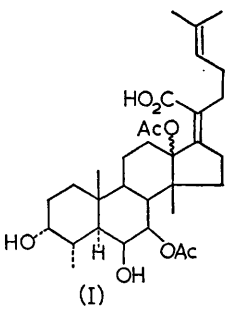
Cephalosporin P_1 and Helvolic Acid

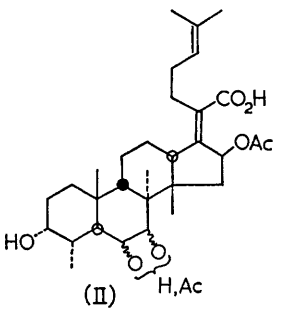
By P. OXLEY

(Research Department, Boots Pure Drug Co. Ltd., Nottingham)

CEPHALOSPORIN P_1 is a triterpenoid antibiotic for which the structure (I) has been suggested,[1] mainly on chemical grounds. However, mass spectrometry[2] indicates the presence of an additional angular methyl group, and the n.m.r. spectrum[3] shows a very close relationship with two other triterpenoid antibiotics, helvolic acid and fusidic acid. The structure of fusidic acid has finally been settled,[4] and on the reasonable assumption that it has the same carbon skeleton all the available evidence is consistent with the partial structure (II) for cephalosporin P_1.



(I)

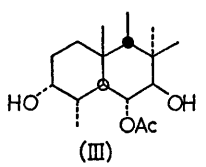


(II)

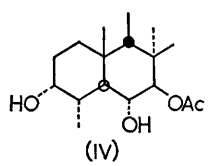
The remaining uncertainty concerns the position and configuration of the hydroxyl and acetoxyl groups in ring-B. In the fusidanes ring-B has the boat conformation, with C-5 and C-8 lying below the plane of C-6, C-7, C-9 and C-10 so that both 6α- and 7α-positions are equatorial, whereas 6β and 7β are axial and very strongly shielded by the angular methyl groups at C-10 and C-14.

The acetoxyl group in ring-B is known[5] to be very rapidly hydrolysed by cold dilute sodium hydroxide. Treatment of cephalosporin P_1 in the cold with either a weak base ($NaHCO_3$, Na_2CO_3, NH_4OH) or a strong acid (HCl) leads to the formation of an equilibrium mixture containing some 40% of cephalosporin P_1 and 60% of an isomer. The same equilibrium mixture is also formed from the isomer, and since both compounds are hydrolysed to deacetylcephalosporin P_1 by sodium hydroxide they must be the 6- and 7-acetates. Similarly easy migration and assisted hydrolysis of the acetyl group has been observed[6] for the monoacetates of several axial/equatorial pairs of steroidal 1,2-diols.

In the n.m.r. spectrum of cephalosporin P_1 methyl ester, the signal corresponding to the B-ring H–$\overset{|}{C}$–OAc proton is a doublet at τ 5·43 (J = 10 c./sec.), and the H–$\overset{|}{C}$–OH proton a singlet at τ 6·50. In the isomer, however, the H–$\overset{|}{C}$–OAc proton appears as a singlet at τ 5·37, the H–$\overset{|}{C}$–OH proton a doublet at τ 6·58 (J = 10 c./sec.) and there is a marked downfield shift of the 4-methyl signal. Clearly the conformation of ring-B is such that there is strong coupling between the protons attached to C-5 and C-6, but negligible coupling between those attached to C-6 and C-7. The spectra are consistent only with a 6α-acetoxy-7β-hydroxy-structure (III) for cephalosporin P_1, the isomer being the 7β-acetate (IV).



(III)



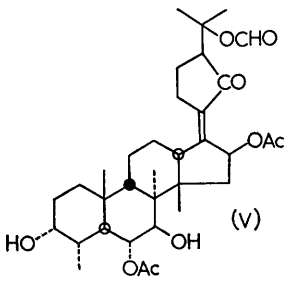
(IV)

We have made certain derivatives, notably the 3-ketones, in which the conformation of ring-B has changed enough to produce increased coupling between the protons attached to C-6 and C-7. In the n.m.r. spectra of these compounds the proton at C-6 appears as a quartet (J = 10 and 5 c./sec.) and that at C-7 as a doublet (J = 5 c./sec.).

Confirmation that the B-ring acetoxyl group is equatorial comes from some experiments on the acetylation of cephalosporin P_1. It has been reported[1] that cephalosporin P_1 is difficult to acetylate, but we find it reacts very rapidly with acetic anhydride to give a surprisingly stable mixed anhydride. 6-Deacetylcephalosporin P_1 similarly yields a mixed anhydride with acetic anhydride alone, and in presence of a basic catalyst it is then smoothly converted into the same mixed anhydride as that obtained from cephalosporin P_1 itself. Acetylation of the axial 3α-hydroxyl group is much slower, and the remaining B-ring hydroxyl group cannot be esterified under any conditions. From inspection of molecular models it seems reasonable that an equatorial 6α- (or 7α-) hydroxyl group

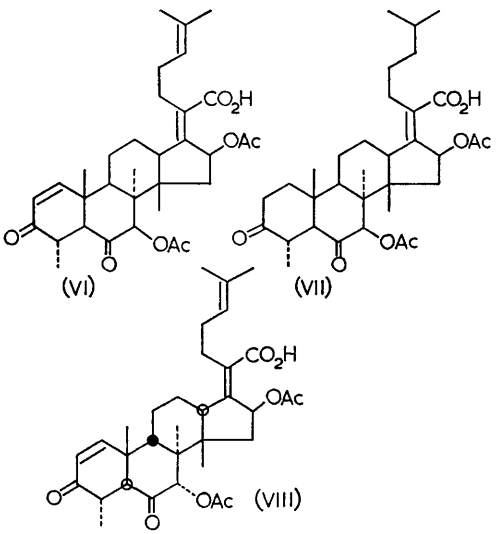
should be more reactive than the axial 3α, and the inertness of the hindered axial 7β (or 6β) is equally understandable.

The mixed cephalosporin P_1/acetic anhydride reacts with alcohols and amines to give exclusively free cephalosporin P_1 and the acetic esters or amides. In the presence of strong acids, a reaction occurs between the anhydride group and the terminal double bond. One such product, that obtained from the anhydride and formic acid, has been fully characterised. The infrared and n.m.r. spectra indicate that it has the structure (V).



(V)

Japanese workers[7] have advanced the structure (VI) for helvolic acid. It is known[8] that helvolic acid on treatment with cold aqueous sodium hydroxide gives the 7-deacetyl compound, which can easily be reacetylated to helvolic acid. For the steric reasons outlined above it seemed unlikely to us that a 7β-hydroxyl group would acetylate readily and the arguments which have been used to assign this configuration are invalidated by the boat conformation of ring-B. We therefore oxidized isocephalosporin P_1 (IV) with Jones' reagent and hydrogenated the terminal double bond to obtain the diketone (VII). This compound differed sharply from tetrahydrohelvolic acid[8] in both melting point and rotation, and on treatment with cold dilute sodium hydroxide it afforded not the 7β-hydroxy-6-ketone but the 6α-hydroxy-7-ketone. Presumably the interaction between an axial hydroxyl and the angular methyls at C-10 and C-14 favours formation of the equatorial ketol. We suggest therefore that if helvolic acid is a fusidane derivative it must be the 7α-acetate (VIII).



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