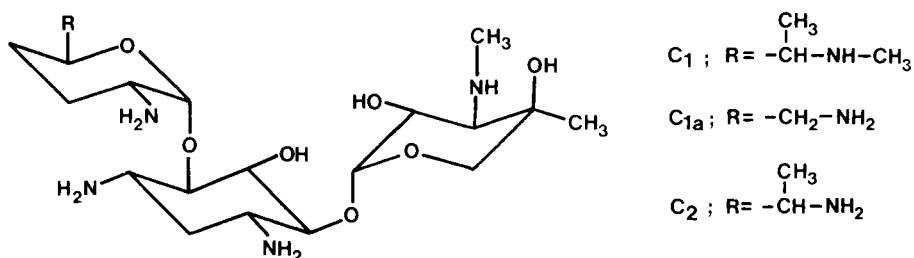


AN INVESTIGATION OF SOME MINOR COMPONENTS OF THE GENTAMICIN C COMPLEX

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The gentamicin C complex of broad spectrum antibiotics was first described by Weinstein and co-workers in 1963. The mixture consists of closely related water soluble basic aminocyclitols isolated from Micromonospora purpurea and M. echinospora.

Fig. 1



Chromatographic separation of the commercial complex shows it to consist of three major components C_1 , C_{1a} , and C_2 with the structures illustrated (fig. 1). The proportions of these and minor components of the complex may vary from batch to batch, but more particularly are dependant upon manufacturing site, (Kraisintu Parfitt and Rowan unpublished). In some batches up to eight minor components have been observed and in total they may constitute up to 20% of the mixture. Clearly, batch variation may have therapeutic implications and the minor components deserve chemical and microbiological investigation.

Daniels et al (1975) have designated two of the minor components as gentamicin C_{2a} and C_{2b} . The former is an isomer of gentamicin C_2 with an inverted asymmetric centre in the side chain. At least six minor components may be observed by thin layer chromatographic (tlc) examination of a commercial mixture by the method of Wilson et al (1973). However, unlike the high pressure liquid chromatographic (hplc) method of Kraisintu et al, (unpublished), this technique does not separate gentamicins C_{2a} and C_{2b} from C_2 .

Tlc proved to be useful analytically but when employed preparatively decomposition of some components was observed. Preparative column and preparative hplc methods were therefore explored. Up to 200mg of gentamicin C base was separated on a column of silica gel H by eluting with the lower phase of a chloroform, isopropanol, 17% ammonia mixture (2:1:1). The fractions collected were monitored by tlc. Seven minor components were obtained in a pure form. These had Rf values of 0.18, 0.31, 0.37, 0.65, 0.75, 0.80 and 0.85 on the tlc system of Wilson and others. Gentamicins C_{1a} , C_2 and C_1 had Rf values of 0.52, 0.61, and 0.69 respectively. This separation formed the basis of a more convenient preparative hplc method. Gentamicin C base (3.5g) was applied to a column of lichroprep Si60 (15-25 μ m) and eluted with identical mobile phase at a pressure of 7.2 bars. The minor components were isolated in sufficient quantities for examination by spectroscopic methods.

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