1-butanol–acetone–water–concentrated ammonium hydroxide (8:1:6:1)[12] revealed only threonine (R_f 0.16) and no allothreonine (R_f 0.10).

Registry No.—O-Benzyl-N-*t*-butyloxycarbonyl-L-threonine, 15260-10-3; O-benzyl-L-threonine benzyl ester hemioxalate, 15260-11-4; O-benzyl-L-threonine, 4378-10-3.

(12) S. W. Fox, *J. Am. Chem. Soc.*, **75**, 3421 (1953).

Glutarimide Antibiotics. XIII. Comment on the Stereochemistry of Streptovitacin-A and E-73

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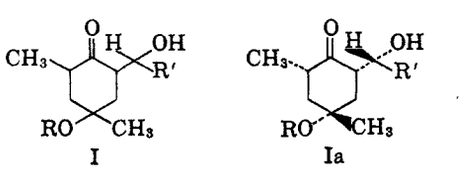
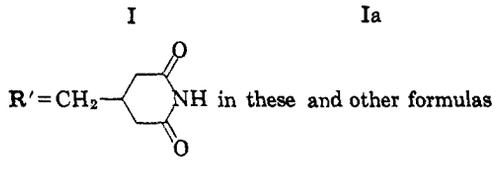
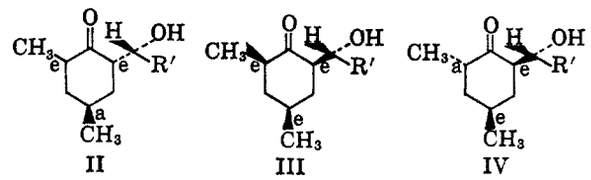
Although the gross structure of E-73[1] and streptovitacin-A,[2] its parent alcohol, have been shown to be I (R = Ac or H, respectively), little stereochemical work has been done on these molecules. Johnson, *et al.*,[3] on the basis of nmr evidence, have suggested that these ketones have the 2-methyl group equatorially oriented and have commented that in view of their biological activity, they are in all probability 4*e*-acetoxy (Ia, R = Ax) and 4*e*-hydroxycycloheximide, respectively (I, R = H). Additional evidence, reported below, now supports this view. The stereochemical problems involved here concern (a) the relative orientation of the substituents at the 2 and 6 positions of the cyclohexanone ring, (b) the orientations of the substituents at the 4 position of the same ring, (c) the relative position of the side-chain hydroxyl group, and (d) the question as to whether streptovitacin-A is related to the *l*- or to the *d*-cycloheximide series.



I Ia



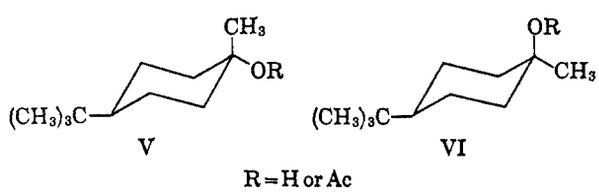
Both a and d can be solved by means of an ORD study. Dispersion curves (See Experimental Section) were obtained for streptovitamin-A and, for comparison purposes, for cycloheximide II and isocycloheximide III. The curve for I (R = H) shows a low intensity negative Cotton effect (λ_{max} 316.0, [α] −206°) and closely resembles that obtained for II (λ_{max} 308, [α] −360°) rather than that found for III which shows a low intensity positive Cotton effect (λ_{max} 320, [α] +150°). This should be contrasted with the ORD



II III IV

curve reported[4] for naramycin B (IV) where there is a very large positive contribution (λ_{max} 312.5, [α] +684°) to the Cotton effect by the 2*a*-methyl group. From these results we can only conclude that streptovitacin-A belongs to the same series as *l*-cycloheximide II, and in addition has both the 2-methyl and 6-hydroxyethyl glutarimide groups equatorially oriented.

The solution to problem b was only possible with the publication of a paper by Shoppee, *et. al.*[5] They found that in compounds of type V, which can be regarded as being essentially conformationally rigid, the nmr line widths at half-height (W_h) of the signals due to the C-1 axial tertiary methyl groups were in the range 1.0–1.3 cps whereas those due to C-1 equatorial tertiary methyl groups in the corresponding isomers (VI) were in the range 0.6–0.7 cps. The measurements were made at a resolution such that the tetramethylsilane signal had a W_h in the range 0.5–0.6 cps.



V VI

R = H or Ac

We have now measured the W_h of the absorptions of the 4-methyl groups of streptovitacin-A and E-73 where the cyclohexanone rings are also essentially conformationally rigid. In each case the value found (~2.0 cps) was twice the W_h value observed for the tetramethylsilane signal of comparable intensity. We conclude from this that the 4-methyl group in these compounds is axially oriented. It must be added that while it would have been desirable to compare the spectra of the 4*e*-methyl-4*a*-hydroxy isomers with those mentioned above, the latter compounds are not available. Thus the evidence is not completely conclusive.

Finally the question of the orientation of the side chain hydroxyl group was solved using the methods developed earlier.[6,7] Reduction of streptovitacin-A using hydrogen and a platinum catalyst afforded a dihydro derivative whose nmr spectrum in pyridine shows absorption[8] at 246 and 237 cps. The former position is characteristic of the side chain CHOH proton a fact testified to by the W_h of the peak (~35 cps). The latter position corresponds to that of a >CHOH proton in a rigid cyclohexanol, where the hydroxyl group is axially oriented. Again corroboration of this comes from the W_h of the peak which is 6.75 cps. These results should be compared with those obtained with the

(1) K. V. Rao and W. P. Cullen, *J. Am. Chem. Soc.*, **82**, 1127 (1960); K. V. Rao, *ibid.*, **82**, 1129 (1960).

(2) T. E. Eble, M. E. Bergy, C. L. Large, R. R. Herr, and W. G. Jackson, *Antibiot. Ann.*, 555 (1959); R. R. Herr, *ibid.*, 560 (1959); R. R. Herr, *J. Am. Chem. Soc.*, **81**, 2595 (1959).

(3) F. Johnson, W. D. Gurowitz, and N. A. Starkovsky, *Tetrahedron Letters*, 1173 (1962); *J. Am. Chem. Soc.*, **87**, 3492 (1965).

(4) T. Okuda, M. Suzuki, and Y. Egawa, *Chem. Pharm. Bull.* (Tokyo), **8**, 335 (1960); **9**, 1014 (1961). M. Suzuki, Y. Egawa, and T. Okuda, **11**, 582 (1963).

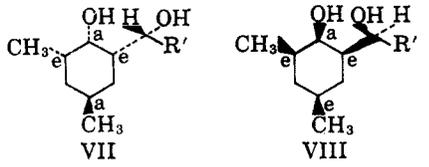
(5) C. W. Shoppee, F. P. Johnson, R. E. Lack, and S. Sternhell, *Chem. Commun.*, 347 (1965).

(6) F. Johnson, N. A. Starkovsky, and A. A. Carlson, *J. Am. Chem. Soc.*, **87**, 4612 (1965).

(7) F. Johnson, A. A. Carlson, and N. A. Starkovsky, *J. Org. Chem.*, **31**, 1327 (1966).

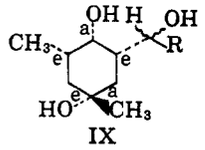
(8) Measured at 60 Mc downfield from TMS taken at 0 cps.

corresponding dihydrocycloheximide[6] VII [peaks at 246 (broad) and 233 cps (sharp)] and dihydro-α-epiisocycloheximide[7] VIII [peaks at 249 (broad) and 235



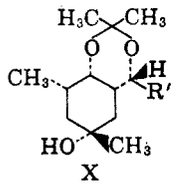
VII VIII

cps (sharp)]. Thus dihydrostreptovitacin A can be written as IX. The side chain hydroxyl orientation



IX

can now be determined by finding whether IX gives an acetonide or not, under mild conditions. Facile acetonide formation would indicate that the side chain hydroxyl group has the (*R*) configuration whereas no, or a very slow, reaction would point to the (*S*) configuration. Detailed arguments in support of this statement have been presented in earlier papers and will not be repeated here. It suffices to say that IX gave a noncrystalline product in almost quantitative yield, whose elemental analysis and spectral data left little doubt that it is the desired acetonide X. Its nmr



X

spectrum in deuteriochloroform for instance shows strong absorption at 83.0 and 84.5 cps characteristic of acetonide–methyl protons[7] whereas the characteristic broad and sharp peaks due to the CHO protons of the acetonide ring appear at 238 and 226 cps. These values again may be compared with those found for the acetonide of VIII[7] which are, respectively, 81.8, 83.9, 237 (broad), and 226 (sharp).

Attempts were also made to obtain the isomer of IX having an equatorial hydroxyl group at C-1 of the cyclohexane ring. If the stereochemistry assigned to X is correct, the isomeric triol should not easily form an acetonide. These efforts however were fruitless. Although this type of reduction could be accomplished successfully in the cycloheximide series using lithium tri-*t*-butoxyaluminum hydride, in the case of I only aluminum-containing complexes could be isolated. All attempts to remove the metal without destroying the molecule failed.

The new information then, while not unequivocal points to Ia as the complete structure for streptovitacin-A (R = H) and E-73 (R = Ac). Virtually the only glutarimide antibiotics whose stereochemistry is not known, are the isomeric streptovitacins-B, -C, and -D. Sufficient quantities of these have not been available for study as yet but it would not be surprising if they also had the same basic stereochemistry as cycloheximide.

Experimental Section

Nmr spectra were measured using a Varian A-60 instrument, and infrared spectra were obtained from a Baird Model 4-55 recording spectrophotometer. Melting points are corrected. ORD spectra were measured using a Bendix polarmatic recording spectropolarimeter Model 4600.

ORD determinations were (a) streptovitacin-A (chloroform, *c* 0.300) $[\alpha]_{400}$ −23°, $[\alpha]_{384.5}$ −32°, $[\alpha]_{370}$ −42°, $[\alpha]_{357}$ −55°, $[\alpha]_{345}$ −70°, $[\alpha]_{333}$ −95°, $[\alpha]_{322.5}$ −157°, $[\alpha]_{317.5}$ −200°, $[\alpha]_{361}$ −206°, $[\alpha]_{312.5}$ −202°, $[\alpha]_{303}$ −162°, $[\alpha]_{294}$ −107°, $[\alpha]_{289.5}$ 0°, $[\alpha]_{285.5}$ +88°, $[\alpha]_{278}$ +568°, $[\alpha]_{270}$ +674°, $[\alpha]_{263}$ +1015°; (b) cycloheximide (chloroform, *c* 0.300) $[\alpha]_{400}$ −20°, $[\alpha]_{384.5}$ −26°, $[\alpha]_{370}$ −37°, $[\alpha]_{357}$ −50°, $[\alpha]_{345}$ −75°, $[\alpha]_{333}$ −110°, $[\alpha]_{322.5}$ −168°, $[\alpha]_{312.5}$ −302°, $[\alpha]_{303}$ −250°, $[\alpha]_{294}$ −55°, $[\alpha]_{293}$ 0°, $[\alpha]_{285.5}$ +290°, $[\alpha]_{278}$ +750°, $[\alpha]_{263}$ +900°; (c) isocycloheximide (chloroform, *c* 0.304) $[\alpha]_{400}$ +53°, $[\alpha]_{384.5}$ +54°, $[\alpha]_{370}$ +56°, $[\alpha]_{357}$ +64°, $[\alpha]_{345}$ +73°, $[\alpha]_{333}$ +97°, $[\alpha]_{322.5}$ +140°, $[\alpha]_{312.5}$ +104°, $[\alpha]_{303}$ +72°, $[\alpha]_{294}$ +40°, $[\alpha]_{285.5}$ 0°, $[\alpha]_{278}$ −78°, $[\alpha]_{263}$ −134°.

Dihydrostreptovitacin-A (IX).—Steptovitacin-A (0.5 g) in acetic acid (25 ml) was stirred at room temperature in the presence of a platinum catalyst (from 0.2 g of PtO_2) under hydrogen at atmospheric pressure. After 2.5 hr, gas absorption (86 ml, 95% of theory) ceased. The catalyst was removed and the solution evaporated to dryness under reduced pressure. The resulting glassy residue was then crystallized from ethyl acetate–petroleum ether (bp 30–60°) to give IX as white needles (0.41 g, 82%), mp 183–184.5°. A sample recrystallized from the same solvent pair had mp 187–187.5°. Its infrared spectrum taken as a Nujol mull showed significant peaks at 2.80, 2.95, 3.01, 3.04, 5.76, and 5.83 μ.

Anal. Calcd for $C_{15}H_{25}NO_5$: C, 60.18; H, 8.42; N, 4.68. Found: C, 60.15; H, 8.38; N, 4.63.

Acetonide of Dihydrostreptovitacin-A (X).—A solution of dihydrostreptovitacin-A (0.2 g) in acetone (20 ml) was refluxed in the presence of anhydrous copper sulfate (2.0 g) for 12 hr. Removal of the copper sulfate and excess acetone in the usual way afforded a colorless glassy residue which refused to crystallize. A sample prepared for analysis became mobile at 75–77°. Its infrared spectrum (KBr disk) showed peaks at 2.92 (OH), 3.12, 3.25 (NH), 5.88 (imide C=O), 7.25, 7.90, 8.30, 8.56, 8.70, 9.26, 9.75, 10.05, 10.25, 10.90, and 11.65 μ.

Anal. Calcd for $C_{18}H_{29}NO_5$: C, 63.69; H, 8.61; N, 4.13. Found: C, 63.45; H, 8.61; N, 4.10.

Registry No.—Ia (R = H), 523-86-4; Ia (R = Ac), 2885-39-4; *l*-II, 4630-76-6; III, 15314-11-1; IX, 15303-44-3; X, 15303-45-4.

Acknowledgment.—The authors wish to thank Dr. C. Smith of the Upjohn Co., who provided a generous sample of streptovitacin-A, and Dr. K. V. Rao of the John L. Smith Memorial for Cancer Research, for a sample of E-73. Special thanks are due to Professor J. Wolinsky and Mrs. H. Hauer of Purdue University who measured the ORD spectra.

Mass Spectrometry of Ubiquinones. Thermal Loss of a Methoxyl Group[1]

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In the study of the chemistry of the ubiquinones, Q-*n* (I), and ubiquinols H_2Q-*n* (II), we have critically examined the mass spectra of numerous samples from both natural and synthetic sources. In the mass spectra of samples of Q-10 (I, *n* = 10) and Q-9 (I, *n* = 9) of

(1) Coenzyme Q. XCIV.