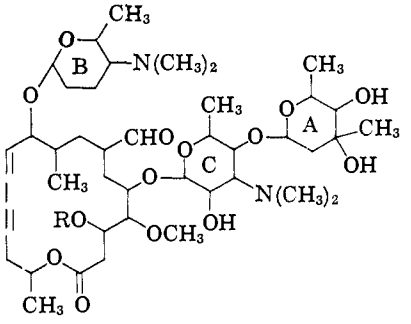
The Structures of the Spiramycins and Magnamycin

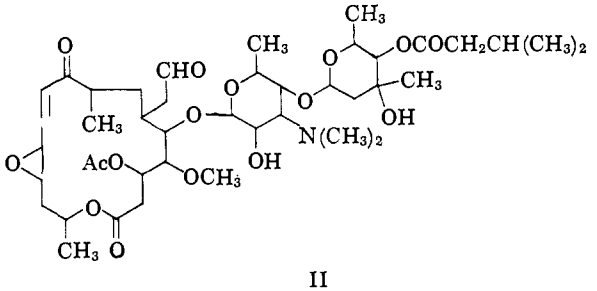
Sir:

The structures Ia–c[1] have been found to represent the spiramycins (foromacidines), a family of macrolide antibiotics isolated from Streptomyces.[2,3] In the course of chemical degradative and spectroscopic studies on these compounds, an interrelation with magnamycin was attempted, which led to evidence requiring a revision of the magnamycin structure. The latter can now be represented as II.[4] Experimental evidence for these structures and against a recent proposal[5] III for the spiramycins and the previous structure[6] IV for magnamycin is presented below.

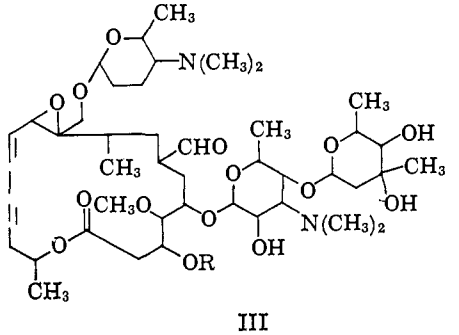


I

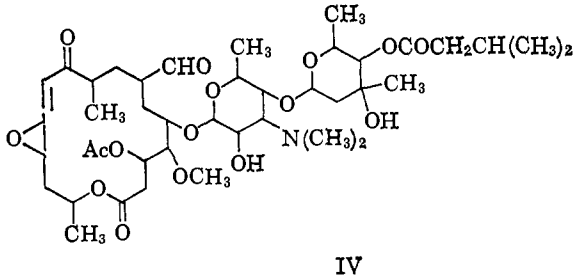
Ia, R = H
b, R = $COCH_3$
c, R = $-COCH_2CH_3$



II



III



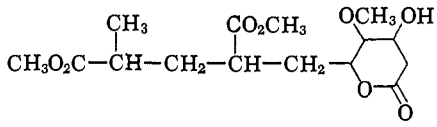
IV

Earlier characterization[2] of the spiramycins had established spiramycin-A as an alcohol (or possibly formate ester) and spiramycin-B and -C as the corresponding acetate and propionate, respectively. Mild acid hydrolysis of the spiramycins yields mycarose (sugar A)[7] and the neospiramycins, which on more vigorous acid hydrolysis give forosamine (sugar B)[8] and the forocidines. From drastic acid treatment mycaminose (sugar C)[9] can be isolated. In addition, the remaining skeleton of the spiramycins was found to bear one methoxyl group, two C-methyl groups, a conjugated diene, a lactone, a carbonyl group recently characterized as an aldehyde,[5] and (erroneously) an epoxide.[5] The empirical formula suggested[2,5,10] (erroneously) for spiramycin B was $C_{47}H_{78}N_2O_{16}$ or $C_{47}H_{80}N_2O_{16}$, with corresponding formulas for derived products.

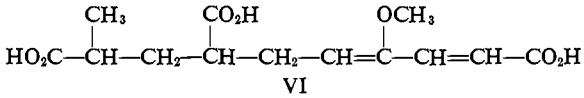
From an exhaustive reductive degradation[10] of the spiramycins the skeletal chain of these macrolides was obtained as an aliphatic acid (erroneously) assigned the empirical formula $C_{21}H_{42}O_2$. Permanganate oxidation[10] of the forocidines, followed by treatment with base, gave fumaric acid, β-hydroxybutyric acid, a noncrystalline lactone dicarboxylic acid ester formulated as V, and the diene triacid VI, which were subsequently degraded to 2-methyl-4-butylglutaric acid and 3-carboxy-5-methyladipic acid, both identified by comparisons with synthetic samples. Linkage of mycarose to mycaminose as indicated and of mycaminose, forosamine, and the ester residue to the lactone nucleus could be established.[5,11] The presence of an unbranched eight-carbon fragment bearing oxygen adjacent to a terminal methyl group was determined through ozonolysis of a reduced forocidine derivative.[5] With arguments for the lactone linkage to the penultimate position of the eight-carbon segment,[5] structure III was proposed for the spiramycins.

The following additional evidence supports the preferred structure I. Catalytic reduction of the forocidines over platinum and refluxing of the resultant hexahydroforocidines with 2 *N* hydrochloric acid gave mycaminose and an olefinic lactone containing one methoxyl group (Zeisel), no acyl residue (hydrolysis), and one double bond (hydrogenation). (Previous definition[10] of this product as a $C_{21}H_{36-38}O_6$ compound derived from structure III would have required an improbable methoxyl elimination or generation of a γ-ketoacrylic lactone system, inconsistent with these results and the failure to show the expected[6] ultraviolet spectrum.) Catalytic reduction over platinum followed by reaction with hydrogen iodide and red

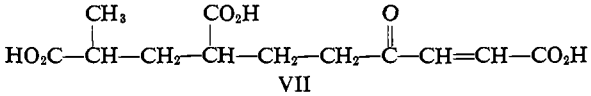
(1) Presented at a Columbia University Colloquium, June 1964.
(2) R. Corbaz, L. Ettlinger, E. Gäumann, W. Keller-Schierlein, F. Kradolfer, E. Kyburz, L. Neipp, V. Prelog, A. Wettstein, and H. Zähner, *Helv. Chim. Acta*, **39**, 304 (1956).
(3) S. Pinnert-Sindico, L. Ninet, J. Preud'Homme, and C. Cosar, *Antibiotics Ann.*, 724 (1954).
(4) After submission of our data, Prof. R. B. Woodward kindly informed us that he had reached the same structure for magnamycin based on parallel and other experiments: *J. Am. Chem. Soc.*, **87**, 4662 (1965).
(5) R. Paul and S. Tchelitcheff, *Bull. soc. chim. France*, 650 (1965).
(6) R. B. Woodward, *Angew. Chem.*, **69**, 50 (1957).
(7) R. Paul and S. Tchelitcheff, *Bull. soc. chim. France*, 443 (1957).
(8) R. Paul and S. Tchelitcheff, *ibid.*, 734 (1957).
(9) R. Paul and S. Tchelitcheff, *ibid.*, 1059 (1957).
(10) R. Paul and S. Tchelitcheff, *ibid.*, 150 (1960).
(11) R. Paul and S. Tchelitcheff, *ibid.*, 189 (1965).



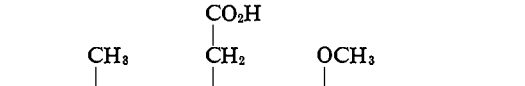
V



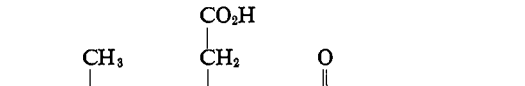
VI



VII



VIII



IX

phosphorous and catalytic hydrogenation yielded a hydrocarbon acid product, which was converted to its methyl ester by diazomethane. Repeated thin layer chromatography gave a mixture of three components containing a $C_{20}H_{40}O_2$ methyl ester as the compound with highest molecular weight (*m/e* 312.5). Elemental analyses of the total ester fraction were in good agreement with the assigned empirical formula and mass spectral result. *This sequence determines the chain size of the lactone nucleus.*

Oxidation of hexahydroforocidine with hot nitric acid gave pimelic acid and 3-carboxy-5-methyladipic acid among other products and oxidation of forocidinol with sodium hypoiodite produced iodoform, showing the presence of an unbranched eight-carbon segment oxygenated at the penultimate carbon. *In conjunction with the twelve-carbon chain degradation product VI these experiments define all carbons of the lactone nucleus, with one carbon atom common to the C_{12} and both C_8 units.*

Oxidation of the forocidines with manganese dioxide furnished dienones (λ_{max} 278 mμ) while the spiramycins and neospiramycins did not undergo this reaction. This finding necessitates an allylic attachment of forosamine in the latter compounds and formulation of the forocidines as allylic alcohols. Confirmation of this assignment is found in the reduction of the forocidines with lithium aluminum hydride, which results in a marked reduction of the diene system as seen in the respective ultraviolet and mass spectra (λ_{max} 231 mμ (ϵ 26,000 changed to ϵ 14,000) and *m/e* 563 and 565.3816) in accord with known reductions[12] of α,β-double bonds in allylic dienols and a parallel observation in the reduction of magnamycin B (desepoxymagnamycin). Furthermore, sublimation of the mixture of reduction products, "forocidinol," results in partial conversion to a triene (λ_{max} 267, 277, and 291 mμ), suggesting facile dehydration due to an allylic hydroxyl group. *On the basis of this evidence structure I can be proposed for the spiramycins.*

The epoxide postulated[5] in III could not be detected through attempts to generate a triene by treatment with sodium iodide in acetic acid, followed by zinc or with chromous chloride.[13] Also, formaldehyde was not formed on sodium bismuthate treatment of forocidinol,[14] as required by the previously proposed[5] structure for this compound.

Finally, a high-resolution mass spectrum of forocidinol showing a mass of 565.3816 necessitates the composition $C_{28}H_{55}NO_{10}$, in agreement with this discussion.

In view of the close similarity of the spiramycin structures (I) to that existing for magnamycin (IV), a correlation was attempted. The most notable difference between the series was the presence of one aldehyde hydrogen signal (δ 9.5) in nuclear magnetic resonance spectra of magnamycin A and B, tetrahydromagnamycin, and carimbose (desmycarosemagnamycin), and two in the spiramycins and forocidines (δ 9.6 and 9.7, Δ 9 c.p.s.). This observation initially suggested a singlet and doublet, respectively, and that the magnamycin structure thus would require revision to a more branched one containing an aldehyde function attached to a quaternary carbon atom. However the following evidence ruled out this postulate:

Removal of the epoxide from magnamycin (λ_{max} 238 mμ (ϵ 15,700)) with chromous chloride in acetic acid gave a dienone (λ_{max} 278 mμ (ϵ 23,000)) in high yield. Removal of mycarose by mild acid treatment and reduction with lithium aluminum hydride furnished a mixture of an allylic dienol and γ-hydroxyolefin. When compared with the lithium aluminum hydride reduction product of the forocidines this showed very similar, if not identical, infrared and ultraviolet spectra and thin layer chromatographic mobilities. The nuclear magnetic resonance spectrum of desepoxycarimbose did not allow for increased branching compared with the spectrum of forocidine (same number of methyl groups), and a high resolution mass spectrum (*m/e* 565.3777, $C_{28}H_{55}NO_{10}$) determined the isomeric nature of the two reduction products and thus eliminated the possibility of an additional ring in the magnamycin structure.

Permanganate oxidation of carimbose gave a C_{13} conjugated diene acid previously assigned structure VI.[6] A nuclear magnetic resonance spectrum of this compound showed three vinyl protons, each as a doublet (δ 6.8, 5.7, and 5.2, DMSO-d_6), thus requiring the presence of a single allylic proton, inconsistent with VI. Hydrolysis of the enol ether produced a ketoacid, previously assigned structure VII.[6] Comparison of its nuclear magnetic resonance spectrum with that of the analogous compound formed in the spiramycin series by the identical reaction sequence showed the ketone α-methylene protons as a poorly resolved doublet (δ 2.6, DMSO-d_6) in the former and an apparent triplet (δ 2.6, DMSO-d_6) in the latter case.[15]

(12) J. Attenburrow, A. Cameron, J. Chapman, R. Evans, B. Hems, A. Jansen, and T. Walker, *J. Chem. Soc.*, 1101 (1952).

(13) J. Cornforth, R. Cornforth, and K. Mathew, *ibid.*, 112 (1959).

(14) W. Rigby, *ibid.*, 1907 (1950).

(15) The dinitrophenylhydrazone of the C_{12} acid derived from magnamycin showed m.p. 192–194° and that from the spiramycin series m.p. 190–192°. A mixture melting point was depressed to 186–190°. The corresponding semicarbazones showed m.p. 285–287 and 158–160°, respectively. Both C_{12} acids were oxidized with nitric acid to 3-carboxy-5-methyladipic acid.

These experiments allow revision of the C_{13} and C_{12} acid structures derived from magnamycin to VIII and IX and thus postulation of the magnamycin structure as II. The expected magnamycinaldehyde proton triplet could not be observed, even with variations in solvent polarity, using deuterated benzene, chloroform, or dimethyl sulfoxide as solvents and a temperature range of -30 to $80°$.[16] However, conversion of tetrahydromagnamycin to an oxime showed the elusive splitting as the expected triplet at δ 6.8, $J = 5$ c.p.s.

A nuclear magnetic resonance spectrum of the spiramycins at 100 Mc. showed a separation of 16 c.p.s. for the two aldehyde signals, clearly ruling out a spin–spin splitting doublet. Direct evidence for two stereoisomeric aldehyde functions attached to an epimerizable center was found in the change of a 31:69% to 34:66% ratio of signals of a sample dissolved in acetic acid and then treated with sodium bicarbonate.

Acknowledgment. We wish to thank Dr. Emil Schlittler and Dr. W. I. Taylor, Ciba, Summit, N. J., for a gift of the spiramycins and valuable discussions, Dr. Hans Grisebach, University of Freiburg, for a procedure for the oxidation of carimbose, Dr. Klaus Biemann, Massachusetts Institute of Technology, for spectral data, and Mr. Grant Warner of our group for nuclear magnetic resonance spectra and Dr. Ross Pitcher of Varian Associates for a 100-Mc. spectrum.

(16) G. J. Karabatsos and N. Hsi, *J. Am. Chem. Soc.*, **87**, 2864 (1965).

(17) Alfred P. Sloan Foundation Fellow.

(18) National Defense Education Act Predoctoral Fellow.

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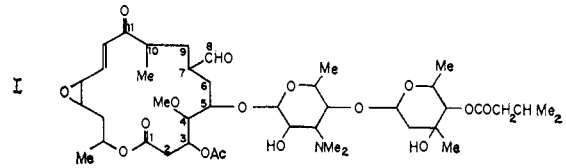
The Structure of Magnamycin

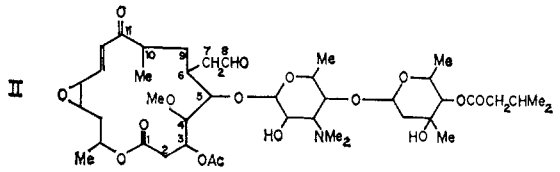
Sir:

Extensive degradative studies led us to propose structure I for the macrolide antibiotic magnamycin *a* in 1957.[1,2] We now wish to record new observations which require that the magnamycin structure be revised to II.[3]

Our previous deductions about the C-1–C-11 system of magnamycin were based in large part upon the degradation of the macrolide, through oxidation, followed by vigorous base treatment, to an $\alpha,\beta,\gamma,\delta$-doubly unsaturated γ-methoxy acid $C_{13}H_{18}O_7$, which was readily hydrolyzed to the corresponding α,β-unsaturated γ-keto acid $C_{12}H_{16}O_7$. Either acid was further oxidized to an *optically active* acid $C_8H_{12}O_6$, m.p. 99–100°, whose

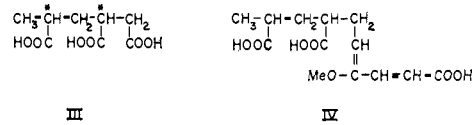


I



II

trimethyl ester was converted by sodium methoxide to a mixture of diastereomers, from which, after hydrolysis, there was obtained a *racemic* acid $C_8H_{12}O_6$, m.p. 154–155°, identical with a sample of 2-methyl-4-carboxyadipic acid (III) prepared by synthesis. Con-

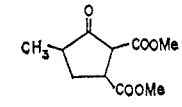


III

IV

version of the $-COCH{=}CHCOOH$ chain of the C_{12} acid to $-CH_2CH_3$ led to a dibasic acid which gave an anhydride (*not* a succinic anhydride) *rather than a cyclopentanone* on treatment with acetic anhydride under pyrolytic conditions; consequently, the C_{13} acid was formulated as IV, and the C_{12} acid as the corresponding ketone.

We were led to re-examine these results when Srinivasan and Gilner[4] reported that, while the trimethyl ester of a synthetic 2-methyl-4-carboxyadipic acid (III) was readily cyclized by sodium in refluxing benzene to the cyclic β-keto ester (V), the ester of the C_8 acid from



V

magnamycin was unchanged under the same conditions. Since these observations brought the structural assignment of the C_8 acid from natural sources into question, we have repeated the degradation and subjected the resulting racemic acid to renewed rigorous scrutiny, in comparison with new samples of synthetic material prepared by our earlier method[1] and by an alternative synthesis[5]; melting points, mixture melting points, and detailed infrared and mass spectrometric studies, buttressed by nuclear magnetic resonance, infrared, mass spectrometric, and vapor chromatographic studies on the corresponding esters, leave no doubt whatsoever that the materials from all three sources are identical and that the racemic acid $C_8H_{12}O_6$ from magnamycin is one of the diastereomers of structure III. On the other hand, we have confirmed the observation

(1) R. B. Woodward, *Angew. Chem.*, **69**, 50 (1957); *Festschr. Arthur Stoll*, 524 (1957).

(2) In the interim the stereochemistry of the sugar components, mycarose and mycaminose, has been completely elucidated. Mycarose: D. M. Lemal, P. D. Pacht, and R. B. Woodward, *Tetrahedron*, **18**, 1275 (1962); F. Korte, U. Claussen, and K. Göhrring, *ibid.*, **18**, 1257 (1962); W. Hofheinz, H. Grisebach, and H. Friebolin, *ibid.*, **18**, 1265 (1962); H. Grisebach, W. Hofheinz, and N. Doerr, *Chem. Ber.*, **96**, 1823 (1963). Mycaminose: A. B. Foster, T. D. Inch, J. Lehmann, M. Stacey, and J. M. Webber, *J. Chem. Soc.*, 2116 (1962); A. C. Richardson, *ibid.*, 2758 (1962); W. Hofheinz and H. Grisebach, *Z. Naturforsch.*, **17b**, 355 (1962).

(3) Professor Martin Kuehne [Vermont] has kindly informed us privately that he has reached a similar conclusion, and concurrent publication has been arranged [M. E. Kuehne and B. W. Benson, *J. Am. Chem. Soc.*, **87**, 4660 (1965)].

(4) D. Gilner, Ph.D. Dissertation, Columbia University, New York, N. Y., 1963; private communication from Dr. P. R. Srinivasan.

(5) E. Hope and W. H. Perkin, Jr., *J. Chem. Soc.*, **99**, 776 (1911).