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Chalcomycin: Single-Crystal X-Ray Crystallographic Analysis; Biosynthetic and Stereochemical Correlations with Other Polyoxo Macrolide Antibiotics.

Peter W. K. Woo* and J. Ronald Rubin*

Parke-Davis Pharmaceutical Research Division of Warner-Lambert Company

2800 Plymouth Road, Ann Arbor, Michigan 48105, U.S.A.

Abstract: The stereochemistry of chalcomycin (**1**), a neutral 16-membered macrolide antibiotic, was established by X-ray crystallography. A correlation model for 16-membered macrolides (**2**), together with its biochemical rationale, is presented and applied to the configurational assignment of seven other analogous neutral macrolides (aldgamycins F, G, CP-61,884 complex, neutramycin, and swalpamycin). The chalcomycin molecule appears to be conformationally restrained in a rigid manner.

INTRODUCTION

The macrolide antibiotics have played an important role in chemistry and medicine over the past four and a half decades. They have stimulated development of synthetic methodologies and led to elegant total syntheses with multiple chiral centers.[1] They have led to biosynthetic and molecular genetic studies which have given extensive insight into the fundamental mechanisms, as well as promise of selective genetic combination to create novel useful compounds.[1,2] They have been targets for structural, configurational, and conformational analyses that fully utilized the constant, rapid advances in methodologies. Some members, like erythromycin and tylosin, have served for many years as important medicinal agents in humans and animals, and novel semisynthetic derivatives, like azithromycin and clarithromycin, are significantly extending the therapeutic utility of the group.[1,3]

Chalcomycin (**1a-c**; **1d** in Fig. 1) is a neutral 16-membered ring macrolide active against gram-positive organisms. It retains effectiveness in the presence of bacterial penicillinases and appears to inhibit protein synthesis via an interference in the transfer of activated amino acids to the cellular s-RNA.[4] Its total structure and partial stereochemistry were previously reported from our laboratories.[5] Attached to the lactone ring are the sugars chalcose (**5**)[6] and mycinose (**6**).[7]

The structure of numerous analogous 16-membered macrolide antibiotics have since been reported by other laboratories (Fig. 1, 2, and 3). In many of these, mycinose is the common sugar attached in an identical manner through a carbinol at C-14 of the lactone. In some others, chalcose is replaced in the same position by other sugars such as desosamine (**7**)[8] and mycaminose as its glycosylated derivatives (**13**, **14**). The complete stereochemical configurations of mycinamicin I (**3**),[9] mycinamicin IV (**4**),[10] rosaramicin (**8**),[11] tylosin (**9**)[12] maridomycin III (**10**),[13] leucomycin A3 (**11**),[14] and acumycin (**12**)[15] have been established, directly or indirectly, by X-ray analysis as shown in Fischer projections in Fig. 1 and 2. Most of these may be considered as representatives of subgroups of closely similar macrolides which have been related chemically. A high

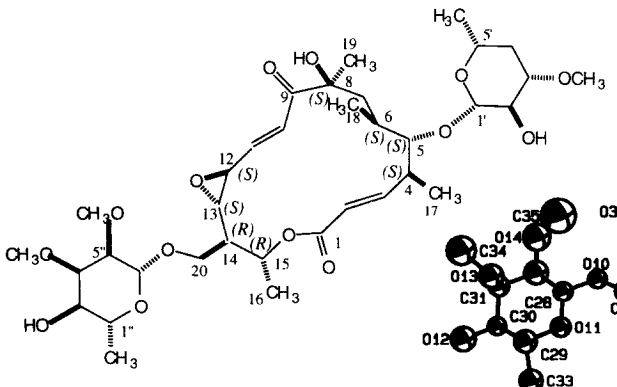
degree of position specific regularity, or structural homology and configurational integrity, are evident among the group.

We have now established the complete stereochemical configuration of chalcomycin by single crystal X-ray analysis. The study confirmed the structure and partial configuration reported earlier from our laboratories. Furthermore it provides a stereochemical link between those 16-membered basic macrolides with fully established configuration (Fig. 1 and 2) to those in the remaining neutral group (Fig. 3), namely, neutramycin (**15**),[16] CP-61,884 complex (**16a-c**, three components including 8-deoxychalcomycin),[17] aldgamicins (e.g., **17**, **18**),[18] and swalpamycin (**19**),[19] for which the configurational assignment in the lactone rings has not been reported. It is also our objective that the structural and stereochemical relationship of the 16-membered macrolides may be correlated and understood in terms of their underlying biosynthetic pathways, especially on the basis of the recent rapid advances in the field.

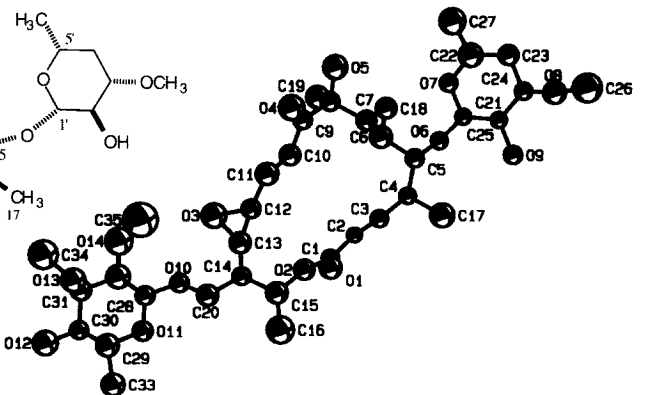
RESULTS AND DISCUSSION

X-Ray Crystallographic Studies

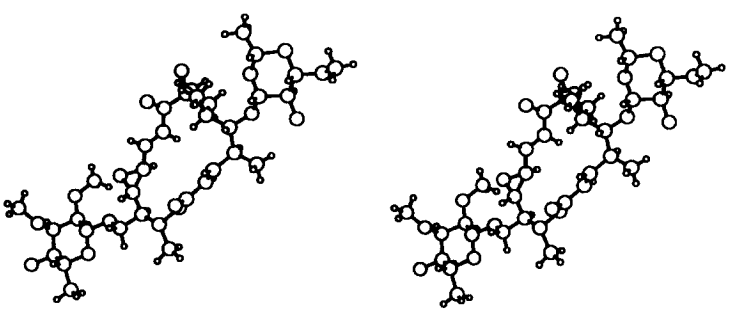
The structure and stereochemistry of chalcomycin are represented by formula **1a**, **1b** (ORTEP plot),



1a



1b



1c

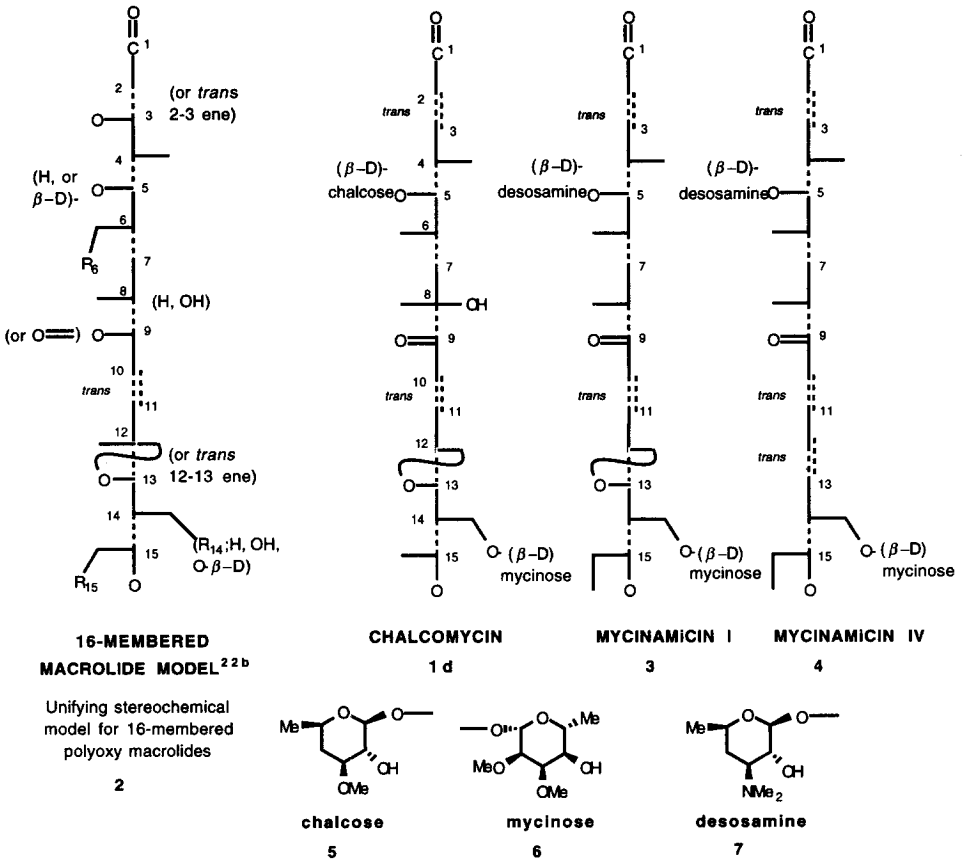


Figure 1

1c (stereo view), and **1d** (in Figure 1, Fischer projection). The heavy atom bond distances and angles and their standard deviations are shown in Tables 1 and 2 respectively.

Based on the known configurations of the chalcose and mycinose sugars and the current crystal structure determination, the absolute configuration of all of the chiral centers in chalcomycin were determined to be: 4-*S*, 5-*S*, 6-*S*, 8-*S*, 12-*S*, 13-*S*, 14-*R*, and 15-*R*. The endocyclic torsion angles for the macrolide ring are shown in Table 3. These values correspond closely to the equivalent torsions observed in the structures of the related 16-membered macrolide ring of mycinamicin.[10] The macrolide ring of chalcomycin is elliptical with major and minor axes of 12Å and 6Å respectively. Two sides including atoms C1-C5 and C8-C12 of the macrolide ring are approximately planar. The bends in the ring chain occur at atoms C5-C7 and C12-C15 adjacent to the glycosidic bonds. Both of the pyranose sugars adopt a similar chair conformation. The displacements of the sugar ring atoms of mycinose and chalcose are shown in Table 4.

The overall dimensions of the chalcomycin molecule are 18Å by 8Å by 6Å. In the crystal lattice translationally related chalcomycin molecules are stacked in pairs with the chalcose and mycinose sugars

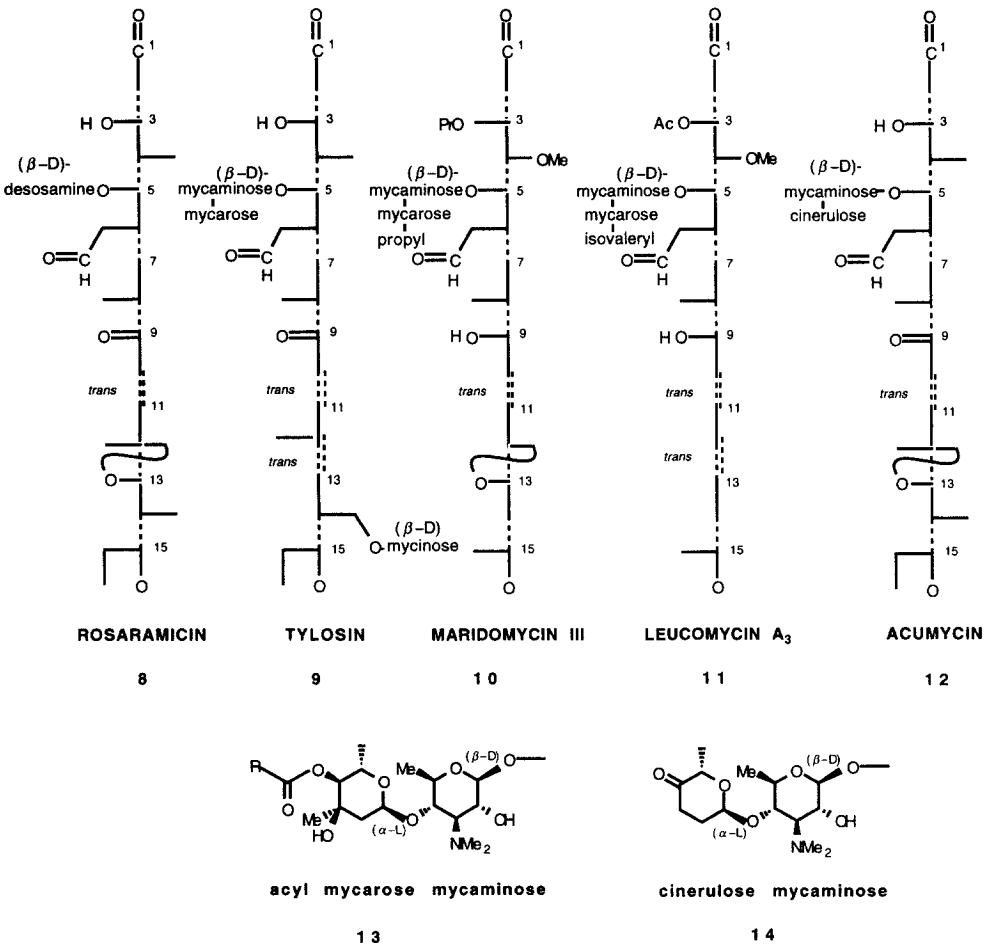
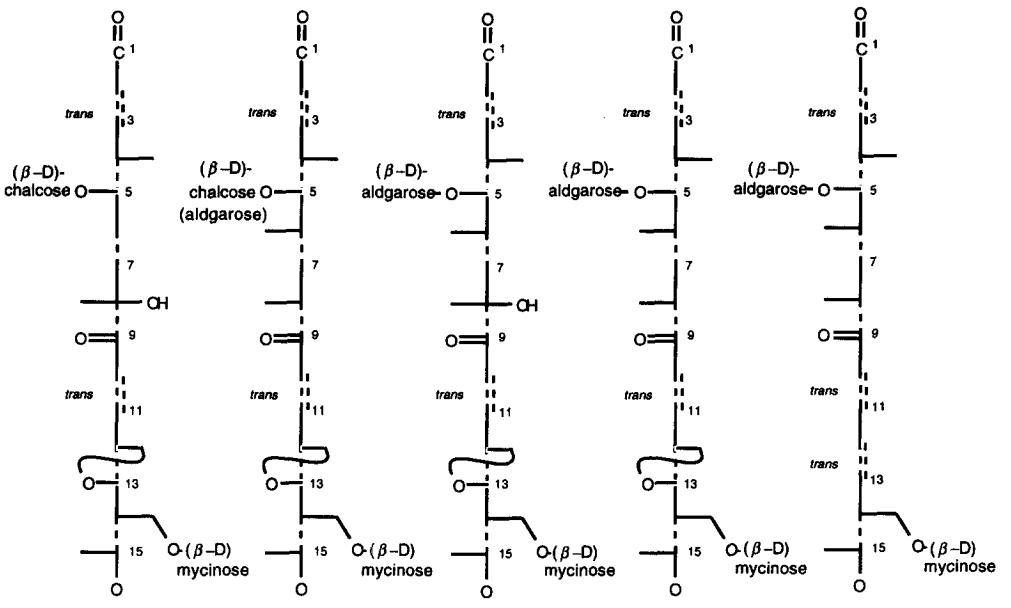


Figure 2

of one molecule stacking over the macrolide ring of an adjacent molecule. Space-filling models of the chalcomycin structure indicate that the molecule, including the exocyclic sugars, is restrained in a rigid conformation. This is further supported by the low temperature factors observed [B(A2) Tables 6], indicating generally small amplitudes of vibration of the atoms. Also, as shown in Table 3, very similar conformations are adopted by the related 16-membered macrolides rosaramicin (**8**),[11] mycinolide IV (aglycone from **4**),[10c] and a derivative of mycinamicin I (**3**),[9b] relatively unaffected by structural variations such as the presence of C12-C13 olefin or epoxide, C-12 methyl, *O*-acetyl, and *O*-glycosyl groups, as indicated by a comparison of the endocyclic torsion angles of these compounds.



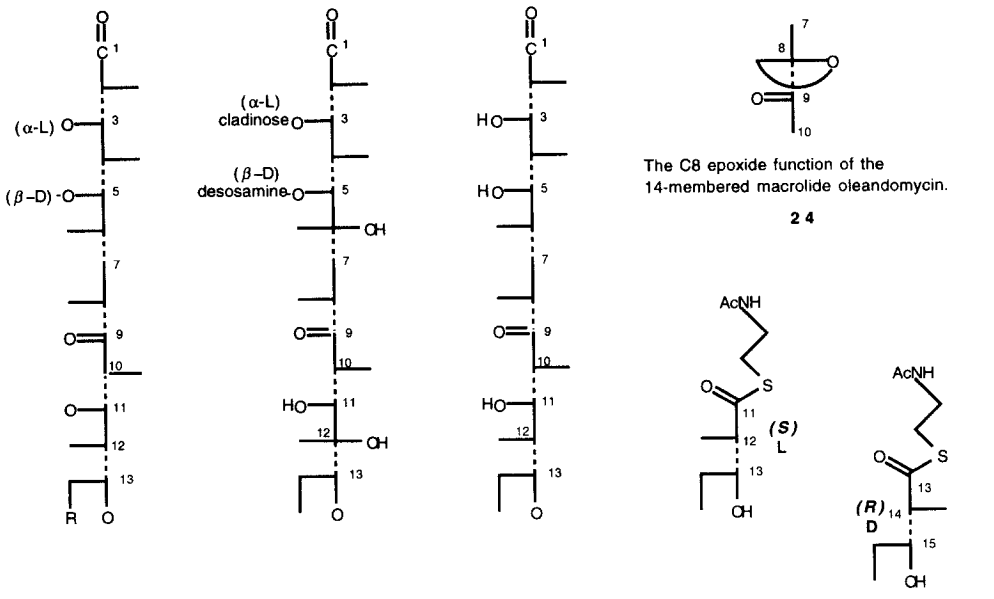
"PREDICTED" CONFIGURATIONS OF VARIOUS NEUTRAL 16-MEMBERED MACROLIDES BASED ON STEREOCHEMICAL MODEL IN FIGURE 1.

Figure 3

Stereochemical Correlations of Chalcomycin and Other Polyoxo Macrolides

It has long been recognized that a remarkable position-specific regularity exists among different polyoxo macrolide antibiotics from actinomycetes. Thus, Celmer's stereochemical model (**21**, Fig. 4), which correlates and predicts the absolute configuration of the glycosidic linkages of the sugars and the chiral centers on the aglycone,[20a] is putatively consistent with every single chiral center in the 14- and 12-membered macrolides.[1b,c,15] It is also applicable to a portion of the 16-membered lactone ring.[20b,c,21b]

Compared with other 16-membered polyoxo macrolides of known configuration (Fig. 1 and 2), chalcomycin is unique in being neutral, lacking a basic sugar, and having a chiral C8 with methyl and hydroxyl substitutions.[22] Among the other chiral centers, the stereochemistry at C12 and C13 of chalcomycin is identical to that in mycinamicin I (**3**), rosaramicin (**8**), maridomycin III (**10**), and acumycin (**12**). Its C14 and C15



CELMER'S MODEL 14(12)-membered [20a] (MODEL A [20b,c]) **21**

ERYTHROMYCIN A **22**

6-Deoxy-erythronolide B (6-dEB) **23**

precursor for 14-membered erythronolide **25**

precursor for 16-membered tylonolide **26**

Figure 4

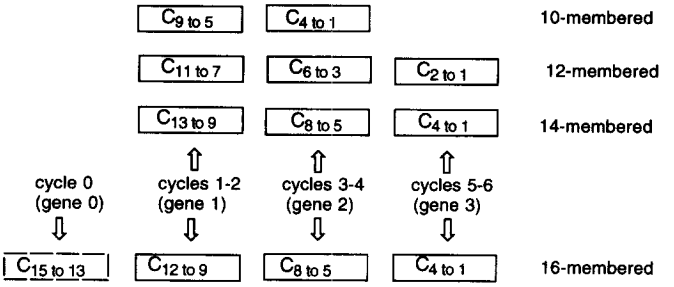
configurations are analogous to that in all of the 16-membered basic polyoxo macrolides of known configuration. The configurations for the segment from C1 to C11 show analogy to the remaining 16-member macrolides of known configuration, as well as with the corresponding segment in Celmer's model for C14-member macrolides (**21**).

The observed structures and stereochemistry of the 16-membered macrolides may be summarized by the presently proposed model (**2**),[23] which would complement Celmer's or Cane-Celmer's model (**21**) for the 14(12)-membered macrolides. The two series differ in one important biosynthetic aspect (see below).

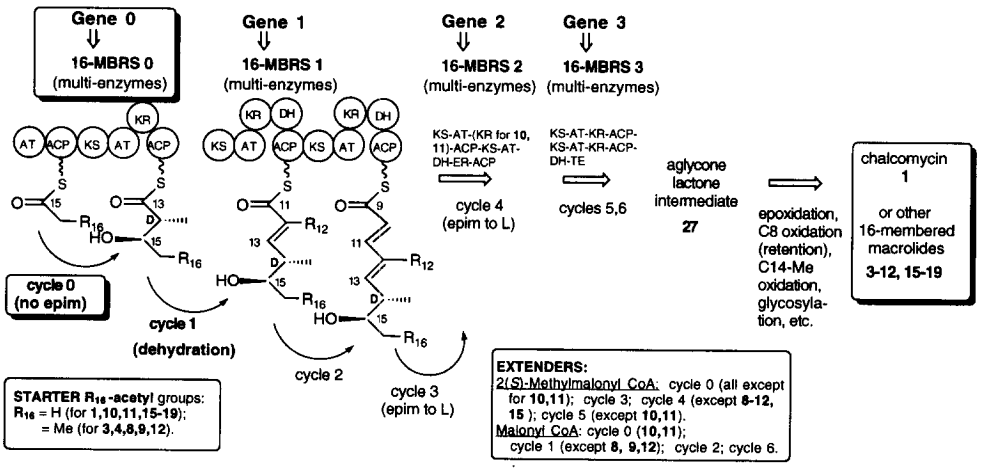
The Biochemical Basis for Stereochemical and Structural Correlations.

The biosynthesis of macrolide antibiotics, members of the "complex" group of polyketides (PKS), is believed to occur in two stages: formation of the polyketide lactone intermediates, such as **23** (Fig. 4) and **27** (Fig. 5b), and their subsequent elaboration to the final antibiotics products. The lactone intermediates are mostly derived from acetates, propionates, or butyrates, wherein the β-carbonyl groups formed in each condensation cycle are processed in variable manners which may include keto reduction, dehydration, enoyl reduction, and other reactions. For the 14-membered erythromycin (**22**, Fig. 4), substantial understanding of the process has been gained from extensive biosynthetic, enzymological, and molecular genetic studies.[1,2,24]

(5a) Correspondence between lactone ring segments and gene or cycle numbers in the biosynthesis of 16-membered vs. 14-, 12- and 10-membered macrolides.



(5b) A possible scheme for the elaboration of representative 16-membered ring macrolides, showing the organization of the genes and enzymes of the antibiotic-producing polyketide synthase, processive chain elongation to an aglycone lactone intermediate (**27**), and final modifications to the individual antibiotics (**1, 3-12, 15-19**). Module 0 is an addition to, and modules 1 and 2 are modifications of, the 14-membered 6-DEB model of Leadlay, et al.,[2c] and Donadio, et al.[2b]



Abbreviations: ACP, acyl carrier protein; KS, β-ketoacyl-ACP synthase; AT, propionyl-CoA, malonyl-CoA or 2(*S*)-methylmalonyl-CoA:ACP acyltransferase; DH, β-hydroxyacylthioester dehydratase; ER, enoyl reductase; KR, β-ketoacyl-ACP reductase; TE, thioesterase or putative cyclase. **Cycle**, chain elongation cycle consisting of condensation with 2(*S*)-methylmalonyl CoA or malonyl CoA (see Fig 5a), with decarboxylative inversion, and modifications such as epimerization and adjustment of oxidation state. **Epim to L**, epimerization which occurs either in 2(*S*)-methylmalonyl Co-A immediately before decarboxylative condensation (which occurs with configuration inversion) or in the ketide product immediately after condensation, resulting in a L-methyl configuration in the ketide chain.[2d] **16-MBRS**, hypothetical polyketide synthase, distinct for individual 16-membered macrolides.

Figures 5a, 5b

The biochemical basis for position-specific regularity among the macrolides is illustrated in Fig. 5. The biosynthetic difference between 16-membered series vs. the 14- to 10-membered series, as shown in Fig. 5a, is consistent with the results from incorporation studies of chain-elongation precursors: (**25**, Fig. 4),[25a] with 2(*S*) chirality, into the C13-C11 segment of erythromycin (**22,** 14-membered), C11-C9 of methymicin (12-membered), and C9-C7 of nargenicin (10-membered); in contrast, (**26**, Fig. 4),[26] with 2(*R*) chirality, into the C15-C13 segment in tylosin (**9**, 16-membered). The C15-C13 segment of the 16-membered macrolides (Fig.

5a) is thus unique, in contrast to the other overlapping segments that are potentially amenable to inter-series correlations.

The scheme of a possible gene and synthase directed biosynthesis, applicable to both chalcomycin and other representative 16-membered macrolides, is shown in Fig. 5b. It is a modified extension of the modular model of synthesis from the Leadlay group[2c] and Katz group for the 14-membered 6-dEB (**23**).[2b] A hypothetical new module (numbered 0), has been added, which corresponds to that shown in Fig. 5a and accounts for the D-configuration at C14 in the 16-membered macrolides. The complement of enzymes in modules 1 and 2 has been modified so that the C12-13 epoxide geometry in the final antibiotics is a result of late-stage modification of the aglycone intermediates (**27**).

The observed conformational stability of the lactone with respect to the presence of either epoxide or olefin at C12-C13 , as shown by X-ray data (see Table 3 and discussion above), is consistent with epoxidation occurring with concerted attack of the olefinic carbons and may be analogous to the microbial epoxidation in the genesis of polyether antibiotics.[20b] As may be evident from the stereo formula **1c**, an oxygen could approach the lactone ring from only one direction, hence leading to one stereochemical consequence, and the resulting epoxide formation should occur with minimum disturbance to the conformation of the lactone ring.

The C-8 hydroxyl (or other "extra" oxygens [21b] on the polyketide chain) is also envisioned as introduced late in the biogenesis, by oxidation without inversion, in a manner analogous to the demonstrated C-6 hydroxylation of 6dEB (**23**) in the biosynthesis of erythromycin (**22**).[21]

Stereochemical Assignment for the Remaining Neutral 16-Membered Polyoxo Macrolides

Based on the 16-membered macrolide model (**2**),[23] and supported by the possible underlying biochemical mechanisms (Fig. 5), the hitherto undefined lactone chirality of the remaining group of neutral macrolides may be assigned as shown (Fig. 3). The likelihood of the correctness of the assignment is further enhanced by considering the origination of some of these macrolides. Thus, chalcomycin has been identified (as aldgamycin D) in the same fermentation broth that produced either aldgamycins (e.g. **17**) or swalpamycin (**19**). The components in CP-61,884 complex (**16**), two of which being 8-deoxychalcomycin and its 5-O-aldgarose analog, respectively, are obviously closely related to both chalcomycin and the aldgamycins. Neutramycin (**15**) and chalcomycin (**1a**) apparently differ in an acetate vs. a propionate precursor unit to C6-C5.

Conclusion

Among the 16-membered macrolides, chalcomycin has served as a link between the basic group with established chirality and the neutral group with undefined lactone chirality. It shows structural and stereochemical consistency with the basic group, while it shares even more profound similarities with the neutral group, both in microbial origin and in structural features, including the tertiary hydroxyl at C8 characteristically found in several members of the group.[22] The stereochemistry of chalcomycin, presently established, serves as another clear demonstration of the position-specific regularity observed among the naturally occurring macrolides; thereby it enables, with substantially enhanced certainty, the stereochemical assignment for the remaining closely related 16-membered neutral macrolides. The structural and stereochemical relationship, among the 16-membered macrolides (**2**) and with the 14(12)-membered macrolides (**21**), could be better comprehended through an understanding of the underlying mechanisms, as demonstrated by the possible

biosynthetic model for the 16-membered macrolides (Fig. 5b), which has been formulated on the basis of insights and findings from diverse literature sources and from the present study.

EXPERIMENTAL

X-ray Structure Determination.[27]

Chalcomycin ($C_{35}H_{56}O_{14}$, F.W. = 700.80) crystallized as colorless rods from ethanol solutions. X-ray data were collected on an Enraf-Nonius CAD-4 diffractometer using CuK_α radiation (λ =1.54184 Å). The cell constants and an orientation matrix for data collection were determined from the centered angles of 12 reflections. X-ray diffraction data were collected at 23° C. using the omega scan technique with a variable omega scan rate from 2° to 20° per minute. The data were collected to a maximum 2Θ of 48.4°. A total of 1698 reflections were collected, of which 1557 were unique and not systematically absent. Lorentz and polarization corrections were applied to the data as well as an empirical absorption correction based on a series of psi scans. Crystallographic parameters for these crystals are given in Table 5.

The crystal structure was determined by direct methods using the SIR-92 programs. A total of 350 reflections with E>1.20 were used to produce a phase set with an absolute figure of merit of 1.30. All 49 heavy atoms in the structure were located from the E map calculated using this phase set. Hydrogen atom positions were located in subsequent difference Fouriers and added to the structure, but their positions were not

Table 1. Table of Bond Distances in Angstroms

Atom 1	Atom 2	Distance	Atom 1	Atom 2	Distance
O1	C1	1.26(2)	O14	C35	1.50(3)
O2	C1	1.34(2)	C1	C2	1.44(2)
O2	C15	1.46(2)	C2	C3	1.29(2)
O3	C12	1.43(2)	C3	C4	1.50(2)
O3	C13	1.47(2)	C4	C5	1.60(2)
O4	C9	1.22(2)	C4	C17	1.50(3)
O5	C8	1.42(2)	C5	C6	1.51(3)
O6	C5	1.45(2)	C6	C7	1.55(3)
O6	C21	1.37(2)	C6	C18	1.51(3)
O7	C21	1.40(2)	C7	C8	1.55(3)
O7	C22	1.40(2)	C8	C9	1.50(3)
O8	C24	1.45(2)	C8	C19	1.50(3)
O8	C26	1.36(3)	C9	C10	1.47(3)
O9	C25	1.43(2)	C10	C11	1.30(3)
O10	C20	1.35(2)	C11	C12	1.58(3)
O10	C28	1.44(2)	C12	C13	1.50(3)
O11	C28	1.36(2)	C13	C14	1.47(3)
O11	C29	1.50(2)	C14	C15	1.52(3)
O12	C30	1.40(2)	C14	C20	1.51(2)
O13	C31	1.38(2)	C15	C16	1.60(3)
O13	C34	1.46(3)	C21	C25	1.54(2)
O14	C32	1.37(3)	C22	C23	1.60(3)
C22	C27	1.44(3)	C29	C30	1.47(3)
C23	C24	1.47(3)	C29	C33	1.53(3)
C24	C25	1.50(2)	C30	C31	1.52(3)
C28	C32	1.55(3)	C31	C32	1.54(3)

Numbers in parentheses are estimated standard deviations in the least significant digits.

refined. The heavy atom parameters including anisotropic temperature factors were refined by full matrix least squares using 1006 reflections with intensity greater than three times their standard deviation. The final R-factor is given in Table 5. The final difference Fourier was essentially featureless. The highest peak in this map had a height of only 0.28 e/Å^3. The atomic coordinates and isotopic temperature factors are given in Table 6. The bond distances and angles between heavy atoms are given in Tables 1 and 2. The observed and calculated structure factors are presented in Table 7 (supplementary material).[28] All calculations were performed on a VAX computer using the MolEN program set .

Table 2. Table of Bond Angles in Degrees

Atom 1	Atom 2	Atom 3	Angle	Atom 1	Atom 2	Atom 3	Angle
C1	O2	C15	117.(1)	C7	C6	C18	111.(2)
C12	O3	C13	62.(1)	C6	C7	C8	117.(2)
C5	O6	C21	118.(1)	O5	C8	C7	108.(2)
C21	O7	C22	112.(1)	O5	C8	C9	107.(1)
C24	O8	C26	116.(2)	O5	C8	C19	107.(2)
C20	O10	C28	113.(1)	C7	C8	C9	113.(2)
C28	O11	C29	110.(1)	C7	C8	C19	109.(2)
C31	O13	C34	114.(1)	C9	C8	C19	111.(2)
C32	O14	C35	119.(2)	O4	C9	C8	122.(2)
O1	C1	O2	120.(2)	O4	C9	C10	119.(2)
O1	C1	C2	128.(2)	C8	C9	C10	119.(2)
O2	C1	C2	112.(2)	C9	C10	C11	122.(2)
C1	C2	C3	120.(2)	C10	C11	C12	121.(2)
C2	C3	C4	126.(2)	O3	C12	C11	115.(1)
C3	C4	C5	116.(1)	O3	C12	C13	60.(1)
C3	C4	C17	110.(1)	C11	C12	C13	128.(2)
C5	C4	C17	109.(2)	O3	C13	C12	58.(1)
O6	C5	C4	105.(1)	O3	C13	C14	118.(2)
O6	C5	C6	114.(1)	C13	C14	C15	103.(2)
C5	C6	C7	111.(1)	C13	C14	C20	104.(1)
C5	C6	C18	112.(2)	C15	C14	C20	117.(2)
O2	C15	C14	105.(1)	C21	C25	C24	108.(1)
O2	C15	C16	107.(1)	O10	C28	O11	110.(1)
C14	C15	C16	109.(2)	O10	C28	C32	105.(1)
O10	C20	C14	112.(2)	O11	C28	C32	110.(2)
O6	C21	O7	107.(1)	O11	C29	C30	109.(1)
O6	C21	C25	110.(1)	O11	C29	C33	99.(1)
O7	C21	C25	110.(1)	C30	C29	C33	111.(2)
O7	C22	C23	111.(2)	O12	C30	C29	109.(1)
O7	C22	C27	113.(2)	C29	C30	C31	109.(2)
C22	C23	C24	107.(1)	O13	C31	C30	107.(1)
O8	C24	C23	107.(1)	O13	C31	C32	109.(2)
O8	C24	C25	107.(1)	C30	C31	C32	112.(2)
C23	C24	C25	113.(2)	O14	C32	C28	111.(2)
O9	C25	C21	110.(1)	O14	C32	C31	109.(2)
O9	C25	C24	113.(1)	C28	C32	C31	106.(2)

Numbers in parentheses are estimated standard deviations in the least significant digits.

Table 3. Endocyclic torsion angles of for 16-membered macrolide ring systems

	ω12	ω23	ω34	ω45	ω56	ω67	ω78	ω89
chalcomycin	**172**	**174**	**134**	**-47**	**-77**	**174**	**-39**	**66**
mycinolide IV (cf. **4**)[9b,10c]	-177	177	144	-62	-69	180	-56	-52
mycinamicin I (**3**) derivative[9b]	-176	171	135	-56	-62	176	-66	-62
rosaramicin (**8**)[11]	149	-165	178	-57	-73	165	-51	-69
leucomycin (**11**) derivative[14a]	65	177	-170	-55	-61	156	-61	-90

	ω910	ω1011	ω1112	ω1213	ω1314	ω1415	ω15(*O*)2	ω(*O*)21
chalcomycin	**-180**	**174**	**144**	**-154**	**100**	**-69**	**130**	**-177**
mycinolide IV (cf. **4**)[9b,10c]	169	-175	163	-170	94	-62	112	-167
mycinamicin I (**3**) derivative[9b]	-178	-174	149	-151	94	-65	127	-175
rosaramicin (**8**)[11]	-171	-170	143	-160	101	-60	112	177
leucomycin (**11**) derivative [14a]	174	180	180	-74	78	-84	170	-173

Mycinamicin I (**3**) derivative[9b]: 5-dedesosaminyl-5,4"-*O*,*O*-diacetylmycinamicin I.
Leucomycin (**11**) derivative [13a]: demycarosyl leucomycin A_3 hydrobromide.

Table 4. Table of Least-Squares Planes

Orthonormal Equation of Plane 1

0.4909 X + 0.8710 Y + -0.0162 Z - 3.9670 = 0

0.0062 0.0035 0.0111 0.0916

Crystallographic Equation of Plane

4.4017 X + 20.0243 Y + -0.2119 Z - 3.9670 = 0

0.0560 0.0807 0.1037 0.0916

Atom	X	Y	Z	Distance	Esd
C21	2.8569	2.8018	7.2578	-0.2414	+- 0.0170
O7	3.0677	3.2813	8.5530	0.2589	+- 0.0105
C22	3.7584	2.3591	9.3492	-0.2182	+- 0.0200
C23	5.2019	2.0449	8.7627	0.2262	+- 0.0192
C24	4.9977	1.5981	7.3687	-0.2407	+- 0.0176
C25	4.1975	2.5572	6.5516	0.2152	+- 0.0166

Chi Squared = 1426.7

Orthonormal Equation of Plane 2

0.3741 X + 0.7499 Y + -0.5457 Z - 1.2066 = 0

0.0075 0.0050 0.0071 0.1117

Table of Least-Square Planes (cont.)

Crystallographic Equation of Plane

3.3537 X + 17.2391 Y + -5.1108 Z - 1.2066 = 0

0.0672 0.1158 0.0668 0.1117

Atom	X	Y	Z	Distance	Esd
C28	-8.9165	9.3380	5.0203	-0.2788	+- 0.0182
O11	-9.4779	9.4777	3.7877	0.2885	+- 0.0113
C29	-10.9724	9.6042	3.9224	-0.2491	+- 0.0206
C30	-11.2623	10.8759	4.5908	0.2313	+- 0.0167
C31	-10.5701	10.9067	5.9360	-0.2206	+- 0.0179
C32	-9.0493	10.6534	5.8071	0.2286	+- 0.0208

Chi Squared = 1501.2

Dihedral Angles Between Planes:

Plane No.	Plane No.	Dihedral Angle
1	2	32.26 +- 0.97

Table 5. Crystallographic Data for Chalcomycin

formula	$C_{35}H_{56}O_{14}$	d_{calc}, gm/cm^3	1.30
formula wt. g/mol	700.8	μ, cm^{-1}	8.3
crystal system	monoclinic	total data	1698
space group	$P2_1$	unique data	1557
a, Å	8.965(3)	R_{merge}	0.035
b. Å	22.989(9)	obs data (F>3σ(F))	1006
c, Å	9.280(2)	R	0.068
ß, deg	90.76(2)		
V, Å^3	1912(1)		
Z	2		
radiation, Å	1.54184		

Table 6. Table of Positional Parameters and Their Estimated Standard Deviations

Atom	x	y	z	B(A2)
O1	-0.433(1)	0.1867(6)	0.441(1)	4.9(4)
O2	-0.410(1)	0.2835(5)	0.407(1)	4.2(3)
O3	-0.628(1)	0.3467(7)	0.746(2)	6.5(4)
O4	-0.260(1)	0.2365(7)	1.067(2)	6.4(4)
O5	0.022(2)	0.2446(7)	1.133(1)	6.2(4)
O6	0.248(1)	0.1633(5)	0.711(1)	3.0(3)
O7	0.356(1)	0.1426(5)	0.922(1)	3.2(3)
O8	0.714(1)	0.0657(6)	0.728(1)	5.4(4)
O9	0.451(1)	0.0917(5)	0.561(1)	3.4(3)
O10	-0.829(1)	0.3978(5)	0.526(1)	3.7(3)
O11	-1.051(1)	0.4121(5)	0.408(1)	3.9(3)

Table of Positional Parameters and Their Estimated Standard Deviations (cont.)

Atom	x	y	z	B(A2)
O12	-1.404(1)	0.4786(6)	0.509(2)	6.4(4)
O13	-1.232(2)	0.4303(5)	0.722(1)	5.8(4)
O14	-0.940(2)	0.4587(7)	0.761(2)	7.8(5)
C1	-0.351(2)	0.2311(8)	0.438(2)	3.0(5)
C2	-0.194(2)	0.2348(7)	0.474(2)	2.1(4)
C3	-0.123(2)	0.1894(8)	0.520(2)	3.0(5)
C4	0.041(2)	0.1852(8)	0.552(2)	2.9(5)
C5	0.088(2)	0.1552(8)	0.701(2)	3.3(5)
C6	0.004(2)	0.1778(8)	0.829(2)	4.8(6)
C7	0.065(2)	0.2381(8)	0.878(2)	4.0(5)
C8	-0.019(2)	0.2702(9)	0.999(2)	3.7(5)
C9	-0.185(2)	0.2656(9)	0.984(2)	3.4(5)
C10	-0.260(2)	0.294(1)	0.861(2)	4.4(6)
C11	-0.403(2)	0.2900(9)	0.838(2)	5.1(6)
C12	-0.482(2)	0.3256(8)	0.712(2)	3.6(5)
C13	-0.613(2)	0.3079(9)	0.620(2)	4.4(6)
C14	-0.630(2)	0.3333(9)	0.475(2)	3.3(5)
C15	-0.570(2)	0.286(1)	0.378(2)	4.8(6)
C16	-0.589(3)	0.306(1)	0.214(3)	7.9(9)
C17	0.117(2)	0.154(1)	0.431(2)	6.4(7)
C18	0.005(2)	0.1353(9)	0.953(2)	4.3(6)
C19	0.030(2)	0.3324(9)	1.004(3)	5.5(6)
C20	-0.795(2)	0.346(1)	0.463(2)	4.8(6)
C21	0.330(2)	0.1216(8)	0.783(2)	2.6(5)
C22	0.433(2)	0.1023(9)	1.008(2)	5.6(6)
C23	0.593(2)	0.0883(9)	0.944(2)	4.2(6)
C24	0.569(2)	0.0692(8)	0.794(2)	2.9(5)
C25	0.478(2)	0.1110(8)	0.706(2)	2.7(5)
C26	0.775(3)	0.012(1)	0.722(3)	8.8(8)
C27	0.438(3)	0.121(1)	1.156(2)	7.2(7)
C28	-0.987(2)	0.4060(8)	0.541(2)	3.6(5)
C29	-1.217(2)	0.418(1)	0.422(2)	5.1(6)
C30	-1.249(2)	0.4733(8)	0.494(2)	3.3(5)
C31	-1.170(2)	0.4742(8)	0.640(2)	4.4(5)
C32	-1.001(2)	0.463(1)	0.626(2)	6.1(7)
C33	-1.261(2)	0.420(1)	0.263(2)	5.4(6)
C34	-1.291(3)	0.449(1)	0.860(2)	8.4(8)
C35	-0.784(3)	0.480(1)	0.786(4)	13(1)
H1	-0.137	0.273	0.471	3.7*
H2	-0.183	0.153	0.529	4.9*
H3	-0.205	0.315	0.782	6.5*
H4	-0.465	0.268	0.905	7.2*
H5	0.090	0.224	0.561	4.7*
H6	0.051	0.115	0.701	4.2*
H7	-0.097	0.187	0.803	7.1*
H9	-0.414	0.357	0.701	6.0*
H10	-0.695	0.281	0.620	11.2*
H11	-0.589	0.376	0.474	8.4*
H12	-0.829	0.345	0.352	5.0*
H13	-0.852	0.312	0.495	5.0*
H14	0.269	0.085	0.781	4.3*
H15	0.382	0.064	1.007	7.5*
H16	0.506	0.033	0.785	4.7*
H17	0.534	0.150	0.711	5.5*
H18	-0.964	0.498	0.564	10.8*
H19	-1.274	0.388	0.457	8.8*
H20	-1.206	0.510	0.440	5.9*
H21	-1.184	0.510	0.699	10.8*
H22	-1.184	0.510	0.699	10.8*

Table of Positional Parameters and Their Estimated Standard Deviations (cont.)

Atom	x	y	z	B(A2)
H23	-0.563	0.278	0.136	10.6*
H24	-0.693	0.319	0.181	10.6*
H25	-0.530	0.341	0.191	10.6*
H26	0.221	0.152	0.435	7.9*
H27	0.075	0.117	0.417	7.9*
H28	0.092	0.175	0.337	7.9*
H29	-0.035	0.097	0.918	5.7*
H30	0.105	0.127	0.985	5.7*
H31	-0.053	0.147	1.030	5.7*
H32	0.337	0.129	1.198	7.4*
H33	0.486	0.097	1.229	7.4*
H34	0.878	0.008	0.691	11.7*
H35	0.488	0.160	1.168	7.4*
H36	0.784	-0.008	0.825	11.7*
H37	0.718	-0.017	0.671	11.7*
H38	-1.248	0.383	0.214	9.1*
H39	-1.370	0.429	0.249	9.1*
H40	-1.211	0.449	0.207	9.1*
H41	-1.337	0.420	0.917	8.1*
H42	-1.212	0.466	0.923	8.1*
H43	-1.365	0.481	0.854	8.1*
H44	-0.754	0.475	0.872	17.2*
H45	-0.723	0.463	0.711	17.2*
H46	-0.785	0.523	0.757	17.2*
H47	0.173	0.243	0.903	6.3*
H48	0.054	0.264	0.792	6.3*
H49	0.134	0.339	1.013	7.0*
H50	-0.003	0.354	0.916	7.0*
H51	-0.017	0.355	1.082	7.0*

Starred atoms were refined isotropically. Anisotropically refined atoms are given in the form of the isotropic equivalent displacement parameter defined as:
(4/3) * [a2*B(1,1) + b2*B(2,2) + c2*B(3,3) + ab(cos gamma)*B(1,2) + ac(cos beta)*B(1,3) + bc(cos alpha)*B(2,3)]

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21. (a) It has been demonstrated that C6 hydroxylation of 6-dEB (**23**) occurred with configuration retention, late in the biosynthetic pathway (Corcoran, J. W.; Vygantas, A. M. *Biochemistry* **1982**, *21*, 263). (b) Similarly, for the stereochemical models **2, 21** and **22**, any "extra" hydroxyl group at a given chiral center are considered introduced with retention of configuration at a late stage (after cycle 6 in Fig. 5b). (c) Seno, E.T.; Baltz, R.H. Antimicrob. Agents Chemother. **1981**, *20,*

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22. (a) The presence a tertiary methyl-substituted C8 hydroxyl grouping in chalcomycin apparently is a feature that has not been reported in the other 16-membered basic macrolides. However, it has been found in the 14-membered lankamycins, Kujimycin A, and 23672RP,[1b] and is analogous to the epoxide structure of the 14-membered oleandomycin (**24**; Fig. 4). (b) The established chirality at C8 of chalcomycin allows assignment to the corresponding center in the other neutral 16-membered macrolides (**15**, **17**, Fig. 3) with certainty.

23. In the application of the empirical models **2** and **21** for predictive purpose, it should noted that their validity depends on the consistency of a hierarchy of biochemical choices during PKS synthesis. In spite of the substantial recent advances, the factors responsible for the sophisticated control of these choices are still unknown. Also, the selective processing of chain elongation intermediates such as **25** and **26** by the different macrolide polyketide synthase proteins suggests the presence of a recognition process. The interplay between molecular recognition and genetic programming in polyketide synthesis remains to be elucidated.[2e]

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27. (a) Some crystallographic properties of chalcomycin, reported previously (Krc. J., Jr.; Scott, R. B. *Microscope* **1975**, *23*, 15; *C.A.* **1975**, *83*. 51293u), were particularly useful in determining the molecular weight of the compound. (b) A previous attempted single-crystal X-ray crystallographic analysis of chalcomycin as its heavy atom derivative was unsuccessful. The derivative, bis-bromoacetyl chalcomycin (crystals from methanol, mp 114-119 ^{o}C) lost its crystallinity under the X-ray beam (George A. Sim, unpublished results, 1966).

28. Deposited at the Cambridge Crystallographic Data Centre.

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