Communications to the editor

SEMISYNTHETIC AMINOGLYCOSIDE ANTIBIOTICS III. NEW DEOXY DERIVATIVES OF PAROMOMYCIN[1]

Sir:

Some problems of bacterial resistance to the aminoglycoside antibiotics have been solved by removing the sites of enzymatic modification.[2] In the case of paromomycin, inactivation is mainly caused by phosphorylation[3] of the hydroxyl groups at position 3′ and 5″. A suggested nucleotidylation at position 4′ of paromomycin and related antibiotics has also been reported.[4] Since it has also suggested[5] that removal of the 4′-hydroxyl group in kanamycin A can prevent the phosphorylation at the 3′ position, we decided to remove the 4′ hydroxyl group. We have also replaced the 5″ hydroxyl group by an amino group. Furthermore, we also succeeded in the dideoxygenation at the 4′, 4‴ positions. The present communication describes the synthesis of 4′-deoxy-4′-epichloroparomomycin (**9a**), 4′-deoxy-paromomycin (**10**), 4′, 4‴-dideoxyparomomycin (**11**), 5″-amino-4′, 5″-dideoxy-4′-epichloroparomomycin (**9b**) and 5″-amino-4′, 5″-dideoxyparomomycin (**12**).*

4′,6′-O-Benzylidene-penta-N-benzyloxycarbonylparomomycin[6] was O-acetylated and then hydrolysed (80% acetic acid, room temperature, 4 days) to give in 93% overall yield compound **1a**: m.p. 116~119°C, $[\alpha]_D^{20°}$+20° (*c* 0.54, $CHCl_3$). Selective benzoylation of the primary hydroxyl group with N-benzoylimidazole at reflux temperature in chloroform afforded the corresponding 6′-O-benzoyl derivative (**2a**): m.p. 115~120°C, $[\alpha]_D^{20°}$+26° (*c* 1.1, $CHCl_3$) in 55% yield. The valuable intermediate **2a**, possessing solely one free hydroxyl group at C-4′, was transformed into the chloro-deoxy derivative **3a**: m.p. 110~115°C, $[\alpha]_D^{20°}$+30° (*c* 1, $CHCl_3$) in 51% yield by treatment with sulfuryl chloride. Conventional O-deacylation and removal of the N-protecting groups by catalytic hydrogenation (cyclohexene, 10% Pd-C in 80% aqueous ethanol, 10 minutes, reflux) afforded 4′-deoxy-4′-epichloroparomomycin (**9a**): m.p. 160~165°C (dec.), Rf 0.39* (63% yield from **3a**). Moreover the intermediate **3a** was reduced with tributyltin hydride (refluxing toluene under nitrogen, 2 hours) affording in 90% yield the 4′-deoxyderivative (**4**): m.p. 125~130°C $[\alpha]_D^{20°}$+33° (*c* 1.2, $CHCl_3$). Usual deprotection sequence gave **10**, in 30% yield from **4**, after column chromatography on Amberlite CG 50 eluting with an aqueous ammonia gradient. The free base was converted into the sulphate form: m.p. ~230°C (dec.), $[\alpha]_D^{20°}$+58° (*c* 1, H_2O), Rf 0.3.*

The intermediate **4** was also obtained in lower yeilds from **2a** *via* the 4′-S-phenyldithiocarbonate (**5**) or the 4′-S-methyldithiocarbonate (**6**) following the procedures described by HAYASHI *et al.*[7] On the other hand treatment of **2a** with a large excess of carbon disulphide and sodium hydroxide in DMSO with further addition of methyl iodide afforded unexpectedly in 37% yield a bis-S-methyldithiocarbonyl derivatives (**7**): m.p. 110~115°C, $[\alpha]_D^{20°}$+43° (*c* 1, $CHCl_3$). Following the BARTON's procedure[8], the intermediate **7** was reduced with tributyltin hydride to give **8** (95% yield): m.p. 110~115°C, $[\alpha]_D^{20°}$+30° (*c* 0.9, $CHCl_3$). The usual deprotecting sequence followed by Amberlite CG 50 column chromatographic purification gave a dideoxyparomomycin: m.p. ~250°C (dec.), $[\alpha]_D^{20°}$+51° (*c* 1.17, H_2O), Rf 0.43** (30% yield from **8**).

The new compound (**11**) was firmly characterized as 4′-4‴-dideoxyparomomycin by ^{13}C-NMR spectroscopy and FD mass spectrometry.*** To rationalize the formation of the di-O-xanthate **7** under our experimental conditions, we tentatively suggest an hydrolysis of the 4‴-O-acetyl group of **2a** intramolecularly assisted by the intermediate 4′-xanthate anion leading to a free hydroxyl group at the 4‴-position and an S-acetyl-dithiocarbonyl group

* All new compounds gave correct microanalyses and exhibited ^1H, ^{13}C NMR and mass spectral characteristics that are in agreement with their structures.

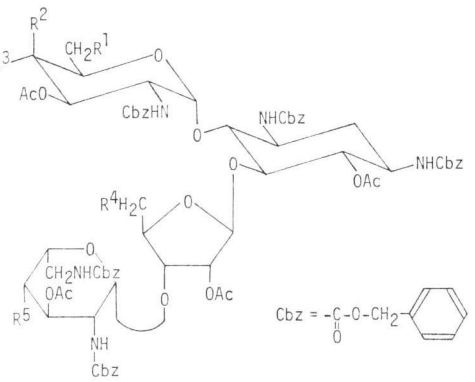
* Silica gel TLC, $CHCl_3$-methanol-18% aqueous ammonia (1:4:3, v/v/v)

** Silica gel TLC, $CHCl_3$-MeOH-32% aqueous ammonia (1:3:2, v/v/v)

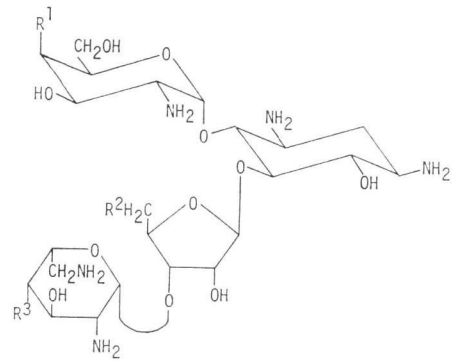
*** Detailed spectroscopic studies will be published.

($R-O-\overset{\overset{S}{\|}}{C}-S-COCH_3$) at the 4′ position. Alkaline hydrolysis of the S-acetyl moiety and O-xanthation at the 4‴-position afforded a dixanthate anion, converted to **7** by methyl iodide.

In order to synthesize the 5″-amino-5″-deoxy analogs, 5″-azido-5″-deoxy-4′-6′-O-benzylidene-penta-N-benzyloxy-carbonyl paromomycin, prepared as described by HANESSIAN *et al.*[9], was utilized as starting material. Following the same reaction sequence used to obtain **9a**, *via* the intermediates **1b**, **2b** and **3b** and the final deprotection reactions, 5″-amino-4′-5″-dideoxy-4′-epichloroparomomycin (**9b**) was obtained as the sulphate: m.p. 240°C (dec.), $[\alpha]_D^{20°}$ +67° (*c* 1, H_2O), Rf 0.35.* Compound **9b** was again N-benzyloxycarbonylated, then reduced with tributyltin hydride (toluene-ethyl acetate 10: 3, reflux, 50 minutes) and finally deprotected to give 5″-amino-4′-5″-dideoxyparomomycin



1a: $R^1=R^3=OH$, $R^2=H$, $R^4=R^5=OAc$
1b: $R^1=R^3=OH$, $R^2=H$, $R^4=N_3$, $R^5=OAc$
2a: $R^1=OBz$, $R^2=H$, $R^3=OH$, $R^4=R^5=OAc$
2b: $R^1=OBz$, $R^2=H$, $R^3=OH$, $R^4=N_3$, $R^5=OAc$
3a: $R^1=OBz$, $R^2=Cl$, $R^3=H$, $R^4=R^5=OAc$
3b: $R^1=OBz$, $R^2=Cl$, $R^3=H$, $R^4=N_3$, $R^5=OAc$
4 : $R^1=OBz$, $R^2=R^3=H$, $R^4=R^5=OAc$
5 : $R^1=OBz$, $R^2=H$, $R^3=OCSSPh$, $R^4=R^5=OAc$
6 : $R^1=OBz$, $R^2=H$, $R^3=OCSSCH_3$, $R^4=R^5=OAc$
7 : $R^1=OBz$, $R^2=H$, $R^3=R^5=OCSSCH_3$, $R^4=OAc$
8 : $R^1=OBz$, $R^2=R^3=R^5=H$, $R^4=OAc$



9a: $R^1=Cl$, $R^2=R^3=OH$
9b: $R^1=Cl$, $R^2=NH_2$, $R^3=OH$
10: $R^1=H$, $R^2=R^3=OH$
11: $R^1=R^3=H$, $R^2=OH$
12: $R^1=H$, $R^2=NH_2$, $R^3=OH$

Table 1. Minimum inhibitory concentration (μg/ml) of paromomycin and its deoxy derivatives as sulphates.*

Test Organism	Inactivating enzyme	9a	10	11	9b	12	Paromomycin
Staph. aureus FDA 209P		6.25	1.56	1.56	25	6.25	1.56
Staph. epidermidis FK 109	AAD (4′)	12.5	3.12	3.12	50	6.25	>50
E. coli K 12		50	12.5	6.25	100	25	6.25
E. coli K 12 (R 112)	APH (3′) I	>200	>200	>200	200	>200	>200
E. coli K 12 (R 148)	APH (3′) II	200	50	100	>200	200	>200

* The MICs were determined *in vitro* with the twofold dilution technique in Antibiotic Medium No. 3 (Difco). The inoculum was about 10^5 cells/ml and the cultures were incubated at 37°C for 24 hours.

* Silica gel TLC, $CHCl_3$ - MeOH - 32% aqueous ammonia (1: 3: 2, v/v/v)

(**12**): m.p. ~265°C (dec.), $[\alpha]_D^{20°}$ +60° (*c* 0.9, H_2O), Rf 0.32*, as the sulphate. This circuitous procedure was applied with gratifying success because an attempted preparation of **12** directly from the more feasible intermediate **3b** was unsuccessful. Probably the presence of the azido group interferred with the radical reduction of the chloroderivative with tributyltin hydride.

Among these novel semisynthetic deoxyparomomycin, both the 4′-deoxy-(**10**) and the 4′-4‴-dideoxyderivative (**11**) exhibited an *in vitro* antibacterial activity against a number of strains which was comparable to the parent antibiotic (Table 1). Compound **10** exhibits also a slightly improved activity against a strain of *E. coli* which is known to produce the inactivating enzyme APH (3′)II. In addition, as expected, they, along with the 4′-epichloro-4′-deoxyderivative (**9a**), were more active against *Staphylococcus epidermidis* FK 109, producer of aminoglycoside-4′-adenyltransferase [AAD(4′)], resistant to neomycins, paromomycins and lividomycins.[4] As reported for lividomycins,[10] the additional replacement of the C-5″ hydroxyl by an amino group led to derivatives (**9b**, **12**) displaying a generally weaker antibacterial activity but still active against *S. epidermidis* FK 109.

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