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THE CHEMISTRY OF CEPHALOSPORIN P_1

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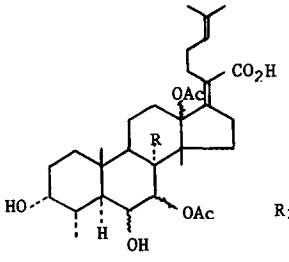
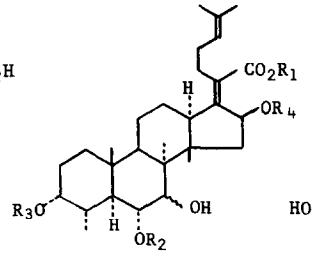
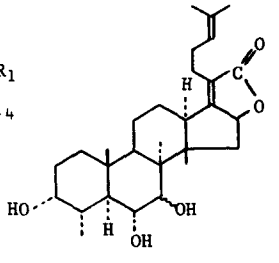
With the availability of large-scale fermentation broths of *Cephalosporium acremonium* cultures, an investigation of the constitutents from butyl acetate extract of filtered broth was begun several years ago. This extract contained steroid antibiotics originally reported as a series of steroid acids called cephalosporin P_1, $_2$, $_3$, $_4$ and $_5$.[2a] These acids possess considerable similarity and present a difficult separation problem. The limited quantities of pure cephalosporin P fractions in the past hampered the study of these compounds, except for cephalosporin P_1, which has been isolated as a pure crystalline fraction. This fraction has received the most attention, and initially structure Ia was proposed.[3]



Ia R = H
Ib R = CH_3



IIa $R_1 = R_3 = H$, $R_2 = R_4 = Ac$
IIb $R_1 = CH_3$, $R_2 = R_4 = Ac$ $R_3 = H$
IIc $R_1 = R_2 = R_3 = H$, $R_4 = Ac$
IId $R_1 = CH_3$, $R_2 = R_3 = H$ $R_4 = Ac$
IIe $R_1 = CH_3$, $R_2 = R_3 = R_4 = Ac$
IIf $R_1 = R_2 = R_3 = R_4 = H$



III

Structure Ia was modified to Ib after mass spectral[4] and nmr[5] studies showed an additional methyl group to be present, but no new chemical evidence has been reported for the more complete and most probable structure and stereochemistry as we now know it. We have been advised that the placement of acetoxy groups in Ib is being revised.[6a,b] We now present evidence supporting the structure and stereochemical assignments of IIa instead of Ib for cephalosporin P_1. Differences in these structures include placement of acetoxy groups at α-C_6 and β-C_{16} instead of positions C_7 and C_{13}. Additional evidence will be cited to support the presence of the extra methyl group at C_8.[4,5]

Employing a benzene solvate procedure,[7] we have now isolated a crystalline steroid acid, monodesacetylcephalosporin P_1 (IIc),[4] mp 197-198°, $[\alpha]_D$ + 37.6° (c = 0.5, CH_3OH), $\lambda_{max}^{C_2H_5OH}$ 220 mμ(ε = 7000), ν^{KBr} 3400, 2920, 1700, 1440, 1370, 1260 cm^{-1} in 18% yield from the solid concentrate of the butyl acetate extraction. Found: C, 69.46; H, 9.26. Cephalosporin P_1 (IIa) has been obtained by direct crystallization from the crude butyl acetate concentrate. The presence of IIc and IIa in the crude extract was shown by comparative gas chromatography of the esterified crude extract and the respective methyl esters IId and IIb using 3% SE-30 on Gaschrom Q at 315°. Further evidence for the presence of IIc in the crude extract was obtained by column chromatography on silica gel, esterification of the partially separated fractions with diazomethane and final separation by preparative thin-layer chromatography on silica gel PF, which produced crystalline IId, mp 225-228°. Its identity was established by comparison of its X-ray diffraction pattern with that of authentic IId. Moreover, there was no depression in melting point when these two samples of IId were mixed. We do not recommend this laborious procedure for isolation, but it did demonstrate that IIc is present in the crude mixture.

The presence of a carboxyl group in IIc was shown by conversion to the methyl ester IId, mp 232-233°, $[\alpha]_D$ + 29° (c = 0.5, $CHCl_3$), $\lambda_{max}^{C_2H_5OH}$ 220 mμ (ε = 8375), ν^{KBr} 3450, 2930, 1720, 1450, 1375, 1269 cm^{-1}. Found: C, 7054; H, 9.22. The mass spectrum of IId showed a parent ion peak at m/e = 546 ($C_{32}H_{50}O_7$). The nmr spectrum of the methyl ester (IId) shows the presence of the following pertinent proton absorptions in ppm from TMS: 1 tertiary methyl group, δ = 1.00(s); 1 secondary methyl group, δ = 1.00(d); 2 tertiary methyl groups, δ = 1.15(s); 1 isopropylidene group (2-methyls), δ = 1.60 and 1.68; 1 acetoxy group, δ = 1.92(s) at C_{16}; 3 hydroxyl groups at C_3, C_6, and C_7, δ = 3.40, 3.47, and 3.68 as a poorly

resolved multiplet; 1 carbomethoxy group, δ = 3.62(s); 1 vinylic proton, δ = 5.10(b) at C_{24}; and 1 secondary proton, δ = 5.83(d) at C_{16}.

Since the mass spectrum of the methyl ester IId showed the presence of an extra methyl group and the nmr spectrum of IId closely resembled that of fusidic acid which has a *trans-syn-trans* B-boat conformation, we placed this methyl group at the α-C_8 position.[8]

We are indebted to Dr. T. G. Halsall for a direct comparison of IId with his monodesacetylcephalosporin P_1 methyl ester.[4] It was found that an admixture did not have a depressed melting point and that identical Rf values were found during thin-layer chromatography. The infrared and nuclear magnetic resonance spectra were also found to be identical.

The dihydro derivative of IId melted at 262° in agreement with the previous report[2b] and showed $[\alpha]_D$ + 28° (c = 1.5, $CHCl_3$), $\lambda_{max}^{C_2H_5OH}$ 220 mμ (ε = 8375). Found: C, 69.71; H, 9.55.

Dihydromonodesacetylcephalosporin P_1, mp 201-202°, $\lambda_{max}^{C_2H_5OH}$ 222 mμ (ε = 8000) was prepared by hydrogenation of IIc in the presence of 2% Pd/C catalyst. Found: C, 69.59; H, 9.39. Treatment with diazomethane gave the dihydro derivative of IId. The reduction of the isopropylidene group was confirmed by the absence of acetone among products of ozonization of this derivative. This ester on prolonged hydrogenation was converted to a tetrahydro derivative, mp 170-171.5°. The nmr spectrum no longer showed a peak due to a vinylic proton. Found: C, 70.05; H, 9.92.

Pyrolysis of IIc at 240° afforded an α,β-unsaturated γ-lactone, III, mp 180-181°, $\lambda_{max}^{C_2H_5OH}$ 224.5 mμ (ε =13500) and ν^{KBr} 1740 cm^{-1}. Found: C, 73.59; H, 9.42. The nmr data showed that this lactone no longer contained an acetoxy group. The ORD curve of this unsaturated lactone showed $[\alpha]_{400}$ + 20°, $[\alpha]_{295}$ + 57°, $[\alpha]_{265}$ -27°, $[\alpha]_{261}$ -14° (c = 0.15, 1 cm, C_2H_5OH).[9] This curve is a mirror image of the ORD curve obtained for 3β-acetoxy-16α-hydroxy-$\Delta^{17(20)}$-bisnor-5α-cholenic 22,16-lactone, which showed $[\alpha]_{400}$ -45°, $[\alpha]_{287}$ -116°, $[\alpha]_{267}$ -67°, and $[\alpha]_{263}$ -76° (c = 0.165, 1 cm, C_2H_5OH).[9,10]

Acidification of the sodium salt of IIc afforded the lactone III, mp 180-181°. This lactone previously prepared by this method was reported to melt at 186°.[2b] The lactone III was also obtained by column chromatography of bisdesacetylcephalosporin P_1 (IIf) on a column of neutral alumina. The acid IIf, mp 125-126.5°, $\lambda_{max}^{C_2H_5OH}$ 226 mμ (ε = 7000), ν^{KBr} 3400, 2880, 1710, 1700 cm^{-1}, was obtained by hydrolysis of IIc in hot dilute alkali. The nmr spectrum of IIf showed complete absence of absorption due to acetoxy group. On the basis of these data and the nmr doublet for the secondary proton at

C_{16}, we assign a β-configuration to the oxygen bond at C_{16} of the α,β-unsaturated lactone III and to monodesacetylcephalosporin P_1 (IIc) at the corresponding position.

The presence of vicinal hydroxyl groups at C_6 and C_7 in IId was confirmed by acetonide formation, mp 158-159.5°, $\lambda_{max}^{C_2H_5OH}$ 220 mμ (ε = 5650), ν^{KBr} 3600, 1730, 1460, 1380, 1368, 1236, 1175 cm^{-1}. Found: C, 71.65; H, 9.25.

Additional evidence for the location of the three unsubstituted hydroxyl groups was gained from the triketones IVa, IVb and IVc obtained on chromic acid oxidation of IIc, IId, and the methyl ester of 23,24-dihydromonodesacetylcephalosporin P_1. These triketones IVa, IVb, and IVc were found to melt at 172-177°, 207-210°,[6c] and 205-208° respectively. Found for: IVa, $C_{31}H_{42}O_7.H_2O$: C, 68.08; H, 8.18. IVb, $C_{32}H_{44}O_7.H_2O$: C, 69.19; H, 8.38. IVc, $C_{32}H_{46}O_7.H_2O$: C, 68.08; H, 8.33.

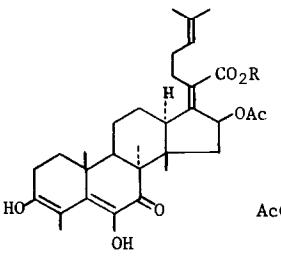
The ultraviolet spectrum of IVc showed no absorption between 260 and 340 mμ. However, the addition of acid produced $\lambda_{max}^{CH_3OH,\ H^+}$ 219 and 282 mμ (ε = 8290 and 4500). Similar absorption was also observed in alkaline solution. We consider this strong evidence for a conjugated triketo system involving carbonyl functions at C_3, C_6, and C_7. These data are similar to the results obtained by Okuda from the triketone obtained from helvolic acid.[11]

To establish whether the alkaline conditions of the benzene solvate procedure could have caused epimerization of monodesacetylcephalosporin P_1 (IIc) during isolation, lithium aluminum hydride reductions of IIb and IId were carried out. The reduction products from these esters (each ester gave two products) showed identical Rf values (0.35 and 0.61). The major products (Rf 0.61), mp 222-223°, from the two sources were proven to be identical by X-ray diffraction studies indicating that no epimerization had occurred. The infrared spectrum showed $\nu_{c=o}^{CHCl_3}$ 1770 cm^{-1}, indicative of a saturated γ-lactone. The mass spectrum showed peaks at m/e = 474 and 391 which represent the parent ion of $C_{29}H_{46}O_5$ and side-chain elimination with hydrogen transfer via an expected McLafferty rearrangement.[12] Found: C, 73.28; H, 9.93. The lack of absorption in the region of 225 mμ in the ultraviolet spectrum and the absence of Cotton effect of the optical rotatory dispersion curve in the vicinity of 266 mμ provided additional evidence that the unsaturated γ-lactone III had been reduced to a saturated γ-lactone during the LAH reduction. This lactone is readily formed from the material with Rf 0.35 on treatment with dilute hydrochloric acid.

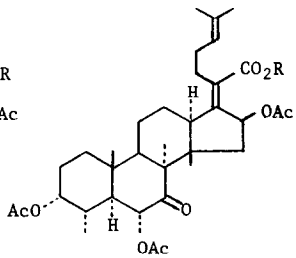
Accordingly, the material with Rf 0.35 is probably the corresponding hydroxy acid. The stereochemistry of this new saturated γ-lactone and the corresponding hydroxy acid is being studied. In a subsequent experiment, the unsaturated γ-lactone III was reduced with lithium aluminum hydride to the identical saturated γ-lactone (Rf 0.61).

Additional evidence for the arrangement of the acetate in ring B results from selective acetylation of IIb to give IIe [6βH, δ = 4.55 ppm ($J_{5,6}$ = 10 cps); 4αCH$_3$, δ = 0.90 (J = 6 cps); 3βH, δ = 3.70], which yields a single new amorphous acetate derivative, IIe. Found: C, 68.50; H, 8.84. The acetate IIe is acetylated at the 3α position, since an upfield shift of the 4-methyl protons [δ = 0.81(d)] is seen. Also, the downfield shift of the broad signal of the 3β-proton (δ = 4.90) is consistent with this attachment.

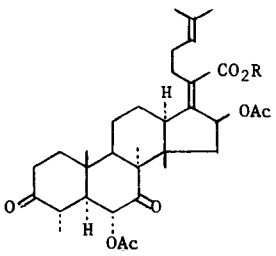
Chromic acid oxidation of the triacetate IIe provides the triacetoxy ketone V. Found: C, 68.27; H, 8.55. The assignment of ketone carbonyl at C_7 is consistent with the downfield shift of the 6βH-*trans* diaxial doublet signal (δ =5.30 ppm, $J_{5,6}$ = 13 cps). Furthermore, the spectrum of the diacetoxy diketone VI (Found for $C_{34}H_{48}O_8.H_2O$: C, 67.09; H, 8.10) obtained by chromic acid oxidation of the methyl ester IIb shows the 4α-CH$_3$ proton signal [δ = 1.28(d)] is also shifted downfield from its initial location. These data lend support to the placement of the acetoxy groups of cephalosporin P_1 (IIa) at the 6α and 16β positions. Additional evidence that the stereochemical assignment of the 6α acetoxy group for V and VI is correct was obtained from the ORD data of these compounds which showed negative Cotton effect ($[\alpha]_{317}$ = -1025° and $[\alpha]_{320}$ = -516°) with decreasing amplitude in the ORD curves obtained from V and VI.



IVa R = H
IVb R = CH_3
IVc R = CH_3, 24,25-dihydro



V



VI

Examination of Dreiding models of V and VI and application of the Octant Rule places the 6α-acetate in a negative octant relative to the carbonyl at C_7, and thus a substantial negative contribution can be expected. A positive contribution to the Cotton effect could be expected from a 3-keto group which appears in a positive octant and therefore changes the negative value from -1025° for IV to -516° for VI.

Thus, our new structure of monodesacetylcephalosporin P_1 (IIc) is consistent with the data presented, and cephalosporin P_1 (IIa) possesses the acetate at the 6α-position. The stereochemistry of the 7-hydroxyl group is being studied.

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10. We are grateful to Prof. Y. Mazur for providing this sample.

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