gel cartridges (325 g each) and the column was eluted at 250 ml/min using the step-gradient technique and collecting 500 ml fractions. The elution was started with chloroform–ethyl acetate–methanol (20 : 79 : 2) (8 l). The methanol concentration was increased in stages (20 : 79 : 3) (4 l), (20 : 79 : 4) (4 l), (20 : 79 : 5) (4 l), and (20 : 79 : 6) (4 l). The combined eluates were evaporated to dryness to afford kijanimicin (1) (13.2 g), as an off-white amorphous solid.* Additional kijanimicin (1) (7.4 g) was obtained (70% pure) by stripping the columns with acetone (4 l).

(iii) Crude kijanimicin complex (3.5 g) was chromatographed on a Waters Prep 500 h.p.l.c. instrument equipped with two silica gel cartridges (325 g each) and the column was eluted at 250 ml/min using dichloromethane–methanol–triethylamine (98 : 1 : 1) as the eluant, 500-ml fractions being collected. The appropriate fractions were combined and evaporated to dryness. The residue was taken up in chloroform and the pH was adjusted to 1 with dilute hydrochloric acid. The chloroform layer was separated and washed thoroughly with distilled water to remove all traces of acid. The chloroform solution was evaporated to dryness to give kijanimicin (1) (2.3 g) as an off-white amorphous solid.*

(iv) Purified kijanimicin (1) (1.78 g) [from (iii) above] was rechromatographed on a Waters Prep 500 h.p.l.c. instrument equipped with two C_{18}-silica gel (reversed phase) cartridges (370 g each) and the column was eluted at 300 ml/min with acetonitrile–aqueous ammonium acetate (3 : 5) (pH 6.8). The appropriate fractions were combined and most of the acetonitrile was distilled off under reduced pressure. The pH was adjusted to 4.5—5.0 with dilute hydrochloric acid and the aqueous suspension was extracted with chloroform. The chloroform layer was washed thoroughly with distilled water and evaporated to dryness to give kijanimicin (1) (846 mg) as a colourless amorphous solid which was judged to be analytically pure by the following criteria: (a) t.l.c. on silica gel plates using 10% methanol in chloroform as the eluant; (b) t.l.c. on silica gel plates using 40% chloroform in acetone as the eluant; (c) analytical reversed phase h.p.l.c. (C_{18}-silica gel) using acetonitrile–aqueous ammonium acetate (3 : 5) (pH 6.8) as the eluant; (d) bioautography against *Sarcina lutea*; (e) bioassay against *Bacillus subtilis*.

The following data were recorded for kijanimicin (1), m.p. 174.5 °C (decomp.) (Found: C, 60.9; H, 8.0; N, 1.9. $C_{67}H_{100}N_2O_{24}$ requires C, 61.08; H, 7.65; N, 2.13%), $[\alpha]_D^{26}$ −124.2° (CH_3OH), pK_a 5.0, $\lambda_{max.}$ (CF_3CH_2OH) 200 (ε 42 832), 241 (ε 8 946), 264sh (ε 9 697), and 274nm (ε 9 446); $\lambda_{max.}$ (CH_3OH + 0.1M HCl), 205 (ε 38 313) and 258 nm (ε 9 881); $\lambda_{max.}$ (CH_3OH + 0.1M NaOH) 236 (ε 14 677), 266sh (ε 12 002), and 276 nm (ε 12 002); $[\theta]_{194}$ −174 152, $[\theta]_{204}$ +22 716, $[\theta]_{216}$ −174 152, $[\theta]_{250sh}$ −25 744, and $[\theta]_{300}$

* Kijanimicin (1) showed a marked tendency to form chelates with heavy-metal ions and consequently batches often varied in colour from off-white, through pale blue-green, to pink. In most instances where some chelates were present they could be removed by dissolving the kijanimicin (1) in chloroform and bubbling hydrogen sulphide through the solution. The solution was filtered and passed over a short silica gel column using 2.5% methanol in chloroform as the eluant to give dechelated kijanimicin (1).

Table 18. ^{1}H N.m.r. data for 26-*O*-methylkijanimicin (2) in $CDCl_3$ at 600 MHz (2D *J* and difference spectra) [a]

Assignment	Multiplicity	δ_H	*J* (Hz)
5ax-H	m	2.03	$J_{5ax,6ax}$ 9.3
6ax-H	m	1.54	$J_{5ax,6ax}$ 9.3, $J_{6ax,7ax}$ 9.3, $J_{6ax,6\text{-}CH_3}$ 6.3
7eq-H	m	1.55	
7ax-H	m	1.43	$J_{6ax,7ax}$ 9.3
8eq-H	m	2.21	$J_{8eq,8\text{-}CH_3}$ 7.1
9ax-H	dd	3.44	$J_{8eq,9ax}$ 5.2, $J_{9ax,10ax}$ 9.6
10ax-H	m	2.03	$J_{9ax,10ax}$ 9.6
11-H	ddd	5.69	$J_{10ax,11}$ 2.0, $J_{11,12}$ 10.2, $J_{11,13}$ 2.0
12-H	ddd	5.42	$J_{10ax,12}$ 2.8, $J_{11,12}$ 10.2, $J_{12,13}$ 5.2
13-H	m	4.00	$J_{12,13}$ 5.2
15-H	m	5.08	$J_{15,14\text{-}CH_3}$ <1.0, $J_{15,16}$ 10.2
16-H	ddd	2.35	$J_{15,16}$ 10.2, $J_{16,16'}$ 16.3
16'-H	m	2.19	
17-H	m	4.18	$J_{16,17}$ 4.0, $J_{16',17}$ 4.0
19-H	m	5.02	$J_{19,20}$ 10.2
20-H	m	3.41	$J_{19,20}$ 10.2
21-H	s	5.51	
23-H	ddq	2.63	$J_{23,24}$ <0.5, $J_{23,24'}$ 7.4, $J_{23,23\text{-}CH_3}$ 7.1
24-H	dd	1.74	$J_{23,24}$ <0.5, $J_{24,24'}$ 14.0
24'-H	dd	2.34	$J_{23,24'}$ 7.4, $J_{24,24'}$ 14.0
32-H [b]			
32'-H [b]			
4-CH_3	s	1.52	
6-CH_3	d	0.62	$J_{6ax,6\text{-}CH_3}$ 6.3
8-CH_3	d	1.04	$J_{8eq,8\text{-}CH_3}$ 7.1
14-CH_3	d	1.32	$J_{15,14\text{-}CH_3}$ <1.0
18-CH_3	d	1.31	$J_{19,18\text{-}CH_3}$ <1.0
23-CH_3	d	1.29	$J_{23,23\text{-}CH_3}$ 7.1
26-OCH_3	s	4.12	
1^A_{eq}-H	dd	4.78	$J_{1^A_{eq},2^A_{eq}}$ <0.5, $J_{1^A_{eq},2^A_{ax}}$ 4.4
2^A_{eq}-H	ddd	2.32	$J_{1^A_{eq},2^A_{eq}}$ <0.5, $J_{2^A_{eq},2^A_{ax}}$ 15.3, $J_{2^A_{eq},3^A_{eq}}$ 3.5
2^A_{ax}-H	ddd	1.71	$J_{1^A_{eq},2^A_{ax}}$ 4.4, $J_{2^A_{eq},2^A_{ax}}$ 15.3, $J_{2^A_{ax},3^A_{eq}}$ 3.5
3^A_{eq}-H	ddd	4.06	$J_{2^A_{eq},3^A_{eq}}$ 3.5, $J_{2^A_{ax},3^A_{eq}}$ 3.5, $J_{3^A_{eq},4^A_{ax}}$ 3.5
4^A_{ax}-H	m	3.30	
5^A_{ax}-H	dq	3.89	$J_{4^A_{ax},5^A_{ax}}$ 9.6, $J_{5^A_{ax},6^A\text{-}CH_3}$ 6.0
6^A-CH_3	d	1.28	$J_{5^A_{ax},6^A\text{-}CH_3}$ 6.0
1^B_{eq}-H	dd	5.10	$J_{1^B_{eq},2^B_{eq}}$ <0.5, $J_{1^B_{eq},2^B_{ax}}$ 4.4
2^B_{eq}-H	ddd	2.21	$J_{1^B_{eq},2^B_{eq}}$ <0.5, $J_{2^B_{eq},2^B_{ax}}$ 15.5, $J_{2^B_{eq},3^B_{eq}}$ 3.7
2^B_{ax}-H	ddd	1.79	$J_{1^B_{eq},2^B_{ax}}$ 4.4, $J_{2^B_{eq},2^B_{ax}}$ 15.5, $J_{2^B_{ax},3^B_{eq}}$ 3.7
3^B_{eq}-H	ddd	4.28	$J_{2^B_{eq},3^B_{eq}}$ 3.7, $J_{2^B_{ax},3^B_{eq}}$ 3.7, $J_{3^B_{eq},4^B_{ax}}$ 3.7
4^B_{ax}-H	dd	3.36	$J_{3^B_{eq},4^B_{ax}}$ 3.7, $J_{4^B_{ax},5^B_{ax}}$ 9.9
5^B_{ax}-H	dq	3.96	$J_{4^B_{ax},5^B_{ax}}$ 9.9, $J_{5^B_{ax},6\text{-}CH_3}$ 6.2
6^B-CH_3	d	1.25	$J_{5^B_{ax},6^B\text{-}CH_3}$ 6.2
1^C_{eq}-H	dd	5.17	$J_{1^C_{eq},2^C_{eq}}$ <0.5, $J_{1^C_{eq},2^C_{ax}}$ 3.1
2^C_{eq}-H	ddd	2.18	$J_{1^C_{eq},2^C_{eq}}$ <0.5, $J_{2^C_{eq},2^C_{ax}}$ 15.4, $J_{2^C_{eq},3^C_{eq}}$ 2.9
2^C_{ax}-H	ddd	1.89	$J_{1^C_{eq},2^C_{ax}}$ 3.1, $J_{2^C_{eq},2^C_{ax}}$ 15.4, $J_{2^C_{ax},3^C_{eq}}$ 3.3
3^C_{eq}-H	m	3.95 [c]	
4^C_{ax}-H	dd	3.14	$J_{3^C_{eq},4^C_{ax}}$ 2.9, $J_{4^C_{ax},5^C_{ax}}$ 9.8
5^C_{ax}-H	dq	4.06	$J_{4^C_{ax},5^C_{ax}}$ 9.8, $J_{5^C_{ax},6^C\text{-}CH_3}$ 6.0
6^C-CH_3	d	1.28	$J_{5^C_{ax},6^C\text{-}CH_3}$ 6.0
1^D_{ax}-H	dd	4.91	$J_{1^D_{ax},2^D_{eq}}$ 2.2, $J_{1^D_{ax},2^D_{ax}}$ 9.8
2^D_{eq}-H	ddd	2.20	$J_{1^D_{ax},2^D_{eq}}$ 2.2, $J_{2^D_{eq},2^D_{ax}}$ 14.2, $J_{2^D_{eq},3^D_{eq}}$ 3.0
2^D_{ax}-H	ddd	1.58	$J_{1^D_{ax},2^D_{ax}}$ 9.8, $J_{2^D_{eq},2^D_{ax}}$ 14.2, $J_{2^D_{ax},3^D_{eq}}$ 3.0
3^D_{eq}-H	ddd	4.24	$J_{2^D_{eq},3^D_{eq}}$ 3.0, $J_{2^D_{ax},3^D_{eq}}$ 3.0, $J_{3^D_{eq},4^D_{ax}}$ 3.0
4^D_{ax}-H	dd	2.78	$J_{3^D_{eq},4^D_{ax}}$ 3.0, $J_{4^D_{ax},5^D_{ax}}$ 9.6
5^D_{ax}-H	dq	3.72	$J_{4^D_{ax},5^D_{ax}}$ 9.6, $J_{5^D_{ax},6^D\text{-}CH_3}$ 6.0
6^D-CH_3	d	1.27	$J_{5^D_{ax},6^D\text{-}CH_3}$ 6.0
4^D-OCH_3	s	3.41	
1^E_{ax}-H	dd	4.45	$J_{1^E_{ax},2^E_{eq}}$ 2.2, $J_{1^E_{ax},2^E_{ax}}$ 10.1
2^E_{eq}-H	dd [d]	2.78	$J_{1^E_{ax},2^E_{eq}}$ 2.2, $J_{2^E_{eq},2^E_{ax}}$ 14.8
2^E_{ax}-H	dd	1.62	$J_{1^E_{ax},2^E_{ax}}$ 10.1, $J_{2^E_{eq},2^E_{ax}}$ 14.8

Table 18. *(continued)*

Assignment	Multiplicity	δ_H	*J* (Hz)
4^E_{eq}-H	ddd [d]	4.38	$J_{2^E_{eq},4^E_{eq}}$ 1.9, $J_{4^E_{eq},5^E_{ax}}$ 1.9, $J_{4^E_{eq},4^E\text{-NHCOOCH}_3}$ 9.9
5^E_{ax}-H	dq	3.30	$J_{4^E_{eq},5^E_{ax}}$ 1.9, $J_{5^E_{ax},6^E\text{-CH}_3}$ 6.2
6^E-CH_3	d	1.16	$J_{5^E_{ax},6^E\text{-CH}_3}$ 6.2
3^E-CH_3	s	1.58	
4^E-N*H*COOCH$_3$	d	5.34	$J_{4^E_{eq},4^E\text{-NHCOOCH}_3}$ 9.9
4^E-NHCOOC*H*$_3$	s	3.71	

[a] Run at Carnegie-Mellon University; NIH Grant RR 00292. [b] Not located under conditions that the n.m.r. was run. [c] Chemical shift could not be accurately ascertained. [d] The long range W-coupling between 4^E_{eq}-H and 2^E_{eq}-H was not observed in the *J*-resolve cross section of δ_H 2.78, but was evident in that of δ_H 4.38.

+11 358 (CF_3CH_2OH); $\nu_{max.}$ ($CHCl_3$) 3 625, 3 550, 3 480, 3 440, 2 980, 2 940, 2 910, 1 755, 1 730, 1 605w, 1 545, 1 510, 1 230, 1 130, and 1 058 cm^{-1}; δ_H ($CDCl_3$) (220 MHz) 0.65 (3 H, d, *J ca.* 5 Hz, 6-CH_3), 1.07 (3 H, d, *J* 7 Hz, 8-CH_3), 1.18 (3 H, d, *J* 6 Hz, 6^E-CH_3), 1.20—1.40 (envelope of CH_3 signals), 1.60 (3 H, s, 3^E-CH_3), 1.64 (3 H, s, 4-CH_3), 3.45 (3 H, s, 4^D-OCH_3), and 3.76 (3 H, s, 4^E-NHCOOC*H*$_3$); *m/z* 552 (2.1) (D_1), 534 (8.4) ($D_1 - H_2O$), 516 (3.3) ($D_1 - H_2O - H_2O$), 498 (1.2), 374 (0.7) (D_9), 356 (1.5) ($D_9 - H_2O$), 231 (8.4) ($B_1{}^b$), 184 (100) ($B_6{}^b$ and $B_{15}{}^b$) 161 (41.0) (D_{19}), 152 (8.4) ($B_{10}{}^b$), 147 (11.4) (D_{20}), 145 (17.6) ($A_1{}^c$), 140 (20.3) ($B_{18}{}^b$), 131 (13.1) ($A_1{}^b$), 128 (38.1) ($B_{12}{}^b$), 127 (27.7) ($A_3{}^c$), 96 (22.1) ($B_{14}{}^b$), and 95 (50.2) (A_5).

Kijanimicin Copper Complex.—Kijanimicin (1) (150 mg) and copper(II) acetate monohydrate (62 mg, 2 equiv.) were dissolved in methanol (25 ml) and the mixture was stirred at 25 °C for 4 h. The solution was evaporated to dryness and the residue was chromatographed on a Waters Prep 500 h.p.l.c. instrument equipped with one reversed phase C_{18}-silica gel cartridge (370 g) using 10% water in methanol as the eluant to give kijanimicin copper complex * (101 mg) as a pale blue-green amorphous solid which crystallized as fine clusters of needles from ethyl acetate–hexane, m.p. 207 °C, $[\alpha]_D{}^{26}$ −208.5° ($CHCl_3$), $\lambda_{max.}$ (CF_3CH_2OH) 200 (E^1_1 301.4), 226 (E^1_1 82.5), and 263 nm (E^1_1 59.3); $\nu_{max.}$ ($CHCl_3$) 3 570, 3 480, 3 440, 2 940, 1 745, 1 735, 1 587, 1 548, 1 515, 1 438, 1 150, and 1 058 cm^{-1}.

Kijanimicin Zinc Complex.—Kijanimicin (1) (150 mg) and zinc(II) acetate dihydrate (50 mg, 2 equiv.) were dissolved in methanol (25 ml) and the mixture was stirred at 25 °C for 4 h; the solution was then evaporated to dryness and the residue chromatographed on a Waters Prep 500 h.p.l.c. instrument equipped with one reversed phase C_{18}-silica gel cartridge (370 g) using 10% water in methanol as the eluant to give kijanimicin zinc complex * (91 mg) as a colourless amorphous solid, $[\alpha]_D{}^{26}$ −115.3° ($CHCl_3$), $\lambda_{max.}$ (CF_3CH_2OH) 199 (E^1_1 276.8), 240 (E^1_1 54.0), and 264 nm (E^1_1 59.5); $\nu_{max.}$ ($CHCl_3$) 3 620, 3 550, 3 490, 3 450, 2 940, 1 747, 1 735, 1 585, 1 545, 1 515, 1 460, 1 132, and 1 060 cm^{-1}.

Kijanimicin Sodium Salt.—Kijanimicin (1) (7.6 g) was added to a mixture of 0.1M sodium hydroxide (58.12 ml, 1.01 equiv.) and distilled water (800 ml) and the mixture was stirred at 25 °C for 5 h. The mixture was filtered through a fine glass sintered funnel and the aqueous filtrate was lyophilized to give kijanimicin sodium salt * (6.3 g, 82%) as a colourless amorphous solid (Found: C, 58.65; H, 7.5; N, 1.95; Na, 2.25. $C_{67}H_{99}N_2NaO_{24}$ requires C, 60.08; H, 7.45; N, 2.09; Na, 1.72%), $[\alpha]_D{}^{26}$ −165.5° (H_2O), $\lambda_{max.}$ (CF_2CH_2OH) 197 (ε 40 788), 236 (ε 8 734), 266sh (ε 8 412), and 272 nm (ε 8 412); $\nu_{max.}$ (KBr) 3 450, 2 970, 2 925, 1 720, 1 625, 1 542, 1 510, 1 405, and 1 050 cm^{-1}.

Kijanimicin Potassium Salt.—Kijanimicin (1) (250 mg) was added to a mixture of 0.1M-potassium hydroxide (0.186 ml, 1.01 equiv.) and distilled water (50 ml) and the mixture was stirred at 25 °C for 2.5 h. The product was worked up as above to give kijanimicin potassium salt * (182 mg, 71%) as a colourless amorphous solid (Found: C, 50.55; H, 6.5; N, 1.65; K, 2.75. $C_{67}H_{99}KN_2O_{24}$ requires C, 59.36; H, 7.36; N, 2.07; K, 2.88%), $[\alpha]_D{}^{26}$ −147.7° (CH_3OH), $\lambda_{max.}$ (CF_3CH_2OH) 201 (ε 31 938), 239 (ε 8 052), 264sh (ε 8 052), and 273 nm (ε 7 862); $\nu_{max.}$ (KBr) 3 440, 2 955, 2 920, 1 710, 1 620, 1 540, 1 402, and 1 050 cm^{-1}.

Kijanimicin Rubidium Salt.—Kijaniminin (1) (250 mg) and rubidium hydroxide hydrate (23 mg, 1.01 equiv.) were dissolved in distilled water (20 ml) and the mixture allowed to remain at 7 °C for 16 h. The product was worked up as above to give kijanimicin rubidium salt * (187 mg, 70%) as a colourless amorphous solid, $[\alpha]_D{}^{26}$ −168.0° (CH_3OH), $\lambda_{max.}$ (CF_3CH_2OH) 201 (ε 41 401), 240 (ε 10 207), 266sh (ε 10 010), and 276 nm (ε 10 319); $\nu_{max.}$ (KBr) 3 430, 2 955, 2 920, 1 715, 1 622, 1 540, 1 400, and 1 050 cm^{-1}.

Determination of the Molecular Composition of Kijanimicin (1).—(i) *Radiochemical molecular weight determination.*[6] (a) Kijanimicin (1) (113 mg) and desoxycholic acid (110 mg) were dissolved in dry tetrahydrofuran (450 ml) and the solution was cooled to 0 °C. Diazomethane prepared in the usual way from [^{14}C]-Diazald (1.1 mg, 0.0051 mmol, 0.05 mCu) and cold Diazald (500 mg, 2.383 mmol) was bubbled through the solution. The mixture was allowed to remain at 24 °C for 17 h. Excess diazomethane was flushed out with a stream of dry nitrogen and the solution was evaporated to dryness. Preparative t.l.c. on silica gel plates using 75% diethyl ether in acetone as the eluant afforded 26-*O*-[^{14}C]methylkijanimicin (2) (74.5 mg) and [^{14}C]methyl desoxycholate (49.1 mg). The 26-*O*-[^{14}C]methylkijanimicin (2) was further purified by preparative t.l.c. on silica gel plates using 50% diethyl ether in acetone as the eluant to give 26-*O*-[^{14}C]methylkijanimicin (2) (50.3 mg) (97.2% radiochemical purity; specific activity 0.013 378). The [^{14}C]methyl desoxycholate was further purified by preparative t.l.c. on silica gel plates using 30% acetone in hexane as the eluant to give [^{14}C]methyl desoxycholate (47.2 mg) (95.4% radiochemical purity; specific activity 0.042 822). Molecular weight of (2) = 0.042 822/0.013 378 × 406.56 = 1 301.4 g/mol.

(b) Kijanimicin (1) (75.7 mg) and desoxycholic acid (76.0 mg) were dissolved in dry tetrahydrofuran (450 ml) and the

* The complexes and salts of kijanimicin gave unsatisfactory analyses and precise compositions of these derivatives could not be determined from the analytical data.

solution was cooled to 0 °C. Diazomethane prepared in the usual way from [^{14}C]-Diazald (3.3 mg, 0.0153 mmol, 0.15 mCu) and cold Diazald (321.3 mg, 1.531 mmol) was bubbled through the solution. The products were worked up as in (a) above to give 26-*O*-[^{14}C]methylkijanimicin (2) (60.1 mg) (95.7% radiochemical purity; specific activity 0.078 352) and [^{14}C]methyl desoxycholate (49.6 mg) (98.8% radiochemical purity; specific activity 0.248 930). Molecular weight of (2) = 0.248 930/0.078 352 × 406.56 = 1 291.7 g/mol.

(ii) ^{252}Cf-*Plasma desorption mass spectrometry*.[7] The ^{252}Cf-p.d.m.s. measurements were made in the following manner. Separate solutions of 26-*O*-methylkijanimicin (2) and *O*-β-D-kijanosyl-(1→17)-26-*O*-methylkijanolide (15) were prepared by dissolving *ca.* 300 μg of the material in 500 μl of acetone (Burdich and Jackson, ' Distilled in Glass '). These solutions (100 μl) were electrosprayed onto a 1.5 μm thick aluminized Mylar film (Steiner Film Corporation) stretched over the 1.9 cm diameter aperture of a stainless-steel target holder.[33] The sample film was irradiated by a ^{252}Cf source giving a nuclear fission fragment flux of 2 000 s^{-1} through the samples. Positive and negative ion spectra were recorded for 1 h and the results analyzed using a procedure described elsewhere.[34]

26-O-*Methylkijanimicin* (2).—Kijanimicin (1) (1.2 g) was dissolved in methanol and an excess of diazomethane in diethyl ether (125 ml) was added. The mixture was allowed to remain at 25 °C for 16 h. The excess diazomethane was blown off with a stream of nitrogen and the solution was evaporated to dryness. The residue was chromatographed on a silica gel column (60 × 3 cm) using 1% methanol in chloroform as the eluant to give 26-*O*-methylkijanimicin (2) (885 mg, 73%) as a colourless amorphous solid (Found: C, 60.15; H, 7.55; N, 1.85. $C_{68}H_{102}N_2O_{24}$ requires C, 61.34; H, 7.72; N, 2.10%), $[\alpha]_D^{26}$ −130.1° (CH_3OH), $\lambda_{max.}$ (CF_3CH_2OH) 200 (ε 42 625) and 254 nm (ε 9 880); $[\theta]_{197}$ −275 124, $[\theta]_{204}$ −154 069, $[\theta]_{212}$ −199 190, $[\theta]_{233}$ +11 005, $[\theta]_{255}$ −11 005, $[\theta]_{280}$ +4 402, and $[\theta]_{335}$ −3 301 (CF_3CH_2OH); ν_{max} ($CHCl_3$) 3 675, 3 600, 3 530, 3 475, 3 425, 3 010, 2 950, 2 925, 1 745, 1 730, 1 662, 1 570, 1 540, 1 510, 1 350, 1 228, 1 130, 1 100, and 1 053 cm^{-1}; δ_H ($CDCl_3$) (600 MHz) see Table 18, δ_H (CD_3CN) (600 MHz) 4.72 (1 H, dd, $J_{1^A_{eq},2^A_{ax}}$ 4.5 Hz, $J_{1^A_{eq},2^A_{eq}}$ <0.5 Hz, 1^A_{eq}-H), 4.88 (1 H, dd, $J_{1^D_{ax},2^D_{ax}}$ 10.0 Hz, $J_{1^D_{ax},2^D_{eq}}$ 1.9 Hz, $H_{1^D_{ax}}$), 4.95 (1 H, ddq, $J_{15,16}$ 10.0 Hz, $J_{15,16'} = J_{14,15\text{-}CH_3}$ = <1 Hz, 15-H), 5.02 (1 H, ddq, $J_{19,20}$ 9.0 Hz, $J_{17,19} = J_{19,18\text{-}CH_3}$ = *ca.* 0.5 Hz, 19-H), 5.06 (1 H, dd, $J_{1^B_{eq},2^B_{ax}}$ 4.0 Hz, $J_{1^B_{eq},2^B_{eq}}$ <0.5 Hz, 1^B_{eq}-H) and 5.13 (1 H, dd, $J_{1^C_{eq},2^C_{ax}}$ 3.3 Hz, $J_{1^C_{eq},2^C_{eq}}$ <0.5 Hz, 1^C_{eq}-H); *m/z* 566 (0.3) (D_1), 548 (0.7) ($D_1 - H_2O$), 530 (0.4) $D_1 - H_2O - H_2O$), 184 (6.5) (B_6[b] and B_{15}[b]), 161 (16.5) (D_{19}), 152 (1.4) (B_{10}[b]), 147 (2.2) (D_{20}), 145 (39.2) (A_1[c]), 140 (5.1) (B_{18}[b]), 131 (27.6) (A_1[b]), 128 (23.4) (B_{12}[b]), 127 (100) (A_3[c]), 96 (22.4) (B_{14}[b]), and 95 (41.2) (A_5).

32-O-(4-*Iodobenzoyl*)*kijanimicin* (3).—Kijanimicin (1) (1.5 g) was dissolved in dry pyridine (300 ml) and 4-iodobenzoyl chloride (1.5 g) (4.7 eq) was added. The mixture was stirred under argon at 80 °C for 4 h. The solution was evaporated to dryness and the residue was azeotroped with toluene and then chromatographed on a silica gel column (30 × 5 cm) using 3% methanol in chloroform as the eluant to give the product. The latter was rechromatographed on a silica gel column (60 × 5 cm) using 40% chloroform in acetone as the eluant to give a product which was chelated. The product was dissolved in chloroform and hydrogen sulphide was bubbled through the solution. The solution was filtered and evaporated to dryness. The residue was rechromatographed on a silica gel column (60 × 2 cm) using 0.5% methanol in chloroform as the eluant to give 32-*O*-(4-iodobenzoyl)kijanimicin (3) (481 mg, 32%) as a colourless amorphous solid (Found: C, 57.27; H, 6.77; I, 11.97; N, 1.56. $C_{74}H_{103}IN_2O_{25}$ requires C, 57.40; H, 6.71; I, 8.21; N, 1.81%), $[\alpha]_D^{26}$ −112.6° (CH_3OH), $\lambda_{max.}$ (CH_3OH) 196 (ε 63 300) and 256 nm (ε 26 247); $\nu_{max.}$ ($CHCl_3$), 3 580, 3 500, 3 460, 2 950, 1 760, 1 730, 1 597, 1 555, 1 520, 1 275, 1 240, 1 130, and 1 062 cm^{-1}; δ_H ($CDCl_3$), (220 MHz) 0.63 (3 H, d, *J ca.* 5 Hz, 6-CH_3), 1.07 (3 H, d, *J* 7 Hz, 8-CH_3), 1.15—1.40 (envelope of CH_3 signals), 1.58 (3 H, s, 6^E-CH_3), 1.61 (3 H, s, 4-CH_3), 3.39 (3 H, s, 4^D-OCH_3), 3.69 (3 H, s, 4^E-NHCOOCH_3), and 7.78 (4 H, bs, 32-$OCOC_6H_4I$); *m/z* 782 (0.2) (D_5), 764 (0.8) ($D_5 - H_2O$), 746 (0.2), 615 (0.1), 562 (0.3), 544 (0.3), 534 (0.4) ($D_5 - IC_6H_4COOH$), 520 (0.6), 516 (0.7) ($D_5 - IC_6H_4COOH - H_2O$), 498 (0.4), 248 (7.0) (D_{21}), 231 (5.5) (B_1[b] and D_{22}), 184 (8.7) (B_6[b] and B_{15}[b]), 152 (3.9) (B_{10}[b]), 145 (12.2) (A_1[c]), 140 (3.9) (B_{18}[b]), 131 (14.9) (A_1[b]), 128 (10.9) (B_{12}[b]), 127 (91.2) (A_3[c]), 96 (53.4) (B_{14}[b]), and 95 (88.1) (A_5).

Methanolysis of Kijanimicin (1).—Kijanimicin (1) (1.68 g) was dissolved in 0.5M-methanolic hydrogen chloride (1 600 ml) and the solution was stirred at 25 °C under argon for 16.5 h. Concentrated ammonium hydroxide (90 ml) was added and the mixture was evaporated to dryness under reduced pressure. The residue was extracted with chloroform and the extract was filtered. The filtrate was evaporated to dryness and the residue was chromatographed on a silica gel column (150 × 2.5 cm) using 5% acetone in hexane as the eluant to give in the order of elution the following compounds. Methyl 2,6-dideoxy-4-*O*-methyl-β-L-*ribo*-hexopyranoside (5) (49 mg) which was further purified by preparative t.l.c. on silica gel plates using 80% ethyl acetate in dichloromethane as the eluant. The resulting gum was subjected to short-path distillation affording pure methyl 2,6-dideoxy-4-*O*-methyl-β-L-*ribo*-hexopyranoside (5) as a colourless solid, m.p. 76.0 °C [Found: *m/z* 176.1060 ($M^{+\cdot}$). $C_8H_{16}O_4$ requires *m/z* 176.1046], $[\alpha]_D^{26}$ −12.4° (CH_3OH), $\nu_{max.}$ ($CHCl_3$) 3 560, 2 930, 2 890, 2 840, 1 135, and 1 050 cm^{-1}.

Overlapping fractions containing (5) and methyl 2,6-dideoxy-4-*O*-methyl-α-L-*ribo*-hexopyranoside (4) (94 mg) were eluted next. Preparative t.l.c. on silica gel plates using 80% ethyl acetate in dichloromethane as the eluant afforded additional (5) (23 mg) and methyl 2,6-dideoxy-4-*O*-methyl-α-L-*ribo*-hexopyranoside (4) (20.6 mg) which was subjected to short-path distillation to give a colourless oil [Found: *m/z* 176.1051 ($M^{+\cdot}$). $C_8H_{16}O_4$ requires: *m/z* 176.1046], $[\alpha]_D^{26}$ −209.2° (CH_3OH), $\nu_{max.}$ ($CHCl_3$) 3 580, 2 930, 1 200, and 1 030 cm^{-1}.

Further elution of the column afforded methyl 2,6-dideoxy-α-L-*ribo*-hexopyranoside (6) (120 mg) which was subjected to short-path distillation to give a colourless oil [Found: *m/z* 131.0721 ($M - OCH_3$)$^+$. $C_6H_{11}O_3$ requires *m/z* 131.0707], $[\alpha]_D^{26}$ −170.7° ($CHCl_3$) [lit.,[17] $[\alpha]_D^{20}$ +174.0° ($CHCl_3$) for α-D], $\nu_{max.}$ ($CHCl_3$) 3 500, 2 930, 1 400, 1 185, 1 123, and 1 050 cm^{-1}.

Further elution of the column afforded methyl 2,6-dideoxy-β-L-*ribo*-hexopyranoside (7) (188 mg) which was subjected to short-path distillation to give a colourless oil [Found: *m/z* 131.0709 ($M - OCH_3$)$^+$. $C_6H_{11}O_3$ requires *m/z* 131.0707], $[\alpha']_D^{26}$ +33.2° ($CHCl_3$) [lit.,[17] $[\alpha]_D^{20}$ −36.0° ($CHCl_3$) for β-D], $\nu_{max.}$ ($CHCl_3$) 3 450, 2 930, 1 385, 1 150, and 1 060 cm^{-1}.

Further elution of the column gave overlapping fractions containing as the major product methyl 2,6-dideoxy-α-L-*ribo*-hexofuranoside (11) contaminated with (7) and methyl 2,6-dideoxy-β-L-*ribo*-hexofuranoside (12) (184 mg). This material was rechromatographed on a silica gel column (100 × 2 cm) using 10% acetone in hexane as the eluant to give material that was then rechromatographed again on a silica gel column (100 × 2 cm) using 5% acetone in hexane as

the eluant to give methyl 2,6-dideoxy-α-L-*ribo*-hexofuranoside (11) (55 mg) which was subjected to short-path distillation to give a colourless oil (Found: *m/z* 131.0714 ($M - OCH_3$)$^+$, $C_6H_{11}O_3$ requires *m/z* 131.0707), $[\alpha]_D^{26}$ −135.8° ($CHCl_3$) [lit.,[17] $[\alpha]_D^{20}$ +140.0° ($CHCl_3$) for α-D], $\nu_{max.}$ ($CHCl_3$) 3 600, 2 930, 1 265, 1 215, 1 070, and 1 035 cm^{-1}.

Further elution of the original column afforded methyl 2,6-dideoxy-β-L-*ribo*-hexofuranoside (12) (54 mg) which was subjected to short-path distillation to give a colourless oil [Found: *m/z* 131.0704 ($M - OCH_3$)$^+$. $C_6H_{11}O_3$ requires *m/z* 131.0707], $[\alpha]_D^{26}$ +98.4° ($CHCl_3$) [lit.,[17] $[\alpha]_D^{20}$ −106.0° ($CHCl_3$) for β-D]; $\nu_{max.}$ ($CHCl_3$) 3 600, 3 450, 2 930, 1 205, 1 095, 1 073, and 1 030 cm^{-1}.

The column was then stripped with 80% acetone in hexane and the eluate was evaporated to dryness. The residue was subjected to preparative t.l.c. on silica gel plates using 30% acetone in hexane as the eluant and the u.v. absorbing band at the origin was collected. The material was then chromatographed on a silica gel column (120 × 1.5 cm) using 1% methanol in chloroform as the eluant to give *O*-β-D-kijanosyl-(1→17)-kijanolide (14) (45 mg) as an off-white amorphous solid (Found: C, 60.65; H, 7.1; N, 3.3. $C_{42}H_{58}N_2O_{12}$ requires C, 64.43; H, 7.47; N, 3.58%), $[\alpha]_D^{26}$ −37.6° (CH_3OH), pK_a 4.8; $\lambda_{max.}$ (CH_3OH) 204 (ε 27 738), 240 (ε 10 366), 266 (ε 8 244), and 276 nm (ε 7 625); $[\theta]_{195}$ −574 816, $[\theta]_{206}$ +120 711, $[\theta]_{218}$ −528 831, $[\theta]_{260sh}$ −86 222, and $[\theta]_{293}$ +77 600 (CF_3CH_2OH); $\nu_{max.}$ ($CHCl_3$) 3 680, 3 615, 3 440, 2 940, 1 755, 1 730, 1 605, 1 545, 1 510, 1 235, and 1 060 cm^{-1}; δ_H ($CDCl_3$) (220 MHz) 0.66 (3 H, bd, *J* 4 Hz, 6-CH_3), 1.03 (3 H, d, *J* 7 Hz, 8-CH_3), 1.15 (3 H, d, *J* 6.2 Hz, 6^E-CH_3), 1.31 (3 H, d, *J* 6 Hz, 23-CH_3), 1.34 (3 H, s, 14-CH_3), 1.38 (3 H, s, 18-CH_3), 1.57 (3 H, s, 3^E-CH_3), 1.62 (3 H, s, 4-CH_3), 3.69 (3 H, s, 4^E-NHCOO-CH_3), 4.36 (1 H, dd, $J_{4^E_{eq},4\text{-NH}}$ 10 Hz, $J_{4^E_{eq},5^E_{ax}}$ *ca.* 1 Hz, 4^E_{eq}-H), 4.43 (1 H, dd, $J_{1^E_{ax},2^E_{ax}}$ 10 Hz, $J_{1^E_{ax},2^E_{eq}}$ 2.5 Hz, 1^E_{ax}-H), 5.47 (1 H, s, 21-H) and 5.98 (1 H, ddd, $J_{11,12}$ 10 Hz, $J_{11,13} = J_{10,11}$ = *ca.* 2 Hz, 11-H), *m/z* 552 (1.8) (D_1), 534 (9.9) ($D_1 - H_2O$), 516 (2.4) ($D_1 - H_2O$-H_2O), 498 (0.8), 374 (0.8) (D_9), 356 (1.6) ($D_9 - H_2O$), 231 (10.2) (B_1[b]), 184 (100) (B_6[b] and B_{15}[b]), 152 (9.7) (B_{10}[b]), 140 (26.8) (B_{18}[b]), 128 (68.2) (B_{12}[b]), and 96 (20.5) (B_{14}[b]).

Methyl 3-O-*Benzoyl*-2,6-*dideoxy*-4-O-*methyl*-β-L-ribo-*hexopyranoside* (8).—Methyl 2,6-dideoxy-4-*O*-methyl-β-L-*ribo*-hexopyranoside (5) (140 mg) was dissolved in dry pyridine (84 ml) and benzoyl chloride (0.56 ml) was added. The mixture was heated under dry nitrogen at 35 °C for 4 h and then at 42 °C for 4 h; it was then evaporated to dryness. The residue was azeotroped with toluene, extracted with chloroform and the chloroform extract washed with saturated aqueous sodium hydrogen carbonate. After being washed with water the chloroform solution was dried and evaporated to dryness. The residue was purified by preparative t.l.c. on silica gel plates using 85% carbon tetrachloride in diethyl ether as the eluant. The methyl 3-*O*-benzoyl-2,6-dideoxy-4-*O*-methyl-β-L-*ribo*-hexopyranoside (8) (75 mg, 34%) was subjected to short-path distillation to give a colourless solid, m.p. 58—60.5 °C (Found: C, 64.95; H, 6.55. $C_{15}H_{20}O_5$ requires C, 64.27; H, 7.19%), $[\alpha]_D^{26}$ −31.4° ($CHCl_3$), $\nu_{max.}$ ($CHCl_3$) 2 930, 1 720, 1 280, 1 115, and 1 020 cm^{-1}.

Methyl 2,6-*Dideoxy*-3,4-*di*-O-*methyl*-α-L-ribo-*hexopyranoside* (9).—Methyl 2,6-dideoxy-α-L-*ribo*-hexopyranoside (6) (161 mg) was dissolved in dry dimethylformamide (10 ml). Sodium hydride (477 mg) was added to the stirred solution and the mixture was stirred under dry nitrogen at 25 °C for 1 h. Methyl iodide (1.79 ml) was added and the mixture was stirred at 25 °C for 16 h. The mixture was filtered through Celite and the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column (15 × 1 cm) using 2% acetone in hexane as the eluant to give methyl 2,6-dideoxy-3,4-di-*O*-methyl-α-L-*ribo*-hexopyranoside (9) (125 mg, 66%) which was subjected to short-path distillation to give a colourless oil (Found: *m/z* 190.1206. $C_9H_{18}O_4$ requires *m/z* 190.1205), $[\alpha]_D^{26}$ −211.0° (CH_3OH), $\nu_{max.}$ ($CHCl_3$) 2 950, 1 100, and 1 020 cm^{-1}.

Methyl 2,6-*Dideoxy*-3,4-*di*-O-*methyl*-β-L-ribo-*hexopyranoside* (10).—Methyl 2,6-dideoxy-β-L-*ribo*-hexopyranoside (7) (200 mg) was dissolved in dry dimethylformamide (10 ml). Sodium hydride (592 mg) was added to the stirred solution and the mixture was stirred under dry nitrogen at 25 °C for 1 h. Methyl iodide (1.8 ml) was added and the mixture was stirred at 25 °C for 16 h. The mixture was filtered through Celite and the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column (15 × 1 cm) using 2% acetone in hexane as the eluant to give methyl 2,6-dideoxy-3,4-di-*O*-methyl-β-L-*ribo*-hexopyranoside (10) (37 mg, 16%) which was subjected to short-path distillation to give a colourless oil (Found: *m/z* 190.1208. $C_9H_{18}O_4$ requires *m/z* 190.1205), $[\alpha]_D^{26}$ −26.6° (CH_3OH), $\nu_{max.}$ ($CHCl_3$) 2 960, 1 100, and 1 020 cm^{-1}.

O-β-D-*Kijanosyl*-(1→17)-26-O-*methylkijanolide* (15).—*O*-β-D-Kijanosyl-(1→17)-kijanolide (14) (953 mg) was dissolved in dry tetrahydrofuran (350 ml) and an excess of diazomethane in diethyl ether was added. The mixture was left at 25 °C for 18 h. The excess diazomethane was removed with a stream of nitrogen and the solution was evaporated to dryness. The residue was chromatographed on a silica gel column (120 × 2 cm) using chloroform as the eluant to give *O*-β-D-kijanosyl-(1→17)-26-*O*-methylkijanolide (15) (334 mg, 34%) as a colourless solid that crystallized as fine needles from chloroform–methanol (Found: * C, 63.5; H, 7.25; N, 4.1. $C_{43}H_{60}N_2O_{12}$ requires C, 64.81; H, 7.59; N, 3.52%), $[\alpha]_D^{26}$ −83.6° (CH_3OH); $\lambda_{max.}$ (CF_3CH_2OH) 199 (ε 40 416) and 254 nm (ε 9 405), $[\theta]_{196}$ −338 013, $[\theta]_{204}$ −177 902, $[\theta]_{212}$ −234 830, $[\theta]_{233}$ +14 232, $[\theta]_{256}$ −10 674, $[\theta]_{285}$ +7 116, $\nu_{max.}$ ($CHCl_3$) 3 675, 3 600, 3 430, 2 950, 1 740, 1 730, 1 660, 1 562, 1 540, 1 350, 1 232, and 1 055 cm^{-1}; δ_H ($CDCl_3$) (600 MHz) 0.64 (3 H, d, *J* 5.7 Hz, 6-CH_3), 1.05 (3 H, d, *J* 6.7 Hz, 8-CH_3), 1.17 (3 H, d, *J* 6.2 Hz, 6^E-CH_3), 1.31 (3 H, s, 18-CH_3), 1.32 (3 H, d, *J* 7.2 Hz, 23-CH_3), 1.37 (3 H, s, 14-CH_3), 1.55 (3 H, s, 4-CH_3), 1.59 (3 H, s, 3^E-CH_3), 1.77 (1 H, d, $J_{24,24'}$ 14.3 Hz, 24-H), 2.36 (1 H, dd, $J_{24,24'}$ 14.3 Hz, $J_{23,24'}$ 7.2 Hz, 24′-H), 2.62 (1 H, dq, $J_{23,23\text{-}CH_3} = J_{23,24'}$ = 7.2 Hz, 23-H), 2.82 (1 H, dd, $J_{2^E_{ax},2^E_{eq}}$ 14.8 Hz, $J_{1^E_{ax},2^E_{eq}}$ 1.9 Hz, 2^E_{eq}-H), 3.40 (1 H, dd, $J_{19,20}$ 10.0 Hz, $J_{20,?}$ *ca.* 1 Hz, 20-H), 3.51 (1 H, dq, $J_{5^E_{ax},6^E\text{-}CH_3}$ 6.2 Hz, $J_{4^E_{eq},5^E_{ax}}$ *ca.* 1 Hz, 5^E_{ax}-H), 3.67 (1 H, dd, $J_{9ax,10ax}$ 9.5 Hz, $J_{8eq,9ax}$ 5.2 Hz, 9_{ax}-H), 3.71 (3 H, s, 4^E-NHCOOCH_3), 4.02 (1 H, ddd, $J_{12,13}$ 5.2 Hz, $J_{11,13} = J_{10,13}$ = <1 Hz, 13-H), 4.13 (3 H, s, 26-OCH_3), 4.20 (1 H, d, $J_{32,32'}$ 13.4 Hz, 32′-H), 4.25 (1 H, d, $J_{32,32'}$ 13.4 Hz, 32-H), 4.40 (1 H, dd, $J_{4^E_{eq},4\text{-NH}}$ 10.0 Hz, $J_{4^E_{eq},5^E_{ax}}$ <1 Hz, 4^E_{eq}-H), 4.46 (1 H, dd, $J_{1^E_{ax},2^E_{ax}}$ 9.5 Hz, $J_{1^E_{ax},2^E_{eq}}$ 1.9 Hz, 1^E_{ax}-H), 5.02 (1 H, ddq, $J_{19,20}$ 10.0 Hz, $J_{18\text{-}CH_3,19} = J_{17,19}$ = <1 Hz, 19-H), 5.12 (1 H, ddq, $J_{15,16}$ 9.1 Hz, $J_{14\text{-}CH_3,15} = J_{15,16'}$ = <1 Hz, 15-H), 5.37 (1 H, d, $J_{4^E_{eq},4\text{-NH}}$ 10.5 Hz, 4^E-N*H*COO-CH_3), 5.48 (1 H, ddd, $J_{11,12}$ 10.0 Hz, $J_{12,13}$ 5.2 Hz, $J_{10,12}$ 1.9 Hz, 12-H), 5.51 (1 H, s, 21-H), and 5.99 (1 H, ddd, $J_{11,12}$ 10.0 Hz, $J_{11,13} = J_{10,11}$ = <1 Hz, 11-H); *m/z* 796 (2.0) ($M^{+\cdot}$), 566 (11.2) (D_2), 548 (12.2) ($D_2 - H_2O$), 534 (7.0) ($D_2 - CH_3OH$), 516 (2.7) ($D_2 - H_2O - CH_3OH$), 498 (1.3), 388 (6.3) (D_{10}), 231 (11.0) (B_1[b]), 184 (100) (B_6[b] and B_{15}[b]), 152 (14.1)

* Chloroform solvate.

(B_{10}^{b}), 140 (33.0) (B_{18}^{b}), 128 (69.8) (B_{12}^{b}), and 96 (18.6) (B_{14}^{b}).

9.32-*Di*-O-*acetyl*-O-β-D-*kijanosyl*-(1→17)-*kijanolide* (16).—*O*-β-D-Kijanosyl-(1→17)-kijanolide (14) (98 mg) was dissolved in dry pyridine (3.3 ml) and acetic anhydride (0.75 ml) was added. The mixture was stirred at 25 °C for 16 h, and then evaporated to dryness and azeotroped with toluene. The residue was chromatographed on a silica gel column (60 × 2 cm) using chloroform as the eluant to give 9,32-di-*O*-acetyl-*O*-β-D-kijanosyl-(1→17)-kijanolide (16) (77 mg, 72%) as an off-white amorphous solid (Found: C, 63.2; H, 7.25; N, 3.35. $C_{46}H_{62}N_2O_{14}$ requires C, 63.73; H, 7.21; N, 3.23%), $[\alpha]_D^{26}$ −39.6° (CH_3OH), $\lambda_{max.}$ (CF_3CH_2OH) 199 (ε 40 966), 242sh (ε 6 997), 262 (ε 8 020), and 276sh nm (ε 7 161); $\nu_{max.}$ ($CHCl_3$) 3 670, 3 420, 2 930, 1 730, 1 540, 1 510, 1 250, 1 230, and 1 055 cm^{-1}; δ_H ($CDCl_3$) (220 MHz) 0.68 (3 H, bd, *J ca.* 5 Hz, 6-CH_3), 1.02 (3 H, d, *J* 7 Hz, 8-CH_3), 1.17 (3 H, d, *J* 6 Hz, 6^E-CH_3), 1.35 (3 H, d, *J* 7 Hz, 23-CH_3), 1.39 (3 H, s, 14-CH_3), 1.43 (3 H, s, 18-CH_3), 1.61 (3 H, s, 4-CH_3), 1.67 (3 H, s, 6^E-CH_3), 2.15 (6 H, s, 9- and 32-OAc), 3.77, (3 H, s, 4^E-NHCOOCH_3), 4.43 (1 H, dd, $J_{4^E_{eq},4\text{-NH}}$ 10 Hz, $J_{4^E_{eq},5^E_{ax}}$ 1 Hz, 4^E_{eq}-H), 4.50 (1 H, dd, $J_{1^E_{ax},2^E_{ax}}$ 10 Hz, $J_{1^E_{ax},2^E_{eq}}$ 1.5 Hz, 1^E_{ax}-H), 5.66 (1 H, s, 21-H), and 5.75 (1 H, ddd, $J_{11,12}$ 10 Hz, $J_{10,11}$ and $J_{11,13}$ N/A, 11-H); *m/z* 867 (0.2) ($M^{+\cdot}$), 806 (0.8) (M − CH_3COOH), 636 (1.1) (D_3), 618 (4.9) (D_3 − H_2O), 576 (29.1) (D_3 − CH_3COOH), 558 (24.1) (D_3 − CH_3COOH − H_2O), 516 (4.2) (D_3 − CH_3COOH − CH_3COOH), 498 (15.9), 416 (3.4) (D_{11}), 356 (6.5) (D_{11} − CH_3COOH), 231 (23.4) (B_1^b), 184 (100) (B_6^b and B_{15}^b), 152 (12.7) (B_{10}^b), 140 (32.4) (B_{18}^b), 128 (60.2) (B_{12}^b), and 96 (9.5) (B_{14}^b).

9,32-*Di*-O-*acetyl*-O-β-D-*kijanosyl*-(1→17)-26-O-*methylkijanolide* (17).—*O*-β-D-Kijanosyl-(1→17)-26-*O*-methylkijanolide (15) (159 mg) was dissolved in dry pyridine (7 ml) and acetic anhydride (1.54 ml) was added. The mixture was stirred at 25 °C for 18 h, and then evaporated to dryness and azeotroped with toluene. The residue was chromatographed on a silica gel column (40 × 1 cm) and eluted with chloroform to give 9.32-di-*O*-acetyl-*O*-β-D-kijanosyl-(1→17)-26-*O*-methylkijanimicin (17) (64 mg, 36%) as a colourless amorphous solid (Found: C, 62.65; H, 7.1; N, 3.0; O, 25.65. $C_{47}H_{64}N_2O_{14}$ requires C, 64.07; H, 7.32; N, 3.18; O, 25.42%), $[\alpha]_D^{26}$ −65.1° (CH_3OH), $\lambda_{max.}$ (CF_3CH_2OH) 200 (ε 41 187) and 254 nm (ε 9 673); $\nu_{max.}$ ($CHCl_3$) 3 430, 2 950, 1 735, 1 665, 1 572, 1 545, 1 260, 1 235, and 1 060 cm^{-1}; δ_H ($CDCl_3$) (220 MHz) 0.65 (3 H, d, *J ca.* 5 Hz, 6-CH_3), 1.01 (3 H, d, *J* 7 Hz, 8-CH_3), 1.17 (3 H, d, *J* 6 Hz, 6^E-CH_3), 1.32 (3 H, d, *J* 7 Hz, 23-CH_3), 1.33 (3 H, s, 18-CH_3), 1.40 (3 H, s, 4-CH_3), 1.57 (3 H, s, 3^E-CH_3), 1.60 (3 H, s, 4-CH_3), 2.13 (3 H, s, 9 or 32-OAc), 2.15 (3 H, s, 9 or 32-OAc), 3.75 (3 H, s, 4^E-NHCOOCH_3), 4.17 (3 H, s, 26-OCH_3), 4.41 (1 H, dd, $J_{4^E_{eq},NH}$ 10 Hz, $J_{4^E_{eq},5^E_{ax}}$ 1 Hz, 4^E_{eq}-H), 4.49 (1 H, dd, $J_{1^E_{ax},2^E_{ax}}$ 10 Hz, $J_{1^E_{ax},2^E_{eq}}$ 1.5 Hz, 1^E_{ax}-H), 4.60 (1 H, d, $J_{32,32'}$ 14 Hz, 32′-H), 4.80 (1 H, d, $J_{32,32'}$ 14 Hz, 32-H), 5.66 (1 H, s, 21-H), and 5.72 (1 H, ddd, $J_{11,12}$ 10 Hz, $J_{10,11}$ and $J_{11,13}$ N/A, 11-H); *m/z* 880 (2.0) ($M^{+\cdot}$), 848 (0.2) (M − CH_3OH), 838 (0.1) (M − CH_2=C=O), 834 (0.3), 820 (2.2) (M − CH_3COOH), 802 (2.6), 788 (<0.1) (M − CH_3COOH − CH_3OH), 650 (20.2) (D_4), 632 (6.1), 628 (<0.1) (D_4 − CH_3OH), 590 (30.1) (D_4 − CH_3COOH), 572 (17.6), 558 (2.8) (D_4 − CH_3COOH − CH_3OH), 530 (3.6) (D_4 − CH_3COOH − CH_3COOH), 430 (14.8) (D_{12}), 370 (3.4) (D_{12} − CH_3COOH), 231 (13.8) (B_1^b), 184 (85.6) (B_6^b and B_{15}^b), 152 (13.2) (B_{10}^b), 140 (24.6) (B_{18}^b), 128 (53.0) (B_{12}^b), and 96 (12.6) (B_{14}^b).

32-O-(4-*Iodobenzoyl*)-O-β-D-*kijanosyl*-(1→17)-*kijanolide* (18).—*O*-β-D-Kijanosyl-(1→17)-kijanolide (14) (550 mg) was dissolved in dry pyridine (50 ml) and 4-iodobenzoyl chloride (3.52 g) was added. The mixture was heated at 80 °C for 6 days. The solution was evaporated to dryness and the residue was azeotroped with toluene. The residue was taken up in dichloromethane and washed with water. The dichloromethane solution was evaporated to dryness and the residue was chromatographed on a silica gel column (160 × 2 cm) using 1.5% methanol in chloroform as the eluant to give 32-*O*-(4-iodobenzoyl)-*O*-β-D-kijanosyl-(1→17)-kijanolide (18) (245 mg, 34%) as a colourless amorphous solid which crystallized as clusters of colourless needles from aqueous acetone, or methanol, m.p. *ca.* 183 °C (Found: C, 56.9; H, 5.95; I, 16.45; N, 2.85. $C_{49}H_{61}IN_2O_{13}$ requires C, 58.09; H, 6.07; I, 12.53; N, 2.76%), $[\alpha]_D^{26}$ −16.3° (CH_3OH), $\lambda_{max.}$ (CF_3CH_2OH) 257 nm (ε 25 370); $\nu_{max.}$ (KBr) 3 520, 3 430, 3 340, 2 940, 1 765, 1 730, 1 595, 1 550, 1 512, 1 275, and 1 060 cm^{-1}; δ_H ($CDCl_3$) (220 MHz) 0.67 (3 H, d, *J ca.* 4 Hz, 6-CH_3), 1.06 (3 H, d, *J* 7 Hz, 8-CH_3), 1.17 (3 H, d, *J* 7 Hz, 6^E-CH_3), 1.36 (3 H, s, 14-CH_3), 1.38 (3 H, d, *J ca.* 7 Hz, 23-CH_3), 1.41 (3 H, s, 18-CH_3), 1.59 (3 H, s, 3^E-CH_3), 1.64 (3 H, s, 4-CH_3), 3.71 (3 H, s, 4^E-NHCOOCH_3), 4.77 (1 H, d, $J_{32,32'}$ 13 Hz, 32′-H), 5.09 (1 H, d, $J_{32,32'}$ 13 Hz, 32-H), 5.68 (1 H, s, 21-H), 6.03 (1 H, ddd, $J_{11,12}$ 10 Hz, $J_{10,11}$ and $J_{11,13}$ N/A, 11-H) and 7.81 (4 H, s, 32-$OCOC_6H_4I$); *m/z* 782 (3.2) (D_5), 764 (10.8) (D_5 − H_2O), 746 (4.5), 604 (0.7) (D_{13}), 534 (2.7) (D_5 − IC_6H_4COOH), 516 (12.6) (D_5 − IC_6H_4COOH − H_2O), 498 (7.4), 356 (4.9) (D_{13} − IC_6H_4COOH), 248 (34.1) (D_{21}), 231 (47.9) (B_1^b and D_{22}), 184 (100) (B_6^b and B_{15}^b), 152 (10.7) (B_{10}^b), 140 (25.2) (B_{18}^b), 128 (83.7) (B_{12}^b), and 96 (38.9) (B_{14}^b).

32-O-(4-*Iodobenzoyl*)-O-β-D-*kijanosyl*-(1→17)-26-O-*methylkijanolide* (19).—32-*O*-(4-Iodobenzoyl)-*O*-β-D-kijanosyl-(1→17)-kijanolide (18) (500 mg) was dissolved in a solution of diazomethane in diethyl ether (125 ml) and the mixture was allowed to remain at 25 °C for 4 h. Excess of diazomethane was removed with a stream of nitrogen and the solvent was evaporated to dryness. The residue was chromatographed on a silica gel column (110 × 2 cm) using 15% ethyl acetate in dichloromethane as the eluant to give 32-*O*-(4-iodobenzoyl)-*O*-β-D-kijanosyl-(1→17)-26-*O*-methylkijanolide (19) (173 mg). Overlapping fractions were further purified by preparative t.l.c. on silica gel plates using 50% ethyl acetate in dichloromethane as the eluant to give an additional 108 mg of (19) (281 mg, 55%) as a colourless amorphous solid (Found: C, 56.75; H, 6.0; I, 11.95; N, 2.45. $C_{50}H_{63}IN_2O_{13}$ requires C, 58.49; H, 6.19, I, 12.36; N, 2.73%), $[\alpha]_D^{26}$ −47.9° (CH_3OH), $\lambda_{max.}$ (CF_3CH_2OH) 256 nm (ε 27 173); $\nu_{max.}$ (KBr) 3 510, 3 440, 2 960, 1 760, 1 730, 1 670, 1 580, 1 550, 1 355, 1 275, 1 062, and 1 018 cm^{-1}; δ_H ($CDCl_3$) (220 MHz) 0.63 (3 H, d, *J ca.* 4 Hz, 6-CH_3), 1.04 (3 H, d, *J* 7 Hz, 8-CH_3), 1.16 (3 H, d, 6 Hz, 6^E-CH_3), 1.30 (3 H, s, 18-CH_3), 1.37 (3 H, d, *J* 7 Hz, 23-CH_3), 1.37 (3 H, s, 14-CH_3), 1.54 (3 H, s, 4-CH_3), 1.57 (3 H, s, 3^E-CH_3), 3.72 (3 H, s, 4^E-NHCOOCH_3), 4.13 (3 H, s, 26-OCH_3), 4.75 (1 H, d, $J_{32,32'}$ 13 Hz, 32′-H), 5.48 (1 H, ddd, $J_{11,12}$ 10 Hz, $J_{12,13}$ 5 Hz, $J_{10,12}$ *ca.* 1.5 Hz, 12-H), 5.67 (1 H, s, 21-H), 5.98 (1 H, ddd, $J_{11,12}$ 10 Hz, $J_{11,13} = J_{10,11}$ = *ca.* 2 Hz, 11-H), and 7.79 (4 H, s, 32-$OCOC_6H_4I$); *m/z* 796 (3.7) (D_6), 778 (4.3) (D_6 − H_2O), 760 (2.4), 746 (1.1) (D_6 − H_2O − CH_3OH), 618 (1.3) (D_{14}), 548 (1.5) (D_6 − IC_6H_4COOH), 530 (2.7) (D_6 − IC_6H_4COOH − H_2O), 516 (3.9) (D_6 − IC_6H_4COOH − CH_3OH), 498 (3.0), 370 (2.1) (D_{14} − IC_6H_4COOH), 248 (49.7) (D_{21}), 231 (100) (B_1^b and D_{22}), 184 (72.9) (B_6^b and B_{15}^b), 152 (14.0) (B_{10}^b), 140 (28.3) (B_{18}^b), 128 (99.1) (B_{12}^b), and 96 (77.1) (B_{14}^b).

9,32-*Di*-O-(4-*iodobenzoyl*)-O-β-D-*kijanosyl*-(1→17)-*kijanolide* (20).—*O*-β-D-Kijanosyl-(1→17)-kijanolide (14) (1.15 g) was dissolved in dry pyridine (350 ml). 4-Iodobenzoyl

chloride (7.39 g) was added and the mixture was heated at 110 °C for 3 days. The solution was evaporated to dryness and the residue was azeotroped with toluene. The residue was dissolved in dichloromethane. The latter was washed with water and evaporated to dryness. The residue was chromatographed on a silica gel column (100 × 2 cm) using chloroform as the eluant. The product was then rechromatographed on a silica gel column (100 × 2 cm) using 0.75% ethanol in dichloromethane as the eluant to give 9,32-di-*O*-(4-iodobenzoyl)-*O*-β-D-kijanosyl-(1→17)-kijanolide (20) (424 mg, 23%) as a colourless amorphous solid which crystallized as fine needles from tetrahydrofuran–hexane (Found: C, 54.2; H, 5.3; I, 20.7; N, 2.25. $C_{56}H_{64}I_2N_2O_{14}$ requires C, 54.15; H, 5.19; I, 20.43; N, 2.26%), $[\alpha]_D^{26}$ +38.8° ($CHCl_3$), $\lambda_{max.}$ (CF_3CH_2OH) 257 nm (ε 43 764); $\nu_{max.}$ (KBr) 3 425, 2 935, 1 760, 1 725, 1 590, 1 545, 1 270, 1 100, 1 060, and 1 013 cm^{-1}; δ_H ($CDCl_3$) (220 MHz) 0.70 (3 H, d, *J ca.* 4 Hz, 6-CH_3), 1.08 (3 H, d, *J* 7 Hz, 8-CH_3), 1.15 (3 H, d, *J* 6 Hz, 6^E-CH_3), 1.38 (3 H, d, *J* 7 Hz, 23-CH_3), 1.38 (3 H, s, 14-CH_3), 1.40 (3 H, s, 18-CH_3), 1.57 (3 H, s, 3^E-CH_3), 1.67 (3 H, s, 4-CH_3), 3.70 (3 H, s, 4^E-NHCOOCH_3), 4.42 (1 H, dd, $J_{1^E_{ax},2^E_{ax}}$ 10 Hz, $J_{1^E_{ax},2^E_{eq}}$ 2 Hz, 1^E_{ax}-H), 4.71 (1 H, d, $J_{32,32'}$ 13 Hz, 32′-H), 5.34 (1 H, ddd, $J_{11,12}$ 10 Hz, $J_{12,13}$ 5 Hz, $J_{10,12}$ *ca.* 1.5 Hz, 12-H), 5.67 (1 H, s, 21-H), 5.69 (1 H, ddd, $J_{11,12}$ 10 Hz, $J_{11,13} = J_{10,11}$ = *ca.* 2 Hz 11-H), and 7.82 (8 H, s, 9- and 32-$OCOC_6H_4I$); *m/z* 764 (0.2) ($D_7 - IC_6H_4COOH$), 746 (1.5), 516 (0.8) ($D_7 - IC_6H_4COOH - IC_6H_4COOH$), 498 (3.4), 480 (1.0), 248 (100) (D_{21}), 231 (65.8) (B_1[b] and D_{22}), 184 (16.6) (B_6[b] and B_{15}[b]), 152 (3.5) (B_{10}[b]), 140 (6.2) (B_{18}[b]), 128 (23.0) (B_{12}[b]), and 96 (12.0) (B_{14}[b]).

9,32-*Di*-O-(4-*iodobenzoyl*)-O-β-D-*kijanosyl*-(1→17)-26-O-*methylkijanolide* (21).—9,32-Di-*O*-(4-iodobenzoyl)-*O*-β-D-kijanosyl-(1→17)-kijanolide (20) (908 mg) was dissolved in a solution of diazomethane in diethyl ether (180 ml) and the mixture was allowed to remain at 25 °C for 46 h. Excess diazomethane was removed with a stream of nitrogen and the solution was evaporated to dryness. The residue was chromatographed on a silica gel column (60 × 5 cm) using 0.5% methanol in chloroform as the eluant. The product was then rechromatographed on a silica gel column (60 × 5 cm) using chloroform as the eluant to give 9,32-di-*O*-(4-iodobenzoyl)-*O*-β-D-kijanosyl-(1→17)-26-*O*-methylkijanolide (21) (427 mg). Additional product was obtained from the overlap fractions by preparative t.l.c. on silica gel plates using 0.8% methanol in chloroform as the eluant to give (21) (137 mg) (564 mg, 61%) as a colourless amorphous solid (Found: C, 54.15; H, 5.4; I, 18.6; N, 2.1. $C_{57}H_{66}I_2N_2O_{14}$ requires C, 54.50; H, 5.30; I, 20.20; N, 2.23%), $[\alpha]_D^{26}$ 0° ($CHCl_3$), $\lambda_{max.}$ (CF_3CH_2OH) 257 nm (ε 49 841); $\nu_{max.}$ (KBr) 3 425, 2 960, 2 940, 1 755, 1 725, 1 667, 1 592, 1 578, 1 548, 1 352, 1 270, 1 108, 1 060, and 1 018 cm^{-1}; δ_H ($CDCl_3$) (220 MHz) 0.67 (3 H, d, *J ca.* 4 Hz, 6-CH_3), 1.07 (3 H, d, *J* 7 Hz, 8-CH_3), 1.14 (3 H, d, *J* 6 Hz, 6^E-CH_3), 1.31 (3 H, s, 18-CH_3), 1.37 (3 H, d, *J* 7 Hz, 23-CH_3), 1.40 (3 H, s, 14-CH_3), 1.58 (3 H, s, 4-CH_3), 1.60 (3 H, s, 3^E-CH_3), 3.70 (3 H, s, 4^E-NHCOOCH_3), 4.13 (3 H, s, 26-OCH_3), 4.36 (1 H, dd, $J_{4^E_{eq},4\text{-NH}}$ 10 Hz, $J_{4^E_{eq},5^E_{ax}}$ 1 Hz, 4^E_{eq}-H), 4.42 (1 H, dd, $J_{1^E_{ax},2^E_{eq}}$ 9 Hz, $J_{1^E_{ax},2^E_{ax}}$ *ca.* 2 Hz, 1^E_{ax}-H), 4.75 (1 H, d, $J_{32,32'}$, 13 Hz, $H_{32'}$), 5.43 (1 H, ddd, $J_{11,12}$ 10 Hz, $J_{12,13}$ *ca.* 5 Hz, $J_{10,12}$ *ca.* 1.5 Hz, 12-H), 5.67 (1 H, s, 21-H), and 7.80 (8 H, s, 9- and 32-$OCOC_6H_4I$); *m/z* 778 (1.0) ($D_8 - IC_6H_4COOH$), 760 (2.0), 746 (0.5) ($D_8 - IC_6H_4COOH - CH_3OH$), 728 (0.1), 530 (0.4) ($D_8 - IC_6H_4COOH - IC_6H_4COOH$), 512 (1.5), 498 (1.1), 480 (0.5), 248 (98.2) (D_{21}), 231 (100) (B_1[b] and D_{22}), 184 (26.7) (B_6[b] and B_{15}[b]), 152 (4.3) (B_{10}[b]), 140 (6.3) (B_{18}[b]), 128 (B_{12}[b]), and 96 (13.6) (B_{14}[b]).

Methanolysis of O-β-D-*Kijanosyl*-(1→17)-*kijanolide* (14).—(i) *Isolation of the sugars.* *O*-β-D-Kijanosyl-(1→17)-kijanolide (14) (433 mg) was dissolved in 5M-hydrogen chloride in methanol (250 ml) and the mixture was heated under reflux at 65 °C for 3 h. The solution was cooled, concentrated ammonium hydroxide (190 ml) was added, and it was then evaporated to dryness. The residue was taken up in chloroform and washed with water. The chloroform layer was evaporated to dryness and the residue was chromatographed on a silica gel column (160 × 2.5 cm) using 5% acetone in hexane as the eluant to give methyl β-D-kijanoside (24) (80 mg). The latter (24) was subjected to preparative t.l.c. on silica gel plates using 5% methanol in chloroform as the eluant to give methyl β-D-kijanoside (24) (17 mg) which was subjected to short-path distillation and then crystallized from hexane to give colourless crystals, m.p. 180.5—181.5 °C (Found: C, 46.0; H, 6.9; N, 10.65; O, 36.5. $C_{10}H_{18}N_2O_6$ requires C, 45.80; H, 6.92; N, 10.68; O, 36.60%), $[\alpha]_D^{26}$ +34.1° (CH_3OH), $\lambda_{max.}$ (CF_3CH_2OH) 199 nm (ε 4 909); $[\theta]_{234}$ −962 and $[\theta]_{282}$ +2 308 (CF_3CH_2OH); $\nu_{max.}$ ($CHCl_3$) 3 440, 3 000, 2 955, 2 900, 2 860, 1 730, 1 550, 1 515, 1 315, 1 235, and 1 065 cm^{-1}. Further elution of the column afforded the α-anomer (25) (60 mg) which was subjected to preparative t.l.c. on silica gel plates using 5% methanol in chloroform as the eluant to give methyl α-D-kijanoside (23) (31 mg) as a colourless oil which was subjected to short-path distillation to give a viscous gum which solidified with time (Found: C, 47.8; H, 7.3; N, 10.15; O, 34.75. $C_{10}H_{18}N_2O_6$ requires C, 45.80; H, 6.92; N, 10.68; O, 36.60%), $[\alpha]_D^{26}$ +130.0° (CH_3OH), $\lambda_{max.}$ (CF_3CH_2OH) 199 nm (ε 4 968); $[\theta]_{234}$ −326 and $[\theta]_{280}$ +340 (CF_3CH_2OH); $\nu_{max.}$ ($CHCl_3$) 3 430, 2 980, 2 950, 2 940, 2 900, 2840, 1 735, 1 550, 1 508, 1 230, 1 123, and 1 055 cm^{-1}. The column was then eluted with 2% methanol in chloroform to give a complex mixture (254 mg) of partially separated aglycone fragments.

(ii) *Isolation of the kijanolide fragments.* O-β-D-Kijanosyl-(1→17)-kijanolide (14) (1.5 g) was dissolved in 5M-hydrogen chloride in methanol (850 ml) and the mixture was allowed to remain at 25 °C for 16 h. Concentrated ammonium hydroxide (575 ml) was added and the solution was evaporated to dryness. The residue was taken up in chloroform and filtered. The chloroform filtrate was evaporated to dryness. The solid was taken up in acetone (90 ml) and the solution was added dropwise to hexane (910 ml) with stirring and cooling to −33 °C. The hexane layer was decanted from an orange coloured oil which separated out. The orange oil (421 mg) was purified by preparative t.l.c. (twice) on silica gel plates using 40% methanol in chloroform as the eluant to give kijanolide (46) (23 mg) as an off-white amorphous solid (see Table 1 for ^{13}C n.m.r. data). The hexane–acetone layer from the precipitation was evaporated to dryness. The residue (962 mg) was chromatographed on a Waters Prep 500 h.p.l.c. instrument using one silica gel (325 g) cartridge and 9% acetone in hexane as the eluant to give methyl β-D-kijanoside (24) and methyl α-D-kijanoside (23) which had to be further purified. Chromatography on a silica-gel column (160 × 3 cm) using 5% acetone in hexane as the eluant, followed by preparative t.l.c. on silica gel plates using 80% ethyl acetate in dichloromethane as the eluant afforded the pure β-anomer (24) (20 mg) and also the α-anomer (23) (55 mg). The h.p.l.c. column was then stripped with acetone to give the kijanolide fractions (467 mg). The latter were chromatographed on a silica gel column (120 × 6 cm) using 20% ethyl acetate in dichloromethane as the eluant to give 32-chloro-32-deoxykijanolide (47) (107 mg) as an off-white amorphous solid (Found: C, 68.15; H, 7.5. $C_{33}H_{43}ClO_6$ requires C, 69.40; H, 7.59%), $\nu_{max.}$ (KBr) 3 450, 2 970, 2 930, 2 880, 1 760, 1 620, 1 060, and 985 cm^{-1}; δ_H (CD_3OD) (200 MHz) 0.66 (3 H, d, *J ca.* 4 Hz, 6-CH_3), 1.03 (3 H, d, *J* 7 Hz, 8-CH_3), 1.30 (3 H, d, *J* 7 Hz, 23-CH_3), 1.42 (3 H, s, 14-CH_3), 1.42 (3 H, s, 18-CH_3),

1.59 (3 H, s, 4-CH_3), 1.87 (1 H, d, $J_{24,24'}$ *ca.* 14.5 Hz, 24-H), 2.42 (1 H, dd, $J_{24,24'}$ 14.5 Hz, $J_{23,24'}$ *ca.* 7 Hz, 24'-H), 2.84 (1 H, dq, $J_{23,23\text{-}CH_3} = J_{23,24'} = 7$ Hz, 23-H), 3.56 (1 H, ddd, $J_{12,13}$ 4.7 Hz, $J_{11,13}$ and $J_{10,13}$ N/A, 13-H), 4.13 (1 H, d, $J_{32,32'}$ 11.7 Hz, 32'-H), 4.27 (1 H, d, $J_{32,32'}$ 11.7 Hz, 32-H), 5.39 (1 H, ddd, $J_{11,12}$ 10.5 Hz, $J_{12,13}$ 4.7 Hz, $J_{10,12}$ *ca.* 2 Hz, 12-H), 5.63 (1 H, s, 21-H), and 6.10 (1 H, ddd, $J_{11,12}$ 10.5 Hz, $J_{11,13}$ and $J_{10,11}$ N/A, 11-H); *m/z* 570 (5.7) ($M^{+\cdot}$), 552 (14.0) ($M - H_2O$), 534 (6.0) ($M - H_2O - H_2O$), 516 (5.5), 498 (2.9), and 461 (5.8).

Methyl 4-O-*p-Tolylsulphonyl*-α-L-*mycaroside* (32) *and Methyl* 4-O-p-*Tolylsulphonyl*-β-L-*mycaroside* (33).—A mixture of methyl α-L-mycaroside (30) and methyl β-L-mycaroside (31) (3.5 g) (*ca.* 1 : 1) was dissolved in dry pyridine (100 ml) containing toluene-*p*-sulphonyl chloride (4.55 g) and the mixture was stirred at 25 °C for 16 h. The solution was evaporated to dryness and then azeotroped with toluene. The residue was taken up in chloroform and washed with water. The chloroform solution was evaporated to dryness and the residue was chromatographed on a silica gel column (160 × 3 cm) using chloroform as the eluant to give methyl 4-*O*-*p*-tolylsulphonyl-α-L-mycaroside (32) and methyl 4-*O*-*p*-tolylsulphonyl-β-L-mycaroside (33). Each anomer was then subjected separately to preparative t.l.c. on silica gel plates using 25% acetone in hexane as the eluant to give pure samples of each anomer. The α-anomer (32) (1.28 g, 39%) was obtained as a colourless oil (Found: C, 52.15; H, 6.65. $C_{15}H_{22}O_6S$ requires C, 54.54; H, 6.71%), $[\alpha]_D^{26}$ −107.0° ($CHCl_3$), $\lambda_{max.}$ (CF_3CH_2OH) 225 nm (ε 11 460); $\nu_{max.}$ (film) 3 510, 2 975, 2 930, 1 365, 1 170, 1 120, and 980 cm^{-1}. The β-anomer (33) (1.6 g, 49%) was obtained as a colourless oil (Found: C, 53.05; H, 6.6. $C_{15}H_{22}O_6S$ requires C, 54.54; H, 6.71%), $[\alpha]_D^{26}$ +7.8° ($CHCl_3$), $\lambda_{max.}$ (CF_3CH_2OH) 225 nm (ε 11 757); $\nu_{max.}$ (film) 3 500, 2 975, 2 940, 1 460, 1 170, 1 085, and 970 cm^{-1}.

Methyl 4-*Azido*-2,4,6-*trideoxy*-3-C-*methyl*-α-L-xylo-*hexopyranoside* (34).—Methyl 4-*O*-*p*-tolyl-α-L-mycaroside (32) (1.02 g) and sodium azide (2.8 g) were dissolved in hexamethylphosphoramide (10 ml) and the mixture was heated at 115 °C under reflux for 66 h. The solution was diluted with water and extracted with diethyl ether. The ethereal layer was evaporated to dryness and the residue was chromatographed on a silica gel column (140 × 1 cm) using 2% acetone in hexane as the eluant to give methyl 4-azido-2,4,6-trideoxy-3-*C*-methyl-α-L-*xylo*-hexopyranoside (34) (154 mg, 25%) as a colourless oil, $[\alpha]_D^{26}$ −175.0° (CH_3OH), $\nu_{max.}$ ($CHCl_3$) 3 640, 3 510, 2 980, 2 940, 2 120, and 1 025 cm^{-1}.

Methyl 4-*Amino*-2,4,6-*trideoxy*-3-C-*methyl*-α-L-xylo-*hexopyranoside* (35).—Methyl 4-azido-2,4,6-trideoxy-3-*C*-methyl-α-L-*xylo*-hexopyranoside (34) (110 mg) was dissolved in methanol (70 ml). 10% Palladium on carbon (200 mg) was added and the mixture was hydrogenated at 4 atm at 25 °C for 22 h. The catalyst was filtered off and washed. The filtrate was evaporated to dryness and the residue was chromatographed on a silica gel column (100 × 1 cm) using chloroform as the eluant to give methyl 4-amino-2,4,6-trideoxy-3-*C*-methyl-α-L-*xylo*-hexopyranoside (35) (30 mg, 31%) as a colourless oil [Found: *m/z* 157.1121 ($M^+ - H_2O$). $C_8H_{15}NO_2$ requires *m/z* 157.1103], $[\alpha]_D^{26}$ −140.9° (CH_3OH), $\nu_{max.}$ ($CHCl_3$) 3 690, 3 510, 2 980, 2 940, 1 120, and 1 050 cm^{-1}.

Methyl 4-*Azido*-2,4,6-*trideoxy*-3-C-*methyl*-β-L-xylo-*hexopyranoside* (36).—Methyl 4-*O*-*p*-tolylsulphonyl-β-L-mycaroside (33) (950 mg) and sodium azide (2.66 g) were dissolved in hexamethylphosphoramide (10 ml) and the mixture was heated at 115 °C under reflux for 66 h. The solution was diluted with water and extracted with diethyl ether. The ethereal layer was evaporated to dryness and the residue was chromatographed on a silica gel column (120 × 1 cm) using chloroform as the eluant to give methyl 4-azido-2,4,6-trideoxy-3-*C*-methyl-β-L-*xylo*-hexopyranoside (36) (203 mg, 35%) as a colourless oil (Found: C, 47.5; H, 7.3; N, 20.55. $C_8H_{15}N_3O_3$ requires C, 47.76; H, 7.51; N, 20.88%), $[\alpha]_D^{26}$ −23.9° (CH_3OH), $\nu_{max.}$ ($CHCl_3$) 3 610, 2 990, 2 940, 2 120, and 1 070 cm^{-1}.

Methyl 4-*Amino*-2,4,6-*trideoxy*-3-C-*methyl*-β-L-xylo-*hexopyranoside* (37).—Methyl 4-azido-2,4,6-trideoxy-3-*C*-methyl-β-L-*xylo*-hexopyranoside (36) (203 mg) was dissolved in methanol (50 ml). 10% Palladium-carbon was added and the mixture was hydrogenated at 4 atm at 25 °C for 16 h. The catalyst was filtered off and washed. The filtrate was evaporated to dryness and the residue was chromatographed on a silica gel column (120 × 1 cm) using 0.5% methanol in chloroform as the eluant to give methyl 4-amino-2,4,6-trideoxy-3-*C*-methyl-β-L-*xylo*-hexopyranoside (37) (142 mg, 80%) as a colourless amorphous solid, m.p. 107—109 °C (Found: C, 53.65; H, 9.5; N, 7.85. $C_8H_{17}NO_3$ requires C, 54.84; H, 9.78; N, 7.99%) [Found: *m/z* 174.1132 ($M^+ - H$). $C_8H_{16}NO_3$ requires *m/z* 174.1130], $[\alpha]_D^{26}$ +38.6° (CH_3OH), $\nu_{max.}$ ($CHCl_3$) 3 610, 2 990, 2 940, and 1 072 cm^{-1}.

Methyl 2,4,6-*Trideoxy*-4-(*methoxycarbonylamino*)-3-C-*methyl*-β-L-xylo-*hexopyranoside* (38).—Methyl 4-amino-2,4,6-trideoxy-3-*C*-methyl-β-L-*xylo*-hexopyranoside (37) (104 mg) was dissolved in a mixture of tetrahydrofuran (15 ml) and water (7.5 ml). Methyl chloroformate (114 mg) and sodium carbonate (127 mg) were added and the mixture was stirred at 25 °C for 2 h. The mixture was evaporated to dryness and the residue was chromatographed on a silica gel column (110 × 1 cm) using chloroform as the eluant to give methyl 2,4,6-trideoxy-4-(methoxycarbonylamino)-3-*C*-methyl-β-L-*xylo*-hexopyranoside (38) (93 mg, 67%) as a colourless amorphous solid, m.p. 132—133 °C [Found: *m/z* 233.1286 ($M^{+\cdot}$). $C_{10}H_{19}NO_5$ requires *m/z* 233.1263], $[\alpha]_D^{26}$ +2.4° (CH_3OH), $\nu_{max.}$ ($CHCl_3$) 3 610, 3 440, 2 980, 1 725, 1 513, 1 315, 1 228, and 1 060 cm^{-1}.

Methyl 3-*Amino*-2,3,4,6-*tetradeoxy*-4-(*methoxycarbonylamino*)-3-C-*methyl*-α-D-xylo-*hexopyranoside* (25).—Methyl α-D-kijanoside (23) (32 mg) was dissolved in ethanol (500 ml) and Raney nickel (No. 28) was added. The mixture was hydrogenated at 4 atm at 25 °C for 20 h. The catalyst was filtered off and washed and the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column (20 × 2 cm) using 1.5% methanol in chloroform as the eluant to give methyl 3-amino-2,3,4,6-tetradeoxy-4-(methoxycarbonylamino)-3-*C*-methyl-α-D-*xylo*-hexopyranoside (25) (28 mg, 99%) as a colourless oil [Found: *m/z* 232.1395 ($M^{+\cdot}$). $C_{10}H_{20}N_2O_4$ requires *m/z* 232.1426], $[\alpha]_D^{26}$ +121.4° (CH_3OH), $\nu_{max.}$ ($CHCl_3$) 3 440, 2 930, 1 715, 1 500, 1 225, and 1 048 cm^{-1}.

Methyl 3-*Acetamido*-2,3,4,6-*tetradeoxy*-4-(*methoxycarbonylamino*)-3-C-*methyl*-α-D-xylo-*hexopyranoside* (26).—Methyl 3-amino-2,3,4,6-tetradeoxy-4-(methoxycarbonylamino)-3-*C*-methyl-α-D-*xylo*-hexopyranoside (25) (30 mg) was dissolved in methanol (8 ml) and acetic anhydride (1 ml) was added. The mixture was stirred at 25 °C for 15 min and was then evaporated to dryness and the residue azeotroped with toluene. The residue was chromatographed on a silica gel column (20 × 2 cm) using 1.5% methanol in chloroform as the eluant to give methyl 3-acetamido-2,3,4,6-tetradeoxy-4-(methoxycarbonylamino)-3-*C*-methyl-α-D-*xylo*-hexopyranoside (26) (23 mg, 65%) as colourless needles from chloroform, m.p. 163.5—164.5 °C [Found: *m/z* 274.1519 ($M^{+\cdot}$). $C_{12}H_{22}N_2O_5$ requires

m/z 274.1531], $[\alpha]_D^{26}$ +160.0° (CH_3OH), $\nu_{max.}$ ($CHCl_3$) 3 470, 2 970, 1 740, 1 683, 1 520, 1 238, 1 135, and 1 060 cm^{-1}.

Methyl 3-Amino-2,3,4,6-tetradeoxy-4-(methoxycarbonylamino)-3-C-methyl-β-D-xylo-hexopyranoside (27).—Methyl β-D-kijanoside (24) (41 mg) was dissolved in ethanol (50 ml) and Raney nickel (No. 28) was added. The mixture was hydrogenated at 4 atm at 25 °C for 16 h. The catalyst was filtered off and washed and the filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column (20 × 2 cm) using 1.5% methanol in chloroform as the eluant to give methyl 3-amino-2,3,4,6-tetradeoxy-4-(methoxycarbonylamino)-3-*C*-methyl-β-D-*xylo*-hexopyranoside (27) (25.2 mg, 69%) as a colourless oil [Found: *m/z* 232.1388 ($M^{+\cdot}$). $C_{10}H_{20}N_2O_4$ requires *m/z* 232.1426], $[\alpha]_D^{26}$ −4.9° (CH_3OH), $\nu_{max.}$ ($CHCl_3$) 3 440, 2 960, 2 930, 1 722, 1 508, 1 317, 1 230, and 1 062 cm^{-1}.

Methyl 3-Acetamido-2,3,4,6-tetradeoxy-4-(methoxycarbonylamino)-3-C-methyl-β-D-xylo-hexopyranoside (28).—Methyl 3-amino-2,3,4,6-tetradeoxy-4-(methoxycarbonylamino)-3-*C*-methyl-β-D-*xylo*-hexopyranoside (27) (24 mg) was dissolved in methanol (8 ml) and acetic anhydride (1 ml) was added. The mixture was stirred at 25 °C for 15 min and was then evaporated to dryness and azeotroped with toluene. The residue was chromatographed on a silica gel column (20 × 2 cm) using 1.5% methanol in chloroform as the eluant to give methyl 3-acetamido-2,3,4,6-tetradeoxy-4-(methoxycarbonylamino)-3-*C*-methyl-β-D-*xylo*-hexopyranoside (28) (3.2 mg, 11%) as a colourless oil [Found: *m/z* 215.1167 (M^+ − 59). $C_{10}H_{17}NO_4$ requires *m/z* 215.1158], $\nu_{max.}$ ($CHCl_3$) 3 440, 2 930, 1 725, 1 680, 1 508, 1 312, 1 230, and 1 068 cm^{-1}.

Methylation of Methyl α-D-Kijanoside (23).—Methyl α-D-kijanoside (23) (100 mg) was dissolved in dry dimethylformamide (6 ml) and hexane-washed sodium hydride (1.33 g) was added. The mixture was stirred under dry nitrogen at 25 °C for 2 h. Methyl iodide (2.87 g) was added and the mixture was stirred at 25 °C for 18 h. The reaction was quenched with methanol–water (9 : 1) and evaporated to dryness. The residue was chromatographed on a silica gel column (100 × 2 cm) using 5% acetone in hexane as the eluant to give the least polar product as a pale yellow oil (22 mg), $[\alpha]_D^{26}$ +92.9° (CH_3OH), $\nu_{max.}$ ($CHCl_3$) 2 990, 2 950, 1 705, 1 550, 1 350, 1 210, 1 133, and 1 155 cm^{-1}. Further elution of the column afforded a more polar product as a pale yellow oil (57 mg) (Found: C, 48.4; H, 7.3; N, 9.95. $C_{11}H_{20}N_2O_6$ requires C, 47.82; H, 7.30; N, 10.14%), $[\alpha]_D^{26}$ +168.7° (CH_3OH), $\nu_{max.}$ ($CHCl_3$) 3 000, 2 960, 2 920, 1 695, 1 550, 1 330, 1 218, 1 200, 1 160, 1 132, and 1 055 cm^{-1}.

Per-N,O-Methylation of Kijanimicin (1).—Kijanimicin (1) (10 g) was dissolved in dry dimethylformanide (500 ml). Hexane-washed sodium hydride (56.6 g) was added and the mixture was stirred under dry nitrogen at 25 °C for 3 h. Methyl iodide (66.5 ml) was added dropwise to the stirred solution at 0 °C during a period of 5 h. The reaction was then stirred at 25 °C for 21 h. The reaction mixture was neutralized to pH 7.0 with dilute hydrochloric acid and then evaporated to dryness under reduced pressure. The residue was partitioned between chloroform and water. The chloroform layer was washed with water and evaporated to dryness to give the per-*N,O*-methylated kijanimicin (15.6 g) which by t.l.c. was shown to be free of kijanimicin. The crude per-*N,O*-methylated kijanimicin was used without further purification.

Methanolysis of Per-N,O-methylated Kijanimicin.—(i) The per-*N,O*-methylated kijanimicin (15.6 g) from the previous experiment was dissolved in 0.5M-hydrogen chloride in methanol (700 ml) and the solution was allowed to remain at 25 °C for 20 h. The reaction mixture was neutralized to pH 7.5 with concentrated ammonium hydroxide and then evaporated to dryness. The residue was extracted with acetone and filtered. The acetone filtrate was evaporated to dryness and the residue was chromatographed on a silica gel column (120 × 5 cm) by gradient elution using 2.5% to 15% acetone in hexane as the eluant to give methyl 2,6-dideoxy-3,4-di-*O*-methyl-α-L-*ribo*-hexopyranoside (9) * (2.07 g) as a pale yellow oil. Further elution of the column afforded an oil which was purified by preparative t.l.c. on silica gel plates using 5% methanol in chloroform as the eluant to give methyl 2,6-dideoxy-3,4-di-*O*-methyl-β-L-*ribo*-hexopyranoside (10) * (0.49 g). Further elution of the column gave methyl 2,6-dideoxy-4-*O*-methyl-β-L-*ribo*-hexopyranoside (5) * (0.84 g) as a pale yellow oil. Further elution of the column gave methyl 2,6-dideoxy-4-*O*-methyl-α-L-*ribo*-hexopyranoside (4) * (0.26 g) as a pale yellow oil. Further elution of the column gave an oil that was further purified by preparative t.l.c. on silica gel plates using 40% chloroform in acetone as the eluant to give methyl 2,6-dideoxy-α-L-*ribo*-hexopyranoside (6) * (240 mg) as a pale yellow oil. Further elution of the column gave an oil which was subjected to preparative t.l.c. on silica gel plates using 5% methanol in chloroform as the eluant to give methyl 2,6-dideoxy-β-L-*ribo*-hexopyranoside (7) * (350 mg) as a pale yellow oil. Further elution of the column gave an oil which was further purified on a Waters Prep 500 h.p.l.c. instrument using one silica gel cartridge and 15% acetone in hexane as the eluant. The product thus obtained was further purified by preparative t.l.c. on silica gel plates using 5% methanol in chloroform as the eluant to give methyl 2,6-dideoxy-α-L-*ribo*-hexofuranoside (11) (137 mg) * as a pale yellow oil and the partially purified β-anomer (12). The latter was further purified by chromatography on a Waters analytical h.p.l.c. instrument using a silica gel column and 15% acetone in hexane as the eluant to give methyl 2,6-dideoxy-β-L-*ribo*-hexofuranoside (12) * (56 mg) as a pale yellow oil. The original preparative h.p.l.c. column was then stripped with methanol to give additional material (6.2 g). The latter was chromatographed on a silica gel column (120 × 6 cm) using 2% methanol in chloroform as the eluant to give impure *O*-(4^E-*N*-methyl-β-D-kijanosyl)-(1→17)-32-*O*-methylkijanolide (mixture of rotamers) (22) (1.14 g) as an off-white amorphous solid (Found: C, 63.3; H, 7.5; N, 1.95. $C_{44}H_{62}N_2O_{12}$ requires C, 65.17; H, 7.71; N, 3.45%), $[\alpha]_D^{26}$ −42.2° (CH_3OH), $\lambda_{max.}$ (CF_3CH_2OH) 245sh (ε 10 320) and 262 nm (ε 11 004); $\nu_{max.}$ (KBr) 3 500, 2 930, 2 880, 1 760, 1 700, 1 545, 1 455, 1 100, 1 060, and 885 cm^{-1}; δ_H ($CDCl_3$) (100 MHz) 0.66 (3 H, d, *J ca.* 4 Hz, 6-CH_3), 1.05 (3 H, d, *J* 7 Hz, 8-CH_3), 1.20 (3 H, d, *J* 6.5 Hz, 6^E-CH_3), 1.31 (3 H, d, *J* 6.5 Hz, 23-CH_3), 1.38 (3 H, s, 14-CH_3), 1.42 (3 H, s, 18-CH_3), 1.54 (3 H, s, 3^E-CH_3), 1.64 (3 H, s, 4-CH_3), 3.11, 3.13 (3 H, s, 4^E-NCH_3COOCH_3), 3.30, 3.35 (3 H, s, 32-OCH_3) and 3.78, 3.80 (3 H, s, 4^E-NCH$_3$COOCH_3) which was not further purified. Further elution of the column afforded 32-*O*-methylkijanolide (48) (987 mg) as an off-white amorphous solid [Found: C, 70.25; H, 8.3%; *m/z* 566.3286 ($M^{+\cdot}$). $C_{34}H_{46}O_7$ requires C, 72.05; H, 8.18%; *m/z* 566.3242], $[\alpha]_D^{26}$ −11.6° (CH_3OH), $\lambda_{max.}$ (CF_3CH_2OH) 245 (ε 7 090) and 262 nm (ε 7 976); $[\theta]_{205}$ +73 145, $[\theta]_{218}$ −108 846, $[\theta]_{240sh}$ −35 702, $[\theta]_{265}$ +3 269, and $[\theta]_{302}$ +21 769 (CF_3CH_2OH); $\nu_{max.}$ ($CHCl_3$), 3 610, 2 960, 2 920, 2 880, 1 748, and 1 058 cm^{-1}; *m/z* 566 (11.3) ($M^{+\cdot}$)[a], 548 (27.9) (M − H_2O)[a], 534 (1.7) (M − CH_3OH), 530 (3.8) (M − H_2O − H_2O)[a], 516 (2.9) (M − CH_3OH − H_2O)[a], 498

* Mixed t.l.c., ^1H n.m.r., and mass spectra were identical with characterized samples described earlier.

(2.1), 457 (3.0), 388 (5.1) (D_{15}), 356 (10.8) ($D_{15} - CH_3OH$)[a], and 338 (4.3) ($D_{15} - CH_3OH - H_2O$)[a].

(ii) Per-*N*,*O*-methylated kijanimicin (prepared as described above from 7.5 g of kijanimicin (1)) was dissolved in anhydrous methanol (2 l) and hydrogen chloride was passed through the solution for 4 h at 25 °C until the solution reached 8.5M. The mixture was allowed to stand at 25 °C for 43 h. The solution was neutralized with concentrated ammonium hydroxide and evaporated to dryness. The residue was extracted with acetone and the latter was filtered. The acetone filtrate was evaporated to dryness and the residue was chromatographed on a silica gel column (120 × 5 cm) using chloroform as the eluant. Only the 32-*O*-methylkijanolide (48) was collected and this was rechromatographed on a silica gel column (120 × 3 cm) using chloroform as the eluant to give pure 32-*O*-methylkijanolide (48) (3.09 g) as an off-white amorphous solid.

26,32-*Di*-O-*methylkijanolide* (49).—32-*O*-Methylkijanolide (48) (303 mg) was dissolved in methanol (5 ml) and a solution of diazomethane in diethyl ether (100 ml) was added. The mixture was allowed to remain at 25 °C for 4 h. The excess of diazomethane was removed with a stream of nitrogen and the solution was evaporated to dryness. The residue was chromatographed on a silica gel column (120 × 2 cm) using 10% acetone in hexane as the eluant to give the product. Further purification by preparative t.l.c. on silica gel plates using 10% methanol in chloroform as the eluant afforded 26,32-di-*O*-methylkijanolide (49) (121 mg, 39%) as a colourless amorphous solid [Found: *m/z* 580.3415 ($M^{+\cdot}$). $C_{35}H_{48}O_7$ requires: *m/z* 580.3399], $[\alpha]_D^{26}$ −69.0° (CH_3OH), $\lambda_{max.}$ (CF_3CH_2OH) 254 nm (ε 9 494), $\nu_{max.}$ ($CHCl_3$) 3 600, 2 950, 2 930, 2 870, 1 740, 1 660, 1 565, 1 435, 1 348, and 1 055 cm^{-1}; *m/z* 580 (43.8) ($M^{+\cdot}$)[a], 562 (17.2) ($M - H_2O$)[a], 548 (6.7) ($M - CH_3OH$)[a], 544 (5.2) ($M - H_2O - H_2O$), 530 (4.1) ($M - CH_3OH - H_2O$), 516 (1.5) ($M - CH_3OH - CH_3OH$), 471 (10.0), and 402 (20.6) (D_{16})[a].

9,17,32-*Tri*-O-*methylkijanolide* (50).—32-*O*-Methylkijanolide (48) (34 mg) was dissolved in dry dimethylformamide (10 ml) and hexane-washed sodium hydride (22 mg) was added and the mixture was stirred under dry nitrogen at 25 °C for 2 h. Methyl iodide (142 mg) was added and the mixture was stirred at 25 °C for 20 h. The mixture was neutralized to pH 7.0 with dilute hydrochloric acid and evaporated to dryness. The residue was taken up in chloroform (200 ml) and washed with water (500 ml) and the chloroform layer was evaporated to dryness. The residue was chromatographed on a silica gel column (20 × 1 cm) using chloroform as the eluant to give 9,17,32-tri-*O*-methylkijanolide (50) (23 mg, 64%) as a colourless amorphous solid [Found: *m/z* 576.3460 ($M^+ - H_2O$). $C_{36}H_{48}O_6$ requires *m/z* 576.3448], $[\alpha]_D^{26}$ −138.2° ($CHCl_3$), $\nu_{max.}$ (CF_3CH_2OH) 200 (ε 20 760) and 253 nm (ε 4 650); $\nu_{max.}$ ($CHCl_3$) 2 980, 2 940, 2 900, 1 755, 1 100, and 885 cm^{-1}; *m/z* 594 (15.8) ($M^{+\cdot}$), 576 (3.5) ($M - H_2O$)[a], 562 (65.8) ($M - CH_3OH$)[a], 544 (3.8) ($M - CH_3OH - H_2O$)[a], 530 (13.4) ($M - CH_3OH - CH_3OH$)[a], 512 (3.0) ($M - CH_3OH - CH_3OH - H_2O$)[a], 498 (4.2), 480 (2.4), 402 (15.8), (D_{17})[a], 384 (2.8) ($D_{17} - H_2O$), 370 (14.4) ($D_{17} - CH_3OH$)[a], 352 (5.6) ($D_{17} - CH_3OH - H_2O$), 338 (5.1) ($D_{17} - CH_3OH - CH_3OH$), and 320 (9.2) ($D_{17} - CH_3OH - CH_3OH - H_2O$).

9,17-*Di*-O-*acetyl*-32-O-*methylkijanolide* (51).—32-*O*-Methylkijanolide (48) (124 mg) was dissolved in dry pyridine (40 ml) and acetic anhydride (10 ml) was added and the mixture was allowed to remain at 25 °C for 17 h. The solution was evaporated to dryness and the residue was azeotroped with toluene and chromatographed on a silica gel column (60 × 3 cm) using 0.5% methanol in chloroform as the eluant (51) (88 mg, 62%) which crystallized as colourless orthorhombic crystals from chloroform–methanol, m.p. 236 °C [Found: *m/z* 590.3272 ($M^{+\cdot}$). $C_{36}H_{46}O_7$ requires *m/z* 590.3243], $[\alpha]_D^{26}$ −13.2° ($CHCl_3$), $\lambda_{max.}$ (CF_3CH_2OH) 199 (ε 34 095), 243 (6 585), and 263 nm (7 316); $\nu_{max.}$ ($CHCl_3$) 2 965, 2 930, 2 880, 1 730, 1 435, 1 365, 1 250, 1 040, and 980 cm^{-1}; *m/z* 590 (0.1) ($M - CH_3COOH$), 558 (0.1) ($M - CH_3COOH - CH_3OH$), 530 (1.0) ($M - CH_3COOH - CH_3COOH$), 512 (0.1), 498 (0.3), 454 (0.4), and 436 (0.2).

Crystal Data for Compound (51).—$C_{38}H_{50}O_9$, M = 650.8, Orthorhombic, a = 11.897(5), b = 28.423(11), c = 10.282(4) Å, U = 3 477 Å^3, Z = 4, D_c = 1.243 g cm^{-3}, $F(000)$ = 1 400. Cu-K_α radiation, λ = 1.5418 Å; μ(Cu-K_α) = 7.2 cm^{-1}. Space group $P2_12_12_1$(D_2^4) from the systematic absences: $h00$ when $h \neq 2n$, 0k0 when $k \neq 2n$, 00l when $l \neq 2n$.

Crystallographic Measurements.—A crystal of maximum dimensions *ca.* 0.30 × 0.05 × 0.70 mm was mounted on the end of a glass fibre. Preliminary unit-cell dimensions and space group information were obtained from oscillation and Weissenberg photographs (Cu-K_α radiation) and precession photographs (Mo-K_α radiation, λ = 0.7107 Å). The crystal was then oriented on an Enraf-Nonius CAD-3 automated diffractometer (Ni-filtered Cu-K_α radiation) when one octant of reciprocal space to θ 67° (3 531 independent intensity measurements) was surveyed by means of the θ—2θ scanning technique. Background measurements were recorded at each end of the scan range for times equal to half the scan duration. Instrument and crystal stability were monitored throughout by remeasuring the intensity of a strong reference reflection after each batch of 99 measurements; no significant variation was noted. Refined unit-cell parameters were derived by least-squares treatment of the diffractometer setting angles for 40 high-order reflections widely separated in reciprocal space. A total of 1 871 reflections for which $I > 2.0\ (I)$ [$\sigma^2(I)$ = scan count + total background count] were considered observed and were corrected for the usual Lorentz and polarization effects prior to their use in the structure analysis and refinement.

Structure Analysis.—The structure was solved by use of the 400 largest $|E|$ values and the MULTAN76 [35] suite of programs incorporating the magic integer approach.[36] An E map, evaluated by use of a set of phase angles which gave one of the highest combined figures-of-merit, yielded approximate positions for 34 non-hydrogen atoms. The remaining 13 non-hydrogen atoms were then located in an F_0 Fourier synthesis phased by the 34 atom fragment (R = 0.34). Full-matrix least-squares adjustment of carbon and oxygen positional and thermal, at first isotropic and subsequently anisotropic, parameters proceeded smoothly. During the later least-squares iterations, hydrogen atoms, save those on C(30), C(31), C(38), C(42), and C(45) which could not be placed with confidence, were included at their calculated positions. The refinement converged at R 0.067.

Final atomic positional parameters are in Tables 14 and 15. Anisotropic thermal parameters for the non-hydrogen atoms have been deposited along with observed and calculated structure amplitudes as Supplementary Publication No. SUP. No. 23569 (20 pages).†

Atomic scattering factors used in all structure-factor calculations were those for oxygen and carbon from ref. 37 and for hydrogen from ref. 38. In the least-squares iterations,

† For details of the Supplementary publications scheme see Instructions for Authors (1983), *J. Chem. Soc., Perkin Trans. 1*, 1983, Issue 1.

$\Sigma w\Delta^2$ was minimized, with weights, w, assigned according to the scheme: $\sqrt{w} = 1$ for $|F_0| < 20.0$, and $\sqrt{w} = 20.0/|F_0|$ for $|F_0| > 20.0$.

9,17-*Di*-O-*acetyl*-26,32-*di*-O-*methylkijanolide* (52).—26,32-Di-*O*-methylkijanolide (49) (100 mg) was dissolved in dry pyridine (40 ml) and acetic anhydride (10 ml) was added and the mixture was allowed to remain at 25 °C for 16 h. The solution was evaporated to dryness and the residue was azeotroped with toluene and chromatographed on a silica gel column (60 × 3 cm) using chloroform as the eluant to give 9,17-di-*O*-acetyl-26,32-di-*O*-methylkijanolide (52) (51 mg, 45%) which crystallized as clusters of fine colourless needles from chloroform–methanol, m.p. 234 °C [Found: *m/z* 664.3621 ($M^{+\cdot}$). $C_{39}H_{52}O_9$ requires *m/z* 664.3611], $[\alpha]_D^{26}$ −66.9° (CH_3OH), $\lambda_{max.}$ (CF_3CH_2OH) 253 nm (ε 10 143); $\nu_{max.}$ ($CHCl_3$) 2 950, 2 925, 2 880, 1 735, 1 660, 1 585, 1 438, 1 344, 1 249, and 1 035 cm^{-1}; *m/z* 664 (10.2) ($M^{+\cdot}$), 604 (45.1) ($M - CH_3COOH$), 586 (6.7) ($M - CH_3COOH - H_2O$), 572 (2.7) ($M - CH_3COOH - CH_3OH$), 544 (49.5) ($M - CH_3COOH - CH_3COOH$), and 512 (9.9) ($M - CH_3COOH - CH_3COOH - CH_3OH$).

Reaction of Kijanimicin (1) *with Sodium Metaperiodate.*—Kijanimicin (1) (8 g) was dissolved in tetrahydrofuran (1.6 l) and a solution of sodium metaperiodate (13 g, 10 equiv.) in distilled water (320 ml) was added dropwise to the stirred solution at 25 °C. The mixture was stirred in the dark at 25 °C for 18 h. Ethylene glycol (4 g) was added and the stirring was continued for 1 h. The solution was diluted with distilled water (500 ml) and the tetrahydrofuran was distilled off under reduced pressure. The residual aqueous suspension was extracted with chloroform and the latter was washed with water and evaporated to dryness. The residue was dried at 78 °C for 16 h and then dissolved in dry tetrahydrofuran (600 ml). Dry Dowex 50W × 8 resin (H^+) (200—400 mesh) (32 g) was added and the mixture was stirred and heated under reflux for 2 h. The mixture was filtered and the resin was washed with dry tetrahydrofuran. The combined filtrates were evaporated to dryness and the residue was chromatographed on a silica gel column (140 × 5 cm) using 3% methanol in chloroform as the eluant to give 3^B-*O*-dedigitoxosylkijanimicin (53) (1 g, 14%) as a colourless amorphous solid (Found: C, 61.15; H, 7.6; N, 2.0. $C_{61}H_{90}N_2O_{21}$ requires C, 61.70; H, 7.64; N, 2.36%), $[\alpha]_D^{26}$ −129.5° (CH_3OH), $\nu_{max.}$ ($CHCl_3$) 3 610, 3 570, 3 500, 3 400, 2 930, 1 747, 1 725, 1 540, 1 505, 1 225, and 1 055 cm^{-1}; $\lambda_{max.}$ (CF_3CH_2OH) 200 (ε 40 175), 236 (12 754), 265sh (10 203), and 275 nm (11 160); $\lambda_{max.}$ (CF_3CH_2OH + 0.1M-HCl) 200 (ε 42 726) and 260 nm (ε 8 928); $\lambda_{max.}$ (CF_3CH_2OH + 0.1M-NaOH) 200 (ε 42 414) and 265 nm (ε 8 483); δ_H ($CDCl_3$) (220 MHz) 0.64 (3 H, d, *J* 5 Hz, 6-CH_3), 1.09 (3 H, d, *J* 7 Hz, 8-CH_3), 1.16 (3 H, d, *J* 6 Hz, 6^E-CH_3), 1.21 (3 H, d, *J* 6.5 Hz, 6^B-CH_3), 1.25 (3 H, d, *J* 6.5 Hz, 6^D-CH_3), 1.27 (3 H, d, *J* 6.5 Hz, 6^A-CH_3), 1.33 (3 H, d, *J* 6.5 Hz, 23-CH_3), 1.34 (3 H, s, 18-CH_3), 1.41 (3 H, s, 14-CH_3), 1.59 (3 H, s, 4-CH_3), 1.62 (3 H, s, 3^E-CH_3), 3.42 (3 H, s, 4^D-OCH_3), 3.72 (3 H, s, 4^E-NHCOOCH_3), 4.33 (1 H, dd, $J_{4^E_{eq},4\text{-NH}}$ 10 Hz, $J_{4^E_{eq},5^E_{ax}}$ *ca.* 1 Hz, 4^E_{eq}-H), 4.46 (1 H, dd, $J_{1^E_{ax},2^E_{ax}}$ 10 Hz, $J_{1^E_{ax},2^E_{eq}}$ 2 Hz, 1^E_{ax}-H), 4.78 (1 H, dd, $J_{1^A_{eq},2^A_{ax}}$ 5 Hz, $J_{1^A_{eq},2^A_{eq}}$ <1 Hz, 1^A_{eq}-H), 4.92 (1 H, dd, $J_{1^D_{ax},2^D_{ax}}$ 9.5 Hz, $J_{1^D_{ax},2^D_{eq}}$ 1.5 Hz, 1^D_{ax}-H), 5.13 (1 H, dd, $J_{1^B_{eq},2^B_{ax}}$ 4 Hz, $J_{1^B_{eq},2^B_{eq}}$ <1 Hz, 1^B_{eq}-H), 5.38 (1 H, ddd, $J_{11,12}$ 10 Hz, $J_{12,13}$ 5 Hz, $J_{10ax,12}$ 2 Hz, 12-H), 5.51 (1 H, s, 21-H) and 5.73 (1 H, ddd, $J_{11,12}$ 10 Hz, $J_{11,13} = J_{10ax,11}$ = *ca.* 2 Hz, 11-H); *m/z* 257 (0.7), (D_{18}), 231 (0.1) (B_1^b), 184 (1.1) (B_6^b and B_{15}^b), 152 (0.8) (B_{10}^b), 145 (8.5) (A_1^c), 140 (3.4) (B_{18}^b), 128 (11.6) (B_{12}^b), 127 (37.3) (A_3^c), 96 (17.7) (B_{14}^b), and 95 (22.9) (A_5).

3^B-O-*Dedigitoxosyl*-26-O-*methylkijanimicin* (54).—3^B-*O*-Dedigitoxosylkijanimicin (53) (150 mg) was dissolved in chloroform (10 ml) and an excess of diazomethane in diethyl ether (30 ml) was added. The mixture was allowed to remain at 25 °C for 18 h. A stream of nitrogen was bubbled through the solution to remove the excess diazomethane and the solution was then evaporated to dryness and the residue was chromatographed on a silica gel column (120 × 1 cm) using first chloroform and then 1% methanol in chloroform as the eluant to give 3^B-dedigitoxosyl-26-*O*-methylkijanimicin (54) (85 mg, 56%) as a colourless amorphous solid (Found: C, 60.1; H, 7.25; N, 2.1. $C_{62}H_{92}N_2O_{21}$ requires C, 61.98; H, 7.72; N, 2.33%), $[\alpha]_D^{26}$ −129.5° (CH_3OH); $\nu_{max.}$ ($CHCl_3$) 3 680, 3 610, 3 560, 3 440, 2 940, 1 750, 1 730, 1 665, 1 575, 1 541, 1 516, 1 230, and 1 060 cm^{-1}; $\lambda_{max.}$ (CF_3CH_2OH) 200 (ε 42 569) and 253 nm (ε 9 755); δ_H ($CDCl_3$) (600 MHz) 0.61 (3 H, d, *J* 5.2 Hz, 6-CH_3), 1.09 (3 H, d, *J* 6.7 Hz, 8-CH_3), 1.16 (3 H, d, *J* 6.2 Hz, 6^E-CH_3), 1.21 (3 H, d, *J* 6 Hz, 6^B-CH_3), 1.25 (3 H, d, *J* 6 Hz, 6^D-CH_3), 1.26 (3 H, d, *J* 6 Hz, 6^A-CH_3), 1.31 (3 H, s, 18-CH_3), 1.32 (3 H, d, *J* 6.7 Hz, 23-CH_3), 1.35 (3 H, s, 14-CH_3), 1.53 (3 H, s, 4-CH_3), 1.58 (3 H, s, 3^E-CH_3), 3.42 (3 H, s, 4^D-OCH_3), 3.72 (3 H, s, 4^E-NHCOOCH_3), 4.12 (3 H, s, 26-OCH_3), 4.38 (1 H, dd, $J_{4^E_{eq},4\text{-NH}}$ 10.0 Hz, $J_{4^E_{eq},5^E_{ax}}$ *ca.* 1 Hz, 4^E_{eq}-H), 4.45 (1 H, dd, $J_{1^E_{ax},2^E_{ax}}$ 10.0 Hz, $J_{1^E_{ax},2^E_{eq}}$ 1.9 Hz, 1^E_{ax}-H), 4.77 (1 H, dd, $J_{1^A_{eq},2^A_{ax}}$ 4.8 Hz, $J_{1^A_{eq},2^A_{eq}}$ <0.5 Hz, 1^A_{eq}-H), 4.92 (1 H, dd, $J_{1^D_{ax},2^D_{ax}}$ 9.5 Hz, $J_{1^D_{ax},2^D_{eq}}$ 1.4 Hz, 1^D_{ax}-H), 5.02 (1 H, ddq, $J_{15,16}$ 10.0 Hz, $J_{15,16}$ *ca.* 2 Hz, $J_{15,14\text{-}CH_3}$ <1 Hz, 15-H), 5.10 (1 H, ddq, $J_{19,20}$ 9.1 Hz, $J_{17,19}$ *ca.* 2 Hz, $J_{19,18\text{-}CH_3}$ <1 Hz, 19-H), 5.14 (1 H, dd, $J_{1^B_{eq},2^B_{ax}}$ 3.8 Hz, $J_{1^B_{eq},2^B_{eq}}$ <0.5, 1^B_{eq}-H), 5.36 (1 H, d, $J_{4^E_{eq},4^E\text{-NH}}$ 10.5 Hz, 4^E-*NH*COOCH$_3$), 5.41 (1 H, ddd, $J_{11,12}$ 10.5 Hz, $J_{12,13}$ 5.2 Hz, $J_{10ax,12}$ 1.4 Hz, 12-H), 5.51 (1 H, s, 21-H), and 5.71 (1 H, ddd, $J_{11,12}$ 10.5 Hz, $J_{11,13} = J_{10ax,11}$ = *ca.* 2 Hz, 11-H); *m/z* 566 (0.2) (D_2), 548 (0.5) ($D_2 - H_2O$), 534 (0.3) ($D_2 - CH_3OH$), 530, (0.3) ($D_2 - H_2O - H_2O$), 257 (4.5) (D_{18}), 231 (1.0) (B_1^b), 184 (9.7) (B_6^b and B_{15}^b), 152 (1.3) (B_{10}^b), 145 (20.0) (A_1^c), 140 (3.0) (B_{18}^b), 128 (21.4) (B_{12}^b), 127 (91.7) (A_3^c), 96 (11.4) (B_{14}^b), and 95 (24.3) (A_5).

Per-N,O-*methylation of* 3^B-O-*Dedigitoxosylkijanimicin* (53) *and Methanolysis of the Product.*—3^B-*O*-Dedigitoxosylkijanimicin (53) (500 mg) was dissolved in dry dimethylformamide (30 ml) and hexane-washed sodium hydride (2.83 g) was added. The mixture was stirred under dry nitrogen at 25 °C for 1.5 h. Methyl iodide (3.35 ml) was added dropwise during 0.5 h and the mixture was stirred at 25 °C for 21 h. The reaction was quenched with methanol–water and the pH was adjusted to 7.0 with dilute hydrochloric acid. The solution was evaporated to dryness, extracted with chloroform, and filtered. The chloroform filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column (60 × 5 cm) using 5% methanol in chloroform as the eluant to give per-*N*,*O*-methylated 3^B-*O*-dedigitoxosylkijanimicin. The latter was dissolved in 0.5M-hydrogen chloride in methanol (100 ml) and the solution was allowed to remain at 25 °C for 16 h. The solution was neutralized to pH 7.2 with concentrated ammonium hydroxide and then evaporated to dryness. The residue was extracted with acetone, filtered, and the acetone filtrate was evaporated to dryness. The residue was chromatographed on a silica gel column (120 × 2 cm) using 6.5% acetone in hexane as the eluant to give methyl 2,6-dideoxy-3,4-di-*O*-methyl-β-L-*ribo*-hexopyranoside (10) * which was further purified by preparative t.l.c. on silica gel plates using 80% ethyl acetate in dichloromethane as the eluant to give

* Mixed t.l.c., ^1H n.m.r., and mass spec. identical with characterized samples described earlier.

pure (10) (14 mg) as a pale yellow oil. Further elution of the column gave methyl 2,6-dideoxy-3,4-di-*O*-methyl-α-L-*ribo*-hexopyranoside (9) * (13 mg) as a pale yellow oil. Further elution gave methyl 2,6-dideoxy-4-*O*-methyl-β-L-*ribo*-hexopyranoside (5) * (27 mg) as a pale yellow oil. Further elution gave methyl 2,6-dideoxy-4-*O*-methyl-α-L-*ribo*-hexopyranoside (4) * (5 mg) as a pale yellow oil. Further elution of the column gave methyl 2,6-dideoxy-3-*O*-methyl-β-L-*ribo*-hexofuranoside (13) which was further purified by preparative t.l.c. on silica gel plates using 40% chloroform in acetone as the eluant to give pure (13) (20 mg) as a pale yellow oil [Found: *m/z* 145.0857 ($M^+ - 31$). $C_7H_{13}O_3$ requires *m/z* 145.0864], $[\alpha]_D^{26}$ −40.2° (CH_3OH), $\nu_{max.}$ ($CHCl_3$) 3 680, 3 600, 2 915, 2 850, and 1 045 cm^{-1}. The column was then stripped with methanol to give the crude kijanolide containing fragments (255 mg) which contained no additional L-digitoxose derivatives.

Ozonolysis of Kijanimicin (1).—Kijanimicin (1) (3.48 g) was dissolved in a mixture of ethanol (250 ml) and chloroform (40 ml) and the solution was cooled to −78 °C. Ozone was bubbled through the solution at −78 °C for 2.5 h. Nitrogen was then bubbled through the solution for 0.5 h. Sodium borohydride (1.88 g) was added and the mixture was stirred at 25 °C for 20 h. The solution was evaporated to dryness and the residue was partitioned between chloroform and 5% acetic acid. The chloroform layer was washed with water. The chloroform layer was evaporated to dryness and the residue was chromatographed on a silica gel column (160 × 2 cm) using 2% methanol in chloroform as the eluant to give three principal cuts.

The first (fractions 30—50) was rechromatographed on a silica gel column (100 × 1 cm) using chloroform as the eluant to give *O*-β-D-kijanosyl-(1→3)-3-hydroxypropanol (57) (39 mg) as a pale yellow oil (Found: C, 48.15; H, 7.25; N, 8.55. $C_{12}H_{22}N_2O_7$ requires C, 47.05; H, 7.24; N, 9.15%), $[\alpha]_D^{26}$ +27.9° (CH_3OH), $\lambda_{max.}$ (CF_3CH_2OH) 197 nm (ε 4 003); $\nu_{max.}$ ($CHCl_3$) 3 620, 3 550, 3 440, 2 940, 2 890, 1 725, 1 545, 1 510, 1 310, 1 230, 1 130, and 1 060 cm^{-1}. Further elution of the column gave material that was twice subjected to preparative t.l.c. on silica gel plates using 0.75% methanol in chloroform as the eluant to give *O*-β-D-kijanosyl-(1→1)-ethylene (58) (16 mg) as a pale yellow oil, $\nu_{max.}$ ($CHCl_3$) 3 440, 3 000, 2 945, 2 900, 1 735, 1 645, 1 548, 1 512, 1 315, 1 233, 1 177, and 1 070 cm^{-1}.

The second cut (fractions 55—100) from the original column afforded *O*-β-D-kijanosyl-(1→3)-3,4-dihydroxypentanol (56) (400 mg) as a pale yellow gum (Found: C, 48.35; H, 7.5; N, 7.65. $C_{14}H_{26}N_2O_8$ requires C, 47.99; H, 7.48; N, 8.00%), $[\alpha]_D^{26}$ +4.0° (CH_3OH), $\lambda_{max.}$ (CF_3CH_2OH) 199 nm (ε 4 146); $\nu_{max.}$ (film) 3 430, 3 300, 2 970, 2 925, 2 875, 1 700, 1 540, 1 510sh, 1 450, 1 440, 1 405, 1 240, 1 172, 1 045, and 900 cm^{-1}.

The third cut (fractions 310—380) from the original column afforded material that was rechromatographed on a silica gel column (100 × 1 cm) using chloroform as the eluant to give the tetrasaccharide fragment (55) (60 mg) as a colourless amorphous solid (Found: C, 57.65; H, 8.2; O, 34.0. $C_{41}H_{72}O_{18}$ requires C, 55.73; H, 8.51; O, 33.76%), $[\alpha]_D^{26}$ −140.5° (CH_3OH), $\nu_{max.}$ ($CHCl_3$) 3 620, 3 480, 2 970, 2 940, 1 765, 1 715, 1 125, and 1 060 cm^{-1}; δ_H ($CDCl_3$) (100 MHz) 0.95—1.05 and 1.10—1.35 (envelope of CH_3 groups), and 3.42 (3 H, s, 4^D-OCH_3); *m/z* 301 (1.2) (D_{23}), 283 (3.7) ($D_{23} - H_2O$), 265 (7.3) ($D_{23} - H_2O - H_2O$), 257 (7.0) ($D_{23} - CO_2$), 247 (2.3) ($D_{23} - H_2O - H_2O - H_2O$), 161 (7.8) ($D_{19}$), 145 (18.8) ($A_1$), and 131 (22) ($A_1$).

Note added in proof:

Recently the syntheses of methyl 2,6-dideoxy-4-*O*-methyl-α-L-*ribo*-hexopyranoside (4) [39] and of methyl α-D-kijanoside (methyl α-D-tetronitroside) (23) [40] have been reported, thus confirming the structures proposed for these novel sugars. The synthesis of L-rubranitrose (43) [41] has also been reported and the synthetic material has been shown to be enantiomeric with the natural sample,[22,42] thus confirming our conclusions that rubranitrose has indeed the D-configuration.

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