Pyrylium-mediated Transformations of Natural Products. Part 6.[1] Preparation of Pyridinium Salts derived from Aminoglycosides

Alan R. Katritzky,* Yu-Kun Yang, Joan Ellison, and Jorge Marquet
Department of Chemistry, University of Florida, Gainesville, Fl. 32611, U.S.A.

Kanamycins A and B react with a variety of water-soluble pyrylium ions in aqueous solution specifically at the 6′-primary-alkyl primary amine functions to give the corresponding pyridinium ions in high yield. Neomycin also reacts at only one amino group. Products were characterised by ^{13}C n.m.r. and elemental analysis.

Kanamycins A (1) and B (2) and neomycin B (3) exemplify the aminoglycoside antibiotics.[2] Studies [3] of resistance show they are enzymically inactivated by several pathways, and suggest that specific modifications of the aminoglycosides might afford useful derivatives, active against resistant strains of bacteria.

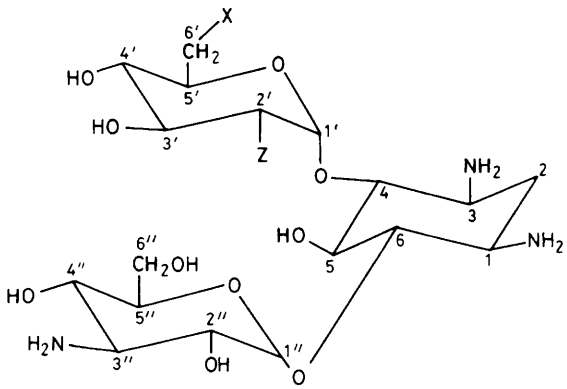
Thus, the discovery that kanamycins and neomycins are commonly inactivated by phosphorylation of hydroxy groups [4] has led to the synthesis of deoxy [5–9] and *O*-alkyl [5,10] antibiotic derivatives, some with significantly changed antibacterial activity. Another important enzymic inactivation procedure is 6′-*N*-acetylation,[11] and modifications of the 6′-amino group have therefore been studied.[12] 3′,4′-Dideoxy-6′-*N*-methylkanamycin B showed significant activity against a *Pseudomonas* species which inactivates kanamycins.

1-*N*-Acyl and 1-*N*-acyl-deoxy derivatives [13,14] reportedly show improved activity against resistant organisms.[15] 1-Alkyl derivatives [16,17] have also emerged as an important group of antibiotics. Modifications of kanamycin B by chemically induced conformational change [18,19] and by additional ring formation [20] have also been described recently. A deamination potentially applicable to aminoglycosides has been tested on 2-amino-2-deoxy-D-glucose.[20]

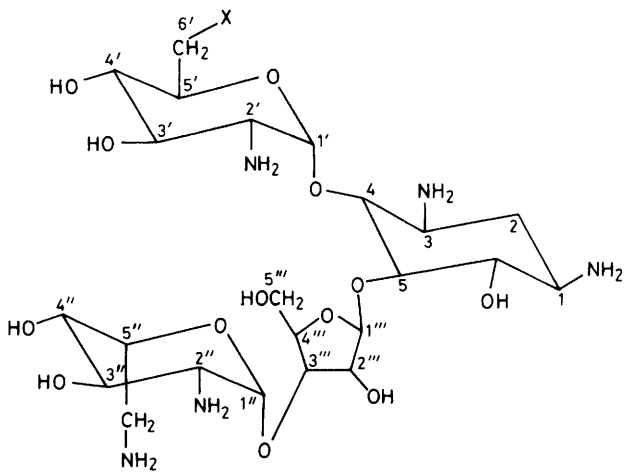
All this previous work on the chemical modification of aminoglycoside antibiotics has required tedious selective protection methods for the hydroxy and/or amino groups (*e.g.* refs. 21 and 22). Application of our pyrylium-mediated transformation of amines appeared to have considerable promise. Pyrylium salts are almost totally unreactive towards most functional groups other than amines, and primary-alkyl primary amines form pyridinium salts more than one hundred times faster than their secondary-alkyl analogues.[23] Thus, we considered that it should be possible to modify suitable aminoglycosides without protection.

Treatment of pyridinium salts (4b) derived from primary amines and 2,4,6-triphenylpyrylium ion (4a) with nucleophiles results in displacement of the *N*-substituent.[23] Pyridinium salts of types (5b) and (6b) are more reactive than (4b) and react with nucleophiles under mild conditions (30—80 °C).[24,25] Triphenylpyridinium salts (4b) also undergo radical reactions with nitronate anions and some other carbon nucleophiles at 25—100 °C leading to *C*-alkylation of the anion by the *N*-substituent.[26,27]

Aminoglycoside antibiotics are completely insoluble in organic solvents; therefore, following our demonstration that arylpyrylium ions [(8a), (8e), (9)] rendered water-soluble by sulphonic and carboxylic acid groups [28,29] would react with amines in aqueous solution to give pyridinium salts,[29,30] we turned our attention to the preparation of pyridinium derivatives of aminoglycoside antibiotics [kanamycins A (1) and B (2) and neomycin B (3)]. These polyfunctional compounds have one primary-alkyl primary amino group [6′-position in kanamycins A (1) and B (2)], or two [6′- and 6″-positions in neomycin B (3)]. We considered that specific reactions of these groups with pyrylium salts (and subsequent displacement of the pyridine by nucleophiles) could lead to novel aminoglycoside antibiotics.[2]



(1) X = NH_2, Z = OH
(2) X = NH_2, Z = NH_2



(3)

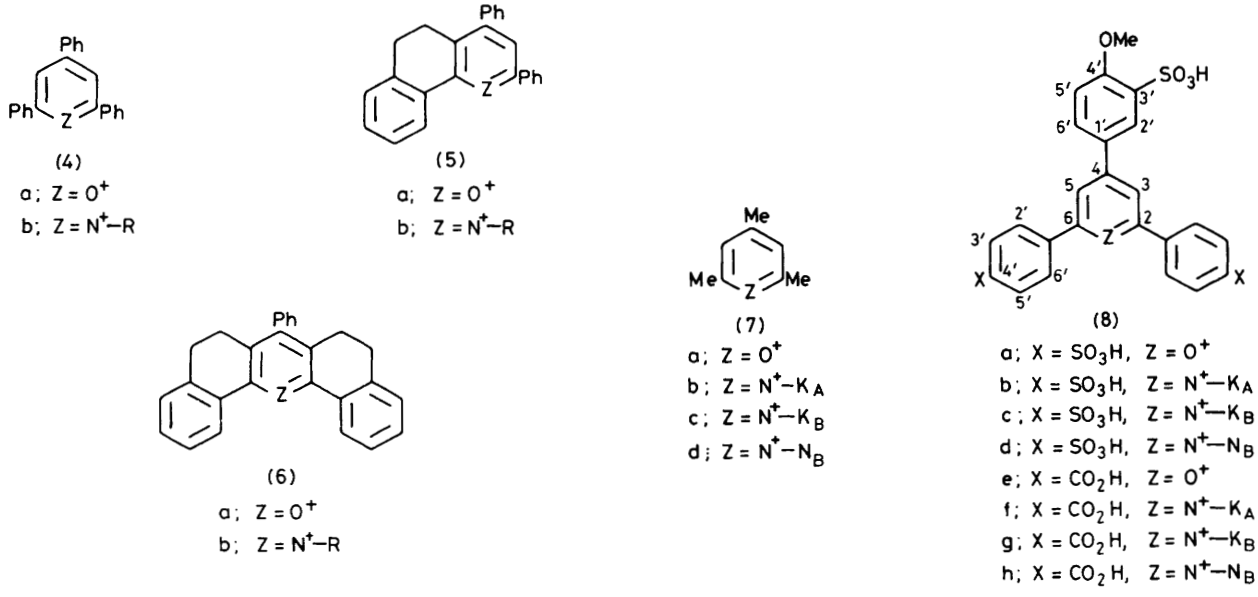
Results

We now report the successful preparation of various monopyridinium derivatives of kanamycins A (1) and B (2) and neomycin B (3) (reaction only at 6′-NH_2).

Table 1. ^{1}H Chemical shifts (δ) [a] of the pyridinium derivatives of aminoglycosides

Cpd. no.	Pyridinium segment: Hetero-cycle 3-, 5-H	Free α-aryl rings 2′-, 3′-, 5′-, 6′-H	γ-Aryl ring 2′-H	5′-H	6′-H	MeO	Aminoglycoside segment: 1′- and 1″-H		2-H_{eq}	2-H_{ax}	Other CH/CH_2	NH_2/OH
(7b) [b]	7.60						5.20 [c]	5.00 [c]	2.10 [c]	*d*	4.00—3.20 [e]	4.70
(7c) [b]	2.60						5.10 [c]	5.10 [c]	2.25 [f]	*d*	4.10—3.20 [e]	4.70
(7d) [g]	2.70						6.15 [f]	5.50 [f]	*d*	*d*	4.50—3.40 [e]	4.25
(8b)	8.20	8.05	8.40	7.35 [c]	7.90—7.70 [e]	3.95	5.10 [f]	5.10 [f]	2.50—2.10 [f]	1.00—1.30 [f]	4.10—2.70 [e]	4.70
(8c)	8.20	8.05	8.40	7.35 [c]	7.90—7.70 [e]	3.95	5.15 [f]	5.15 [f]	2.40—1.90 [f]	1.80—1.20 [f]	4.10—2.40 [e]	4.8—4.7
(8d)	*d*	8.10	*d*	7.35 [c]	*d*	3.93	5.95 [f]	5.45 [f]	*d*	*d*	4.60—2.50 [e]	4.8—4.7
(8f)	8.06	8.13—7.90 [h]	8.36	7.31 [c]	*d*	3.96	5.05 [f]	5.05 [f]	2.60—2.20 [f]	2.00—1.40 [f]	4.10—2.70 [e]	4.8—4.7
(10b) [i]	*d*	7.80—8.40 [j]	8.55	7.50 [f]	*d*	3.93	*d*	*d*	2.70—2.20 [f]	2.10—1.50 [f]	4.10—2.70 [e]	*d*
(10c) [i]	*d*	7.80—8.40 [j]	8.55 [c]	7.50 [f]	*d*	3.95	5.65 [f]	5.65 [f]	2.70—2.20 [f]	2.10—1.50 [f]	4.30—2.70 [e]	*d*
(10d)	*d*	7.70—8.40 [j]	*d*	7.50 [f]	*d*	3.95	5.3—5.5 [f]	5.3—5.5 [f]	2.50—1.90 [e]	1.60—1.10 [e]	4.10—2.50 [e]	*d*

[a] In D_2O, DSS internal reference; singlets unless indicated otherwise. [b] Others: 2.80 (for α-Me) and 2.50 (for γ-Me). [c] Doublet. [d] Signal could not be distinguished. [e] Multiplet. [f] Broad singlet. [g] Others: 2.90 (for α-Me) and 2.60 (for γ-Me). [h] 8.13 [c] for 2′-, 6′-H, and 7.90 [c] for 3′-, 5′-H. [i] The integration showed the presence of 0.5 mol equiv. of ethanol of crystallization [δ 1.23 (1.5 H, t, *J* 7 Hz) and 3.77 (1 H, q, *J* 7 Hz)]. [j] Multiplet, involving 2′-, 3′-, 5′-, 6′-H of 6-aryl ring, 4′-, 5′-, 6′-, 7′-H of 2-benzothiazolyl substituent and 3-, 5-H of pyridinium ring.



Scheme. $N^+–K_A$ is as in (1) with the pyridinium ring as X, $N^+–K_B$ is as in (2) with the pyridinium ring as X, and $N^+–N_B$ is as in (3) with the pyridinium ring as X.

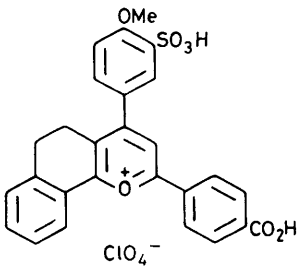
N-Substituted 2,4,6-*Trimethylpyridinium Salts*.—Trimethylpyrylium salts (7a) are water-soluble and react in this medium with amines.[31–35] Although the corresponding pyridinium salts are inert to nucleophilic attack, the easy availability of the starting material makes it a good model for the reactions with the newly developed water-soluble arylpyrylium salts,[28,29] expected to lead to pyridinium salts susceptible to nucleophilic displacement.

Reactions of 2,4,6-trimethylpyrylium perchlorate (7a) with kanamycins A (1) and B (2) and neomycin B (3) were carried out in water by stirring at room temperature or at 60 °C. Pyridinium salts (7b, c, and d) were obtained in yields of 88, 90, and 76%, respectively. N.m.r. spectra (^{1}H and ^{13}C) and elemental analyses were consistent with the pyridinium structures. The elemental analysis indicated that (7b) crystallized as a dication (anions HSO_4^- and ClO_4^-) whereas (7c) and (7d) crystallized as trications (anions $2HSO_4^-$, ClO_4^-). Each of (7a, b, and c) contained 1 mol equiv. of ethanol of crystallization.

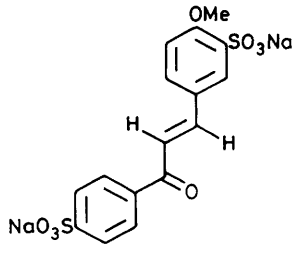
^{1}H N.m.r. integration, ^{13}C n.m.r., and elemental analysis indicated that only the monopyridinium derivative (7d) was formed (reaction at C-6′) in the case of neomycin B. Attempts to introduce a second pyridinium moiety by prolonged heating of neomycin sulphate with an excess of trimethylpyridinium perchlorate (7a) proved unsuccessful.

N-Substituted 2,4,6-*Triarylpyridinium Salts*.—Several water-soluble triarylpyrylium salts have been developed and their reactions with simple amines reported.[29,30]

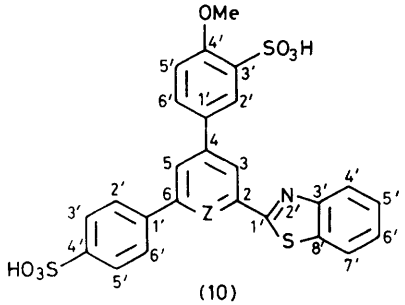
4-(4-Methoxy-3-sulphophenyl)-2,4-bis-(4-sulphophenyl)-pyrylium perchlorate (8a) was treated in water at room temperature with kanamycins A (1) and B (2), and with neomycin (B) (3) affording the corresponding 6′-monopyridinium derivatives (8b, c, and d) in 86, 74, and 77% yields, res-



(9)



(11)



(10)

a; Z = O^+
b; Z = $N^+—K_A$
c; Z = $N^+—K_B$
d; Z = $N^+—N_B$

pectively.* The finely powdered solid pyrylium salt was added in portions to a solution of the aminoglycoside in water at pH *ca.* 7 (this pH was found to give best results). The pyridinium salts were precipitated with acetone. The elemental analyses show that (8b) separates as a mono(hydrogen sulphate), whereas (8c) and (8d) each contain both hydrogen sulphate and perchlorate anions: these compositions correspond to complete protonation of each NH_2 group in the products at the sulphonic acid groups of the pyridinium rings. N.m.r. spectra (^1H and ^{13}C) were consistent with the proposed structures.

In the same way 2,4-bis-(4-carboxyphenyl)-4-(4-methoxy-3-sulphophenyl)pyrylium perchlorate (8e) reacted with kanamycin A (1) giving the corresponding 6′-monopyridinium derivative (8f) (59%), which is also a complex salt, as indicated by elemental analysis. However, although the reactions of (8e) with kanamycin B (2) and with neomycin B (3) gave the corresponding 6′-monopyridinium derivatives (8g and h), the products were mixed with appreciable amounts of starting aminoglycoside (as shown by the ^{13}C n.m.r. spectra), which could not be eliminated by crystallization.

The reaction of 2-(4-carboxyphenyl)-4-(4-methoxy-3-sulphophenyl)-5,6-dihydrobenzo[*h*]chromenylium perchlorate (9) with the aminoglycosides, expected to produce 6′-monopyridinium derivatives which are more susceptible to nucleophilic attack, failed in aqueous solution at various pH values, giving only the pseudobase and other decomposition products of (9). The aminoglycoside was unchanged after ten days at room temperature or two days at 60 °C.

* Kinetic work [36] supports the formation of pyridinium salts. The values of k_3 and k_{va} for the reactions with kanamycins A and B are comparable with those for the reaction with lysine. But the values of k_{va}/k_{OH} are about 50 times lower than with lysine.[36] For definitions of k_{va}, k_{OH}, and k_3 see ref. 30.

Table 2. Chemical shifts (δ) [a] of the pyridinium salts (pyridinium segment)

	Heterocycle				
Cpd. no.	C-2	C-3	C-4	C-5	C-6
(7b) [b]	160.30	129.70	156.10	129.70	160.30
(7c) [c]	160.00	129.60	155.90	129.60	160.30
(7d) [d]	160.10	129.40	156.30	129.40	160.10
(8b)	155.20	126.50	156.10	126.50	155.20
(8c)	155.60	126.50	158.10	126.50	155.60
(8d)	155.50	126.50	158.10	126.50	155.50
(8f) [e]	158.70	125.50	154.30	125.50	158.70
(10b) [f]	156.30	126.90	155.00	126.90	156.30
(10c) [g]	156.50	126.60	155.20	126.60	156.50
(10d) [h]	157.60	126.10	155.30	126.10	156.90

	γ-Aryl groups					
Cpd. no.	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
(8b)	125.20	132.30	129.20	160.80	114.50	134.30
(8c)	124.50	132.30	129.10	161.00	114.90	134.60
(8d)	125.10	132.50	129.70	161.10	114.80	134.80
(8f) [e]	126.40	131.10	129.50	160.50	114.30	132.30
(10b) [f]	126.90	131.00	129.40	160.80	114.60	132.40
(10c) [g]	126.60	129.60	129.40	160.90	114.70	132.50
(10d) [h]	126.10	130.70	129.40	160.90	114.60	132.50

	Free α-aryl group				
Cpd. no.	C-1′	C-2′, -6′	C-3′, -5′	C-4′	OMe
(8b)	135.80	131.00	127.20	145.90	57.30
(8c)	135.70	131.10	126.90	146.10	57.40
(8d)	136.60	131.10	127.00	146.10	57.40
(8f) [e]	135.90	130.20	130.20	139.50	57.40
(10b) [f]	136.10	131.00	127.40	146.00	57.40
(10c) [g]	136.40	131.20	127.40	146.00	57.30
(10d) [h]	136.60	130.95	127.35	146.10	57.40

[a] In D_2O (ref. dioxane, δ 67.4). [b] 22.10, 21.80, 21.50 for the γ- and α-methyls. [c] 22.10, 21.90, 21.60 for the methyls. [d] 22.20, 22.00, 21.70 for the methyls. [e] 174.60 (for carboxy group); 131.10 and 129.50 (unassigned). [f] 156.30 (1′), 155.00 (3′), 125.40 (4′ and 6′), 127.40 (5′ and 7′), 134.50 (8′) for the α-benzothiazole group (tentative assignment by comparison with benzothiazole). [g] 157.70 (1′), 155.20 (3′), 125.00 (4′ and 6′), 127.40 (5′), 127.70 (7′), 134.60 (8′) for the α-benzothiazole group (tentative assignment by comparison with benzothiazole). [h] 158.70 (1′), 155.30 (3′), 125.00 (4′ and 6′), 127.20 (5′ and 7′), 133.10 (8′) for the α-benzothiazole group (tentative assignment by comparison with benzothiazole).

N-*Substituted* 2-(*Benzothiazol-2-yl*)-4,6-*diarylpyridinium Salts*.—The new water-soluble 2-(benzothiazol-2-yl)-4-(4-methoxy-3-sulphophenyl)-6-(4-sulphophenyl)pyrylium perchlorate (10a), which was expected to produce 6′-monopyridinium derivatives susceptible to nucleophilic attack, has been prepared in 49% yield from 2-acetylbenzothiazole and the disodium salt of 4-sulphophenyl-2-(4-methoxy-3-sulphophenyl)vinyl ketone (11). The ketone (11) was obtained in 70% yield from sodium 3-formyl-6-methoxybenzenesulphonate and sodium 4-acetylbenzenesulphonate. The pyrylium salt (10a) reacted readily in water (pH 7—8) with the aminoglycosides to give the corresponding 6′-monopyridinium derivatives (10b—d) in yields of 77, 77, and 67%, respectively. The elemental analyses showed that (10b) separates as a mono(hydrogen sulphate), whereas (10c and d) contain both hydrogen sulphate and perchlorate anions. N.m.r. spectra (^1H and ^{13}C) were consistent with the proposed structures.

^1H *and* ^{13}C *N.m.r. Spectra*.—^1H N.m.r. spectra of the aminoglycosides and their pyridinium salts are presented in Table1.

Table 3. ^{13}C Chemical shifts (δ) of the aminoglycosides and aminoglycoside segment in the pyridinium salts (continued in Table 4)

Cpd. no.	*N*-Substituent	C-1	C-2	C-3	C-4	C-5	C-6	C-1′	C-2′	C-3′	C-4′	C-5′	C-6′
(1) [a]		50.40	28.10	48.30	78.80	73.40	84.30	96.10	71.40	72.80	71.40	69.30	41.00
(1) [b]		51.00	36.10	49.60	87.60	74.80	88.40	99.00	72.40	73.50	71.70	72.90	42.00
(2) [c]		50.20	28.90	48.90	78.70	74.70	84.20	96.30	54.20	69.80	71.20	69.70	40.70
(2) [d]		50.70	35.80	49.60	86.80	74.70	88.20	100.50	55.60	73.80	71.70	73.00	41.90
(3) [e]		50.90	31.40	49.40	78.80	85.70	*f*	96.20	54.50	68.60	69.60	73.80	40.90
(7b) [e]	K_A	51.40	37.40	49.50	84.90	74.60	88.10	101.10	72.40 [g]	73.60	69.40	73.10	53.70
(7c) [e]	K_B	50.20	28.40	49.10	80.30	74.60	84.60	97.20	54.40	68.90	68.90	73.70	53.90
(7d) [e]	N_B	50.70	28.60	49.30	78.30	85.00	74.50	97.00	54.40	68.50	70.40	74.00	54.40
(8b) [e]	K_A	50.80	31.10	49.30	84.40	74.00	85.10	98.90	72.50	73.70	71.60	72.10	58.30
(8c) [e]	K_B	51.50	32.80	49.90	86.00	74.50	87.60	100.90	55.80	73.30	71.90	73.00	56.90
(8d) [e]	N_B	51.40	33.10	50.70	*h*	85.30	74.40	100.10	55.30	68.60	68.60	69.80	55.30
(8f) [e]	K_A	50.50	29.40	48.50	86.00	73.90	87.30	98.00	71.90	73.60	71.70	77.90	55.90
(10b) [e]	K_A	50.90	29.80	49.00	83.10	74.70	84.50	99.80	72.40	73.80	69.30	71.80	58.30
(10c) [e]	K_B	50.60	30.40	49.40	82.00	74.60	85.10	101.10	55.80	73.80	71.40	71.90	56.80
(10d) [e]	K_B	51.70	30.10	49.90	78.40	85.90	70.80	96.80	54.60	68.80	69.60	73.90	56.80

[a] Ref. 33, pH 3.6. [b] Ref. 33, pH 9.6. [c] Ref. 33, pH 5.5. [d] Ref. 33, pH 10.6. [e] In D_2O; ref. dioxane (δ 67.40). [f] Other signals at 70.9 and 60.8. [g] Or δ 72.6. [h] Signal could not be distinguished.

Table 4. ^{13}C Chemical shifts (δ) of the aminoglycosides and aminoglycoside segments in the pyridinium salts (continued from Table 3)

Cpd. no.	*N*-Substituent	C-1″	C-2″	C-3″	C-4″	C-5″	C-6″	C-1‴	C-2‴	C-3‴	C-4‴	C-5‴
(1) [a]		100.70	68.70	55.70	66.20	72.80	62.70					
(1) [b]		100.40	72.40	54.90	70.00	72.90	61.10					
(2) [c]		100.80	68.50	55.50	66.10	73.40	60.50					
(2) [d]		100.10	75.00	54.50	69.80	72.40	60.70					
(3) [e]		96.60	52.00	71.80	68.30	74.20	40.90	110.50	25.40	81.60	*f*	60.80
(7b) [e]	K_A	101.30	72.60 [g]	56.10	66.60	73.10	60.80					
(7c) [e]	K_B	101.30	72.30	55.70	66.40	73.80	60.80					
(7d) [e]	N_B	97.40	51.80	72.50	68.50	73.40	41.40	110.60	76.40	82.40	95.50	60.70
(8b) [e]	K_A	100.50	69.30	55.80	66.70	72.90	60.00					
(8c) [e]	K_B	99.60	72.10	55.20	69.40	72.70	61.30					
(8d) [e]	N_B	99.10	53.40	71.60	66.50	69.80	41.60	110.80	76.90	82.40	*h*	61.50
(8f) [e]	K_A	100.40	69.10	55.10	66.70	72.30	61.00					
(10b) [e,j]	K_A	101.20	71.80	55.90	66.60	73.00	61.10					
(10c) [e,j]	K_B	96.20	69.90	54.30	66.50	73.00	61.13					
(10d) [e,j]	N_B	96.10	71.10	53.20	68.30	74.40	41.50	111.30	74.80	82.40	*i*	62.20

[a] Ref. 33, pH 3.6. [b] Ref. 33, pH 9.6. [c] Ref. 33, pH 5.5. [d] Ref. 33, pH 10.6. [e] In D_2O, ref. dioxane (δ 67.40). [f] Other signals at 21.3 and 61.2. [g] Or δ 72.4. [h] Signal could not be distinguished. [i] Other signal at 72.80. [j] Other signals at 17.70 and 58.30 for ethanol of crystallization.

Comparisons with ref. 37 show that in general pyridinium formation has little effect on chemical shifts of the aminoglycosides. The presence of signals associated with a pyridinium ring [29] is the only indication that such a ring has been formed.

Tables 2—4 list ^{13}C n.m.r. spectral data for kanamycins A and B and neomycin B, and their pyridinium derivatives. The assignments were made by comparison with literature values for similar pyridinium salts [28,29] (the pyridinium part) and with the literature values for kanamycins A (1) and B (2) [38] (for the aminoglycoside segment). In the case of neomycin B derivatives [(7d),(8d),(10d)], the resonances due to the aminoglycoside rings have been tentatively assigned after structural comparison with kanamycin B (2). No literature ^{13}C data for neomycin B (3) are available.

Signals due to aminoglycoside carbon atoms of the novel kanamycin pyridinium derivatives [(7b),(7c),(8b),(8c),(8f),-(10b),(10c)] show marked similarities, and comparison of chemical shifts with literature values for the parent aminoglycosides [38] shows that pyridinium salt formation leads to a downfield shift of 12—15 p.p.m. for C-6′ (the carbon bearing the pyridinium ring), while leaving the other signals virtually unchanged. This is a useful test for pyridinium formation. Of the remaining aminoglycoside carbon atoms, the signals due to the anomeric C-1′ and C-1″ occur at lowest field (*ca.* 100 p.p.m.) and are readily identified. The signals due to the pyridinium ring carbon atoms and the aryl substituents occur at δ >100 and the chemical shifts are in good agreement with those reported for similar pyridinium derivatives with simple amines.[28,29] Signals due to the pyridinium ring and methyl or aryl substituents in neomycin B derivatives [(7d),(8d),(10d)] are found at values virtually identical with those observed for the kanamycin analogues. Neomycin B pyridinium salts show one unchanged CH_2NH_2 signal at δ 41.0—42.0; the second (next to the pyridinium ring) is found at δ *ca.* 54—56. The downfield shift of 12—14 p.p.m. is similar to the downfield shifts in the kanamycin series. We have arbitrarily assigned the unchanged CH_2NH_2 signal to C-6″ and the modified one to C-6′, but further work is needed on this point.

Experimental

^1H N.m.r. spectra were recorded with a Varian EM 360 spectrometer and a JEOL FX-100 instrument was used for ^{13}C n.m.r. spectra. Elemental analyses were carried out by Atlantic Microlab Inc., Atlanta, Georgia. M.p.s were determined with a Bristolscope microscopic hot stage.

Reaction of 2,4,6-Trimethylpyrylium Perchlorate with Kanamycin A.—To kanamycin A sulphate (1) (0.72 g, 1.2

mmol) dissolved in water (10 ml) was added 2,4,6-trimethylpyrylium perchlorate (7a) (0.33 g, 1.5 mmol). After stirring at 25 °C for 9 days, H_2O was removed (60 °C and 1 mmHg) and the resulting syrup triturated with absolute EtOH (10 ml) to yield the *pyridinium derivative* (7b) as cream granules (0.88 g, 88%), m.p. >330 °C (decomp. begins above 240 °C) (Found: C, 40.1; H, 6.3; N, 6.7. $C_{26}H_{46}N_4O_{11}\cdot ClO_4\cdot HSO_4\cdot C_2H_5OH$ requires C, 40.4; H, 6.4; N, 6.7%).

Reaction of 2,4,6-*Trimethylpyrylium Perchlorate with Kanamycin B*.—To kanamycin B sulphate (2) (0.72 g, 1.2 mmol) dissolved in water (10 ml), 2,4,6-trimethylpyrylium perchlorate (7a) (0.33 g, 1.5 mmol) was added. After stirring at 25 °C for 95 h, H_2O was removed (60 °C and 1—5 mmHg), and the resulting syrup triturated with absolute EtOH (10 ml) to yield the *pyridinium derivative* (7c) (0.95 g, 85%) as a yellow powder, m.p. > 300 °C (decomp. begins above 240 °C) (Found: C, 35.8; H, 6.0; N, 7.3. $C_{26}H_{48}N_5O_{10}\cdot ClO_4\cdot 2HSO_4\cdot C_2H_5OH$ requires C, 36.2; H, 6.1; N, 7.5%).

Reaction of 2,4,6-*Trimethylpyrylium Perchlorate with Neomycin*.—To neomycin sulphate (3) (1.2 g, 1.7 mmol) dissolved in water (20 ml) was added 2,4,6-trimethylpyrylium perchlorate (7a) (0.44 g, 2.0 mmol). After stirring at 60 °C for 20 h, the volume of water was reduced to 5 ml (60 °C and 1 mmHg), and absolute EtOH (10 ml) was added to yield the *pyridinium derivative* (7d) (1.41 g, 76%) as cream granules, m.p. > 300 °C (decomp. begins above 240 °C) (Found: C, 36.4; H, 6.1; N, 7.4. $C_{31}H_{57}N_6O_{13}\cdot ClO_4\cdot 2HSO_4\cdot C_2H_5OH\cdot 2H_2O$ requires C, 36.1; H, 6.3; N, 7.7%).

Reaction of 4-(4-*Methoxy*-3-*sulphophenyl*)-2,4-*bis*-(4-*sulphophenyl*)*pyrylium Perchlorate* (8a) *with Kanamycin A* (1).—The pyrylium perchlorate (8a) (0.58 g, 1.0 mmol) in water (80 ml) was mixed with kanamycin A sulphate (0.58 g, 1.0 mmol) in water (20 ml). After stirring at 25 °C for 3 days, water was evaporated off (at 60 °C and 10 mmHg; to 5 ml). The *pyridinium derivative* (8b) was preciptated by addition of acetone (100 ml) as a pale yellow powder (1.0 g, 86%), m.p. >320 °C (decomp.) (Found: C, 41.5; H, 5.3; N, 4.6; S, 10.5. $C_{42}H_{52}N_4O_{21}S_3\cdot HSO_4\cdot 4H_2O$ requires C, 41.5; H, 5.0; N, 4.6; S, 10.5%).

Reaction of the Pyrylium Perchlorate (8a) *with Kanamycin B* (2).—To kanamycin B sulphate (0.58 g, 1.0 mmol) and sodium hydrogen carbonate (0.17 g, 2 mmol) in water (10 ml) was added the pyrylium perchlorate (0.58 g, 1.0 mmol) dissolved in water (100 ml). After stirring at 25 °C for 4 days, the mixture was acidified with $HClO_4$ (to pH 2—3) and was concentrated (60 °C and 10 mmHg) to 10 ml. The *pyridinium derivative* (8c) was precipitated by acetone (150 ml) and purified by dissolving in water and precipitating with methanol, to yield an off-white powder (0.86 g, 74%), m.p. >320 °C (decomp.) (Found: C, 37.7; H, 5.0; N, 5.2; S, 9.5. $C_{42}H_{55}N_5O_{20}S_3\cdot ClO_4\cdot HSO_4\cdot 5H_2O$ requires C, 37.9; H, 5.0; N, 5.3; S, 9.6%).

Reaction of the Pyrylium Perchlorate (8a) *with Neomycin B* (3).—The pyrylium perchlorate (0.58 g, 1.0 mmol) dissolved in water (100 ml) was mixed with neomycin B sulphate (0.69 g, 1.0 mmol) in water (10 ml), and sodium hydrogen carbonate (0.17 g, 2 mmol) was added to adjust to pH 7. After stirring at 25 °C for 4 days, the mixture was acidified with $HClO_4$ (to pH 2—3) and concentrated (at 60 °C and 10 mmHg; to 10 ml). The *pyridinium derivative* (8d) was precipitated by addition of acetone (130 ml) and purified by dissolving in water and precipitating with acetone, to yield a pale yellow powder (1.04 g, 77%), m.p. > 320 °C (decomp.) (Found: C, 40.1; H, 5.2; S, 9.4. $C_{47}H_{63}N_6O_{23}S_3\cdot ClO_4\cdot HSO_4\cdot 2H_2O$ requires C, 40.1; H, 4.8; S, 9.1%).

Reaction of 2,4-*Bis*-(4-*carboxyphenyl*)-4-(4-*methoxy*-3-*sulphophenyl*)*pyrylium Perchlorate* (8e) *with Kanamycin A* (1).—To kanamycin A sulphate (0.58 g, 1.0 mmol) and sodium hydrogen carbonate (0.376 g, 4.0 mmol) dissolved in water (30 ml) was added the pyrylium salt (8e) (0.61 g, 1.0 mmol) (pH *ca.* 7.0). After stirring at 25 °C for 4 days and at 60 °C for 8 h, the mixture was acidified with $HClO_4$ (to pH *ca.* 2—3) and concentrated (at 60 °C and 10 mmHg) to 10 ml. The *pyridinium derivative* (8f) was precipitated by addition of acetone (75 ml) and purified by dissolving and reprecipitating with water and acetone, to yield an off-white powder (0.69 g, 59%), m.p. >320 °C (decomp.) (Found: C, 40.9; H, 4.8; S, 5.3. $C_{47}H_{52}N_4O_{23}S\cdot ClO_4\cdot HSO_4\cdot 3H_2O$ requires C, 41.0; H, 4.6; S, 5.0%).

Disodium Salt of 2-(4-*Methoxy*-3-*sulphophenyl*)*vinyl* 4-*Sulphophenyl Ketone* (11).—Sodium 5-formyl-2-methoxybenzenesulphonate (7.14 g, 30 mmol) and sodium 4-acetylbenzenesulphonate (6.66 g, 30 mmol) were dissolved in methanol (100 ml) and water (25 ml) at 50—60 °C. Sodium hydroxide (1.2 g, 30 mmol) in water (5 ml) and methanol (20 ml) was added at 20—30 °C. The mixture was filtered immediately and a small precipitate discarded. The filtrate was stirred at 25 °C for 24 h; a yellow *product* separated which crystallized from methanol (120 ml) (Found: C, 41.6; H, 3.1. $C_{16}H_{12}Na_2O_8S_2\cdot H_2O$ requires C, 41.7; H, 3.0%); $\nu_{max.}$ ($CHBr_3$) 1 655 (C=O), 1 590, 1 560, 1 490, 1 440, 1 400, 1 180—1 200 (SO_3^-), 1 088, 1 030, 1 002, and 808 cm^{-1}; δ [CF_3CO_2H, ref. sodium 3-trimethylsilylpropane-1-sulphonate (DSS)] 8.50 (1 H, d, *J* 2.0 Hz), 8.30 (4 H, s), 8.10 (1 H, d, *J* 9.0 Hz), 8.05 (1 H, d, *J* 15 Hz), 7.72 (1 H, d, *J* 15 Hz), 7.37 (1 H, d, *J* 9 Hz), and 4.15 (3 H, s).

2-(*Benzothiazol*-2-*yl*)-4-(4-*methoxy*-3-*sulphophenyl*)-6-(4-*sulphophenyl*)*pyrylium Perchlorate* (10a).—The disodium salt (11) (6.9 g, 15 mmol), 2-acetylbenzothiazole [39] (1.77 g, 10 mmol), and perchloric acid (22 g) were stirred at 90—100 °C for 1 h. Addition of acetone (220 ml) precipitated a pale brown product.This was purified by dissolving in water (2 ml) and precipitating with acetone (40 ml) to give prisms (4.24 g, 49%), m.p. > 300 °C (decomp.) (Found: C, 44.5; H, 3.6; N, 2.1. $C_{25}H_{18}ClNO_{12}S_3\cdot H_2O$ requires C, 44.5; H, 3.3; N, 2.1%); $\nu_{max.}$ (film) 1 625 (pyrylium), 1 580, 1 560, 1 505, 1 485, 1 420, 1 385, 1 310, 1 245, 1 190, 1 120, 1 090, 1 030, 1 002, 950, 708, and 618 cm^{-1}; $\lambda_{max.}$ (H_2O) 408 (ε 29 600), 277 (16 300), and 242 nm (20 000); δ (D_2O–CF_3CO_2H, ref. DSS) 8.85 (1 H, d, *J* 2 Hz), 8.80 (2 H, s), 8.10—8.75 (9 H, m), 7.50 (1 H, d, *J* 9 Hz), and 4.20 (3 H, s).

Reaction of 2-(*Benzothiazol*-2-*yl*)-4-(4-*methoxy*-3-*sulphophenyl*)-6-(4-*sulphophenyl*)*pyrylium Perchlorate* (10a) *with Kanamycin A* (1).—The pyrylium salt (10a) (0.66 g, 1.0 mmol) was added to kanamycin A sulphate (1) (0.58 g, 1.0 mmol) and sodium hydrogen carbonate (0.25 g) in water (30 ml) (pH 7.45). After stirring at 25 °C for 84 h, the mixture was acidified with $HClO_4$ (to pH 2—3) and concentrated (at 60 °C and 10 mmHg; to 8 ml). The *pyridinium derivative* (10b) precipitated upon addition of acetone (150 ml). The product was purified by dissolving in water (4 ml) and precipitating with ethanol (4 ml), to yield a pale yellow powder (0.95 g, 77%), m.p. >300 °C (decomp.) (Found: C, 43.3; H, 5.4. $C_{43}H_{53}N_5O_{18}S_3\cdot SO_4H\cdot 0.5C_2H_5OH\cdot 4H_2O$ requires C, 43.5; H, 5.4%). (The ^1H and ^{13}C n.m.r. spectra showed the presence of 0.5 mol equiv. of ethanol of crystallization; see Tables 1 and 4.)

Reaction of the Pyrylium Perchlorate (10a) *with Kanamycin B* (2).—To kanamycin B sulphate (2) (0.58 g, 1.0 mmol), sodium hydrogen carbonate (0.25 g), and sodium carbonate (0.06 g), in water (30 ml) (pH 7.0), was added the pyrylium salt (10a) (0.796 g, 1.2 mmol). After stirring at 25 °C for 90 h, the mixture was acidified with $HClO_4$ (to pH 2—3) and concentrated (at 60 °C and 10 mmHg; to 6 ml). The *pyridinium derivative* (10c) was precipitated with acetone (150 ml) and purified by dissolving in water (5 ml) and precipitating with ethanol (5 ml) to yield a yellow powder (0.95 g, 77%), m.p. > 300 °C (decomp.) (Found: C, 40.9; H, 5.1. $C_{43}H_{52}N_6O_{17}S_3 \cdot SO_4H \cdot ClO_4 \cdot 0.5C_2H_5OH \cdot 3H_2O$ requires C, 40.8; H, 4.8%). (The ^1H and ^{13}C n.m.r. spectra showed the presence of a 0.5 mol equiv. of ethanol of crystallization; see Tables 1 and 4.)

Reaction of the Pyrylium Perchlorate (10a) *with Neomycin B* (3).—The foregoing procedure, with neomycin B sulphate (3) (0.685 g, 1.0 mmol) and the pyrylium salt (10a) (0.796 g, 1.2 mmol) gave the *pyridinium derivative* (10d), after purifying by treatment with water (4 ml) and methanol (5 ml), as a pale yellow powder (0.90 g, 67%), m.p. > 300 °C (decomp.) (Found: C, 41.6; H, 5.0. $C_{47}H_{63}N_7O_{20}S_3 \cdot SO_4H \cdot ClO_4 \cdot H_2O$ requires C, 41.6; H, 4.9%).

References

1 Part 5, A. R. Katritzky, J. L. Mokrosz, and M. L. Lopez-Rodriguez, preceding paper.
2 S. Umezawa, *Adv. Carbohydr. Chem. Biochem.*, 1974, **30**, 111.
3 H. Umezawa, *Adv. Carbohydr. Chem. Biochem.*, 1974, **30**, 183.
4 H. Umezawa, M. Okanishi, S. Kondo, K. Hamana, R. Utahara, K. Maeda, and S. Mitsuhashi, *Science*, 1967, **157**, 1559.
5 S. Umezawa, T. Tsuchiya, R. Muto, Y. Nishimura, and H. Umezawa, *J. Antibiot.*, 1971, **24**, 274.
6 S. Umezawa, Y. Nishimura, H. Hineno, K. Watanabe, S. Koike, T. Tsuchiya, and H. Umezawa, *Bull. Chem. Soc. Jpn.*, 1972, **45**, 2847.
7 I. Watanabe, T. Tsuchiya, S. Umezawa, and H. Umezawa, *J. Antibiot.*, 1973, **26**, 802.
8 R. J. Reid, S. A. Mizsak, L. M. Reineke, G. E. Zurenko, K. F. Stern, and J. Magerlein, *J. Med. Chem.*, 1981, **24**, 1487.
9 T. Yoneta, T. Matsumo, and S. Fukatsu, *Chem. Pharm. Bull.*, 1981, **29**, 3464.
10 H. Umezawa, T. Tsuchiya, R. Muto, and S. Umezawa, *Bull. Chem. Soc. Jpn.*, 1972, **45**, 2842.
11 H. Umezawa, M. Okanishi, R. Utahara, K. Maeda, and S. Kondo, *J. Antibiot.*, 1967, **A20**, 136.
12 H. Umezawa, Y. Nishimura, T. Tsuchiya, and S. Umezawa, *J. Antibiot.*, 1972, **25**, 743.
13 H. Kawaguchi, T. Naito, S. Nakagawa, and K. Fujisawa, *J. Antibiot.*, 1972, **25**, 695.
14 S. Kondo, K. Iinuma, H. Yamamoto, K. Maeda, and H. Umezawa, *J. Antibiot.*, 1973, **26**, 412.
15 D. A. Cox, K. Richardson, and B. C. Ross, *Topics Antibiot. Chem.*, 1977, **1**, 1.
16 J. J. Wright, *J. Chem. Soc., Chem. Commun.*, 1976, 206.
17 (*a*) K. Richardson, S. Jevons, J. W. Moore, B. C. Ross, and J. R. Wright, *J. Antibiot.*, 1977, **30**, 843; (*b*) K. Richardson, K. W. Brammer, S. Jevons, R. M. Plews, and J. R. Wright, *ibid.*, 1979, **32**, 973.
18 Y. Nishimura, H. Umezawa, and S. Umezawa, *Tetrahedron Lett.*, 1981, 77.
19 Y. Nishimura and S. Umezawa, *Tetrahedron Lett.*, 1982, 81.
20 D. H. R. Barton, G. Bringmann, G. Lamotte, W. B. Motherwell, R. S. Hay Motherwell, and A. E. A. Porter, *J. Chem. Soc., Perkin Trans. 1*, 1980, 2657.
21 T. Tsuchiya, Y. Takagi, and S. Umezawa, *Tetrahedron Lett.*, 1979, 4951.
22 M. B. Thomas and M. T. Williams, *Tetrahedron Lett.*, 1980, 4981.
23 A. R. Katritzky, *Tetrahedron*, 1980, **36**, 679.
24 A. R. Katritzky and S. S. Thind, *J. Chem. Soc., Perkin Trans. 1*, 1980, 1895.
25 A. R. Katritzky, J. M. Lloyd, R. H. Manzo, and R. C. Patel, *Angew. Chem., Int. Ed. Engl.*, 1980, **19**, 306.
26 A. R. Katritzky, G. de Ville, and R. C. Patel, *Tetrahedon Lett.*, 1980, 1723.
27 A. R. Katritzky, G. de Ville, and R. C. Patel, *Tetrahedron*, 1980, **37**, Suppl. 1, p. 25.
28 Part 1, A. R. Katritzky, M. De Rosa, and N. Grzeskowiak, *J. Chem. Soc., Perkin Trans. 2*, 1984, 841.
29 Part 3, A. R. Katritzky, Y.-K. Yang, B. Gabrielsen, and J. Marquet, *J. Chem. Soc. Perkin Trans. 2*, 1984, 857.
30 Part 2, A. R. Katritzky, J. L. Mokrosz, and M. De Rosa, *J. Chem. Soc., Perkin Trans. 2*, 1984, 849.
31 A. T. Balaban and C. D. Nenitzescu, *Liebigs Ann. Chem.*, 1959, **625**, 74.
32 A. N. Narkevich, G. N. Dorofeenko, and Y. A. Zhdanov, *Dokl. Akad. Nauk. S.S.S.R.*, 1967, **176**, 103 (*Chem. Abstr.*, 1968, **68**, 78605).
33 C. Toma and A. T. Balaban, *Tetrahedron*, Suppl., 1966, **7**, 27.
34 Y. A. Zhdanov, G. N. Dorofeenko, and A. N. Narkevich, *Zh. Obshch. Khim.*, 1963, **33**, 2418 (*Chem. Abstr.*, 1963, **59**, 14105).
35 M. H. O'Leary and G. A. Samberg, *J. Am. Chem. Soc.*, 1971, **93**, 3530.
36 A. R. Katritzky and J. L. Mokrosz, unpublished work.
37 R. U. Lemieux, T. L. Nagabhushan, K. J. Clemetson, and L. C. N. Tucker, *Can. J. Chem.*, 1973, **51**, 53.
38 G. Kotowycz and R. U. Lemieux, *Chem. Rev.*, 1973, **73**, 669.
39 T. Caronna, G. P. Gardini, and F. Minisci, *Chem. Commun.*, 1969, 201.

Received 29*th April* 1983; *Paper* 3/681