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THE STRUCTURE AND STEREOCHEMISTRY OF ERYTHROMYCIN A.

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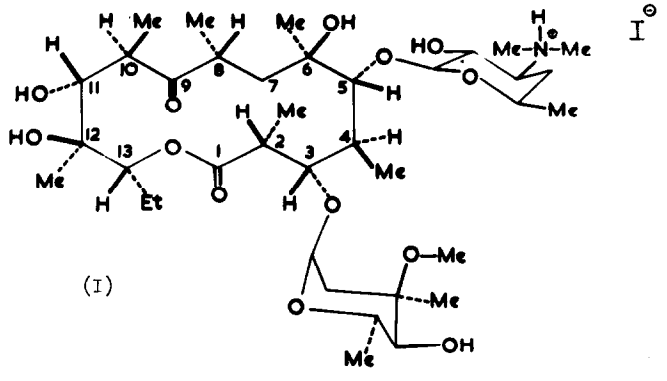
An X-ray crystallographic analysis of erythromycin A hydroiodide dihydrate has defined the structure and stereochemistry, both relative and absolute, of this therapeutically important macrolide[1] antibiotic as in (I). Assignment of configuration to each asymmetric centre of the lactone ring is given under the Cahn-Ingold-Prelog system[2a] and also under that due to Klyne in Table 1.



(I)

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TABLE 1

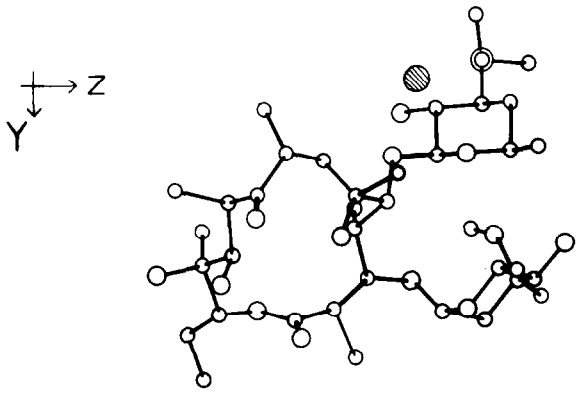
Carbon Atom	2	3	4	5	6	8	10	11	12	13
C.I.P.[2a] Nomenclature	R	S	S	R	R	R	R	R	S	R
Klyne[2b] Nomenclature	D-Me	L-OH	D-Me	L-OH	L-Me D-OH	L-Me	D-Me	L-OH	L-Me D-OH	L-Et

Crystal Data; Erythromycin A hydroiodide dihydrate, $C_{37}H_{68}O_{13}NI.2H_2O$; Mol. wt., 897.9 ;m.p. 193-195°C. Orthorhombic, a= 17.17, b= 18.36, c= 14.39 Å , U= 4536 Å^3, D_m= 1.34, Z= 4, D_c= 1.314, Space Group - $P2_12_12_1$.

Suitable crystals of this compound were grown from methanol-ethyl acetate-water in the form of prisms elongated in the c-axis direction. Preliminary crystallographic work on this derivative had been reported previously[3]. A Single-Crystal Orienter mounted on an XRD-3 diffraction Unit generating CuKα radiation(λ = 1.5418 Å) was used throughout for the collection of X-ray diffraction data. Since the intensities of the reflexions fall off rapidly with increasing sin θ , only data accessible within the sphere given by the spacing 1.2 Å were measured. Of the 1587 reflexions within this range, 1516 had measurable intensities. The position of the iodine atom was found from the three-dimensional Patterson function to be (0.102, 0.000, 0.196). We were unable to solve the structure using the conventional heavy-atom method[4] as the iodine atoms are so located that a mirror plane of symmetry was introduced into the first electron-density distribution. Attempts to destroy the pseudo-symmetry by selecting reasonable atomic positions not related by this symmetry met with failure. The structure was solved

by the systematic use of the anomalous dispersion effect of the iodine atoms. The small differences in the intensities of hkl and $\bar{h}\bar{k}\bar{l}$ reflexions were measured and used to give a set of phase angles[5]; these gave an electron-density distribution in which 24 atoms could be positioned correctly. The normal procedure of structure-factor and electron-density synthesis then led smoothly to the complete structure. This method of phase determination ensures that the structure obtained has the correct absolute configuration. The R-factor for all reflexions is 0.153 at this stage. Fig. 1 shows the a-axis projection of the structure.

FIG. 1



This study has confirmed in full the structure deduced earlier from chemical degradation by Gerzon et al[6]. It is in agreement with the structure and stereochemistry which have already been proposed for the sugar components cladinose[7] and desosamine[8], attached at C(3) and C(5) of the lactone ring respectively, on evidence from a combination of chemical degradation[6c,6a], N.M.R. spectroscopic measurements[7a,8a],

and stereospecific syntheses[7b,8b]. Evidence has been presented which was interpreted as indicating β-glycosidic linkages for both sugars[9]. Our results show that while true for desosamie it is not so for cladinose which has an α-linkage. The evidence in question was the observation from the N.M.R. spectrum of erythromycin in D_2O-acetone(1:1) solution of coupling constants of 2.0 and 9.5 cps. in a 1H quartet situated at lowest field. This signal was assigned to the C(1') proton of cladinose since it apparently was absent in desosaminyldihydroerythronolide. These constants were taken to indicate axial-equatorial and axial-axial spin interactions among the three protons and hence, on the assumption of the conformationally more stable of the chair forms for the pyranose ring, a β-glycosidic linkage (equatorial oxygen at C(1')). We have re-examined the N.M.R. spectrum of erythromycin in $CDCl_3$ solution at 100 Mc. and have similarly observed at low field three 1H signals, a quartet (J= 2.0 and 9.8 cps.) at τ 4.89, a slightly broadened doublet (J= 4.4 cps.) at τ 5.10, both coupled to protons in the methylene region, and a doublet (J=7.0 cps) at τ 5.57 coupled to a proton at ca. τ 6.7. These were previously assigned[9] to the C(1') proton of cladinose, an aglycone proton and the C(1') proton of desosamine respectively. From our results it would appear that the cladinose in erythromycin has the same conformation in both D_2O-acetone and $CDCl_3$ solutions. It is probable that this is the same as that observed in the crystal structure. It would therefore seem more reasonable to re-assign the doublet at τ 5.10 to the C(1') proton of cladinose and the quartet to the aglycone proton (possibly that on C(3)). In support of this are the reports that the C(1) proton of methyl α-mycaroside* [7a] appears as a triplet ($J_{1,2e} = J_{1,2a} = 2.4$ cps.)

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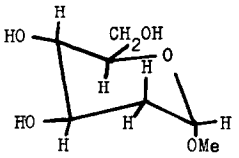
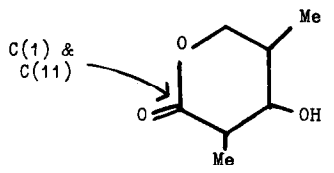
* Cladinose is the 3-O-methyl ether of mycarose.

at τ 5.23 in $CDCl_3$ and of methyl 2-deoxy-α-D-arabohexopyranoside (II) appears as a quartet ($J_{1,2e}$= 3.8 and $J_{1,2a}$= 1.4 cps.) at τ 5.05[10]. In the present instance the appearance of the C(1') proton of cladinose as a slightly broadened doublet would be consistent with the expected small deformation of the ring system by the 1,3-axial oxygen substituents which would result in an increase of the $H_{1,2e}$ and decrease of the $H_{1,2a}$ dihedral angles with opposite effects on the coupling constants.

In addition configurations have already been assigned to certain centres of dihydroerythronolide[6a], the product which results from borohydride reduction of the carbonyl function at C(9) in erythromycin followed by hydrolytic removal of both sugars. Gerzon and coworkers isolated C(1) to C(5) of this derivative as the lactone (III) which on



(II)

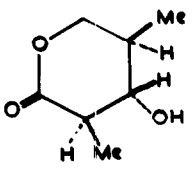


(III)

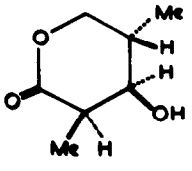
lithium aluminium hydride reduction afforded one of the optically inactive meso forms of 2,4-dimethylpentane-1,3,5-triol. This clearly established that C(2) and C(4) of dihydroerythronolide have opposite configurations. These authors also obtained a second stereoisomer of this lactone (III) which contained C(11) to C(7). Since this gave an optically active 2,4-dimethylpentane-1,3,5-triol on reduction it was apparent that C(10) and C(8) have identical configurations. On the basis of somewhat circumstantial evidence they tentatively suggested the complete stereochemistry of these lactones to be (IIIa) and (IIId), (or their enantiomers) respectively. Djerassi et al have isolated C(8)

as (+)-α-methyl levulinic acid which has been correlated with D-glyceraldehyde by way of (+)-α-methyl succinic acid thus establishing its configuration as R[11].

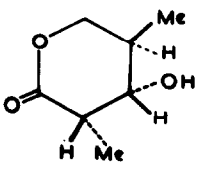
From the present analysis it is clear that the lactone which comprises C(1) to C(5) must have the stereochemistry (IIIa) and that the other is either (IIIc) or (IIId). We have no evidence on the



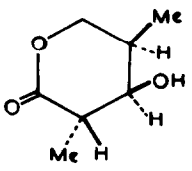
(IIIa)



(IIIb)



(IIIc)



(IIId)

configuration at C(9) in dihydroerythronolide but it is interesting to speculate that if the lactone ring of erythromycin retains in solution the conformation it exhibits in the crystal and borohydride reduction occurs from the less hindered side of the carbonyl then the new asymmetric centre created at C(9) would be S. This in turn would require stereochemistry (IIIc) for the derived lactone. Our findings confirm also the assignment of the R configuration for C(13) proposed by these authors[6d].

From a biosynthetic viewpoint it is interesting to note that the steric arrangement of substituents on the identically substituted fragments C(4) to C(6) and C(10) to C(12) is the same.

Acknowledgement.

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References.

1. R. B. Woodward, Festschrift Arthur Stoll, p. 524. Birkhauser, Basel (1957).
2. (a) R. S. Cahn, C. K. Ingold and V. Prelog, Experientia, 12, 81 (1956). (b) W. Klyne, Chem. and Ind., 1022 (1951).
3. H. A. Rose, Analyt. Chem., 25, 1571, (1953).
4. e.g. J. M. Robertson, Proc. Chem. Soc., 229 (1963).
5. G. N. Ramachandran and S. Raman, Current Sci. India, 25, 348 (1956).
6. (a) E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley and K. Gerzon, J. Amer. Chem. Soc., 76, 3121 (1954); (b) M. V. Sigal, Jr., P. F. Wiley, K. Gerzon, E. H. Flynn, U. C. Quarck and O. Weaver, ibid, 78, 388 (1956); (c) P. F. Wiley and O. Weaver, ibid, 78, 808 (1956); (d) K. Gerzon, E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley, R. Monahan and U. C. Quarck, ibid, 78, 6396 (1956); (e) P. F. Wiley, K. Gerzon, E. H. Flynn, M. V. Sigal, Jr., O. Weaver, U. C. Quarck, R. R. Chauvette and R. Monahan, ibid., 79, 6062 (1957).
7. (a) W. Hofheinz, H. Grisebach and (in part) H. Friebolin, Tetrahedron, 18, 1265 (1962). (b) D. M. Lemal, P. D. Pacht and R. B. Woodward, Tetrahedron, 18, 1275,(1962).
8. (a) P. W. K. Woo, H. W. Dion, L. Durham and H. S. Mosher, Tetrahedron Letters, 735 (1962); W. Hofheinz and H. Grisebach, ibid, 377 (1962). (b) A. C. Richardson, Proc.Chem. Soc., 131 (1963).
9. W. Hofheinz and H. Grisebach, Chem. Ber., 96, 2867 (1963).
10. R. U. Lemieux and S. Levine, Canad. J. Chem., 42, 1473 (1964).
11. C. Djerassi, O. Halpern, D. I. Wilkinson and E. J. Eisenbraun, Tetrahedron, 4, 369 (1958).