AN INVESTIGATION OF THE MICHAEL ADDITION OF MESNA TO CLAZAMYCINS A AND B

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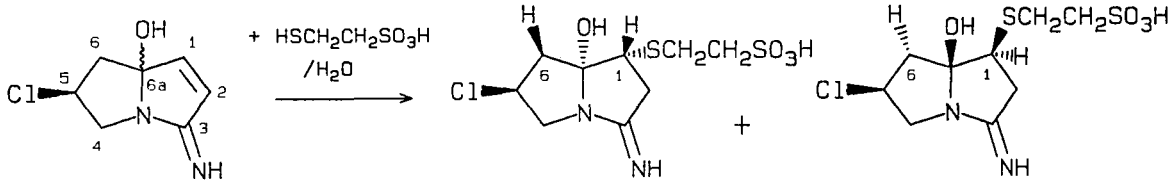
Clazamycin is a highly functionalised pyrrolizidine from Streptomyces species that possesses antitumour, antiviral and antibacterial activity. It exists as a mixture of two diasteriomers, clazamycin A (**6a=R**) and clazamycin B (**6a=S**). Clazamycin reacts with nucleophiles via a Michael-type addition to the C1-position of the conjugated amidine system (Thurston & Buechter 1987). This has been used to reduce the skin toxicity of clazamycin in a rabbit model through co-administration of 2-mercaptoethane sulphonate (mesna), a thiol-containing nucleophile (Green et al 1988).



Clazamycins A and B Adduct A Adduct B

The object of this investigation was to study the stereochemistry of nucleophilic attack of mesna at C1 of clazamycin by isolating and characterising the reaction products. A solution of clazamycin and an equimolar amount of mesna was allowed to stand at room temperature for several days, after which time thin layer chromatography (tlc) indicated a complete loss of clazamycin and the formation of two new compounds (Rf: 0.57 and 0.46; [ethanol (95%)/acetic acid (33%), 99:1; silica gel). The reaction mixture was lyophilised to give a crystalline residue. Flash chromatography afforded the faster running adduct (A) in a pure form, and preparative tlc was used to isolate the slower running adduct (B). The structure of each adduct was characterised by one- and two-dimensional ^{1}H- and ^{13}C-NMR including nOe experiments. Adduct A gave a significant nOe between the H1- and H6β-protons, while a similar nOe was observed between the H1- and H6α-protons of adduct B. Based on previous assignments (Buechter & Thurston 1987) of the 6α- and 6β-protons, the only possible configurations consistent with these results are **C1(R),C6a(R)** (adduct A) and **C1(S),C6a(S)** (adduct B). FAB mass spectrometry and IR data were also consistent with the assigned structures. The adducts were stable in aqueous solution at room temperature in contrast to clazamycins A and B which rapidly interconvert under the same conditions.

These results indicate that Michael addition occurs in a highly stereospecific manner, with the nucleophile approaching from the same face as the C6a-hydroxyl in both clazamycin A and B diasteriomers. This is unusual, as Michael addition usually leads to scrambling of stereochemistry at the site of nucleophilic attack. The most probable explanation for this phenomenon is nucleophilic attack at the least hindered exo-face of both clazamycins A and B. Adducts A and B were shown to have negligible anti-Pseudomonal activity or skin irritancy when compared to clazamycin. It is therefore likely that the formation of clazamycin-mesna adducts accounts for the reduced skin irritancy of combinations of clazamycin and mesna.

The Upjohn Company, Kalamazoo (USA) are thanked for providing clazamycins A and B.

Thurston, D.E., Buechter, D.D. (1987) J. Pharm. Pharmacol. 39: 111P
Green, K.L. et al (1988) Ibid. 40: 22P
Buechter, D.D., Thurston, D.E. (1987) J.Nat.Prod. 50:360-367