STEREOCHEMICAL PROBLEMS IN MACROLIDE ANTIBIOTICS

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ABSTRACT

Since 1950, well over fifty macrolides have been derived from various *Actinomycetes* in screening laboratories located at many corners of the world. Among the most intensely studied macrolides are those produced on a commercial scale (erythromycin, oleandomycin, tylosin, leucomycin and spiramycin) as well as examples of particular historical/scientific importance (Magnamycin, pikromycin, narbomycin, methymycin). As a result of chemical studies by numerous investigators, many macrolides are now structurally defined. Their overall constitutional structures reveal an unusual wealth of stereochemical features involving numerous asymmetric centres and conformational possibilities among 12-, 14- or 16-membered lactone rings containing an array of substituents including one, two or three glycoside units.

Fruitful approaches to the special macrolide sugar problems have followed classical carbohydrate stereochemistry while numerous aglycone asymmetric centres have been successfully defined through localization in a variety of diagnostic fragments. X-ray studies are now complete on three macrolide derivatives. Total absolute configurations are ascribed to oleandomycin, erythromycin, Magnamycins, leucomycins, spiramycins, methymycin and pikromycin. Moreover, considerable configurational data are available on the aglycones of narbomycin, neomethymycin, lankamycin and chalcomycin.

Recent structural revisions have greatly simplified the overall stereochemical problem by bringing 'into line' certain biogenetically 'out-of-step' proposals that have involved unusual 17-, 18- and 22-membered rings as well as misplaced sugars. Growing stereochemical knowledge has largely confirmed an earlier biogenetically-based hypothesis that all antibacterial macrolides can be viewed as following the same configurational model regardless of ring size or degree of carbon branching in the aglycone chain. Application of the model to specific macrolides in need of further configurational definition affords likely insight to the chirality at many experimentally unprobed asymmetric centres. Recent conformational analyses of the 14-membered ring system in erythromycin aglycones using 100 MHz nuclear magnetic resonance and circular dichroism techniques indicate that the shape of the molecule in solution is relatively stable and similar to that in the crystal. The conformation most evident for erythronolide B is a modification of the diamond-lattice section model, taken from the geometry of cyclotetradecane. Available evidence suggests that 'isosteric' macrolides (erythromycin, lankamycin and oleandomycin) have similar conformations.

Although the configurational and conformational models for macrolide antibiotics are still undergoing further refinements, they have proven useful during the entire course of their development in testing certain biogenetic and physico-chemical theories. At present, they are finding increasing applica-

tions as aids in explaining and/or predicting a wide variety of macrolide phenomenology involving stereospecificity in biosynthesis, chemical modifications, mode of action and mechanism of drug resistance.

INTRODUCTION

Our work on oleandomycin led us to take an active interest in the structures and stereochemistry of related macrolide antibiotics[1–21]. Typical of the macrolide group[22] under discussion is the growing number of structurally-defined members listed in *Table 1*. All of the examples possess, in common, a

Table 1. Macrolides of defined constitutional structure

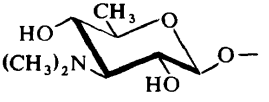
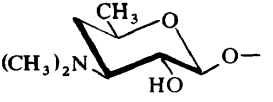
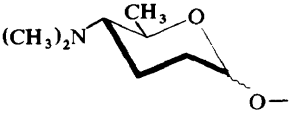
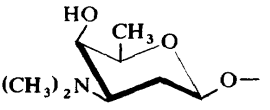
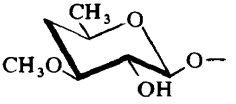
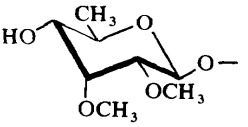
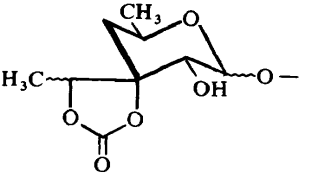
Examples	*Year of definition and references*
Methymycin	(1956)[69]
Erythromycin A† & B	(1957)[80, 81]
Erythromycin C	(1957)[47] (Revised, 1962)[48]
Magnamycin A & B (Carbomycins A & B)	(1957)[50] (Revised, 1965)[51, 52]
Pikromycin	(1957)[72, 73] (Revised, 1968)[74, 75]
Neomethymycin	(1958)[70]
Oleandomycin†	(1960)[5]
Narbomycin	(1962)[71]
Niddamycin	(1962)[53]
Chalcomycin	(1964)[41]
Spiramycins†	(1964)[56] (Revised, 1965)[51] (Re-revised, 1969)[57]
Lankamycin	(1964)[58] (Revised, 1970)[59]
Leucomycins (Kitasamycins)†	(1967)[54] cf. Josamycin† (1970)[55]
Neutramycin	(1969)[42]
Cirramycin A_1	(1969)[44a]
Megalomicin A	(1969)[49]
Kujimycin A	(1969)[60]
B-58941	(1970)[25]
Tylosin†	(1970)[43]
O-Demethyloleandomycin	(1971)[20]

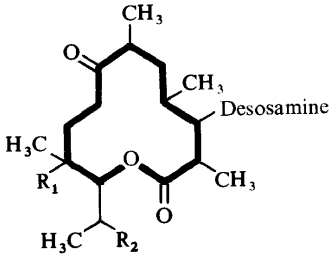
† Produced in various geographical areas on a commercial scale for application in human and/or veterinary medicine.

glycosidically substituted large ring lactone, *Actinomycetes* origins, as well as distinctive activity against bacteria (chiefly Gram-positive) and mycoplasma. Many such macrolides are produced commercially as the free base, various salts, and/or as certain semi-synthetic ester derivatives. There is a continued search for new macrolide products among fermentations, by chemical modifications and through directed biosynthesis.

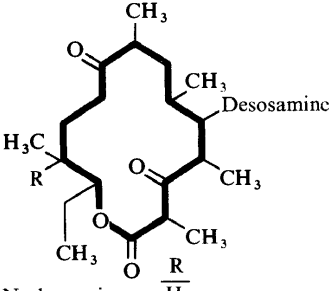
The recent influx of new and revised structural determinations of various macrolides is indicative of widespread chemical interest in the field. The present survey of macrolide constitutional structures (illustrated in *Figure 1*) calls attention to a gradual gradation of molecular features among 12-, 14-, and 16-membered rings and varying degrees of carbon branching, oxygenation, unsaturation and glycoside substitution. Recent revisions to more conventional structures of certain incorrect proposals (some involving 'unorthodox' 17- or 22-membered rings or 'misplaced' sugars) now permit

Table 2. Macrolide D-sugar moieties

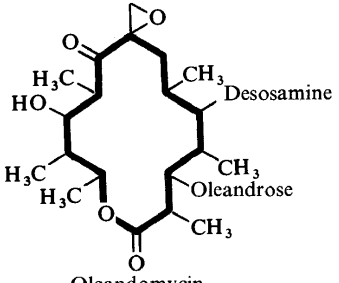
Name	Stereo-structure	Macrolide origin
D-Mycaminose		Magnamycins Leucomycins Cirramycin A_1 Tylosin Niddamycin
D-Desosamine		Methymycins Erythromycins Pikromycin Oleandomycin Narbomycin Megalomicin A *O*-Demethyloleando-mycin
D-Forosamine		Spiramycins
D-Rhodosamine		Megalomicin A
D-Chalcose (Lankavose)		Chalcomycin Lankamycin Neutramycin
D-Mycinose		Chalcomycin Neutramycin Tylosin
D-Aldgarose		Aldgamycin E



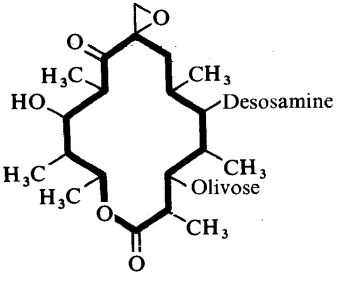
	R_1	R_2
Methymycin:	OH	H
Neomethymycin	H	OH



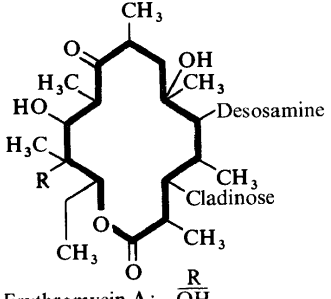
	R
Narbomycin:	H
Pikromycin:	OH



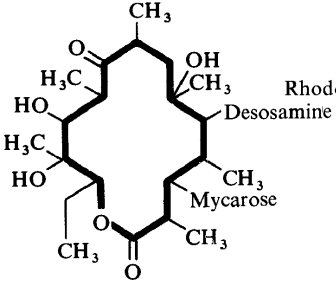
Oleandomycin



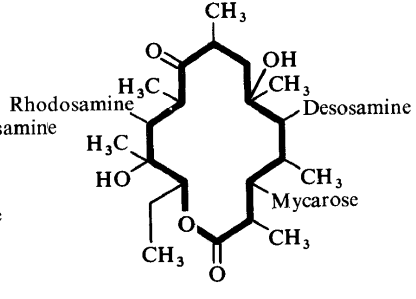
O-Demethyl-oleandomycin



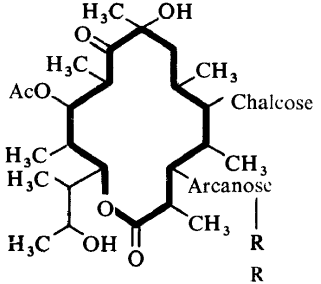
	R
Erythromycin A:	OH
Erythromycin B:	H



Erythromycin C



Megalomicin A



	R
Lankamycin:	Acetyl
Kujimycin A:	H

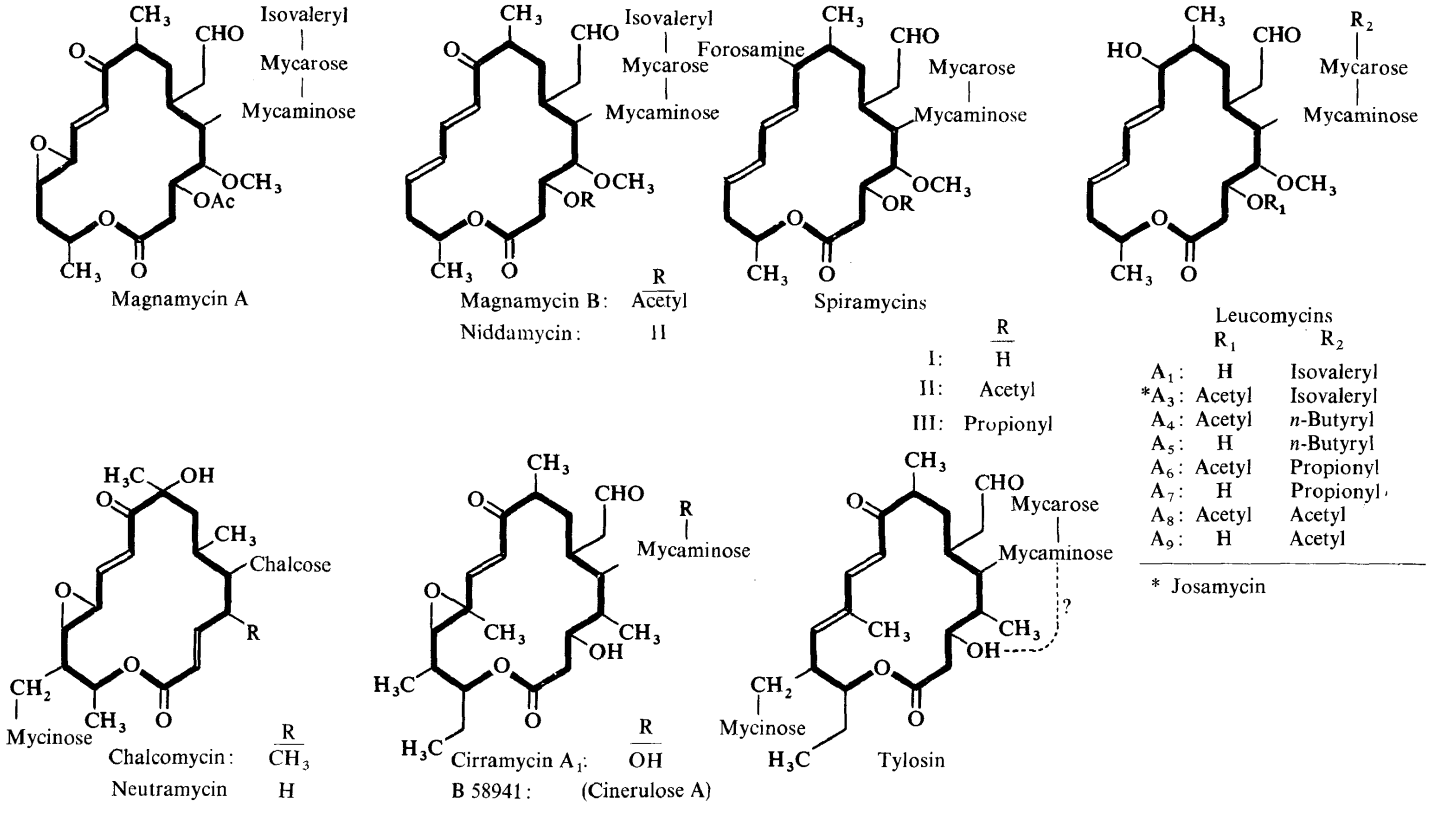
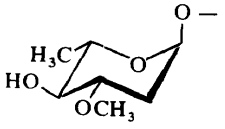
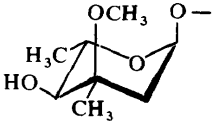
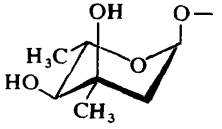
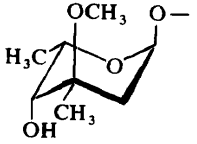
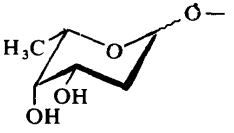
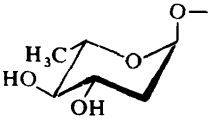


Figure 1. Macrolide constitutional structures

Table 3. Macrolide L-sugar moieties

Name	Stereo-structure	Macrolide origin
L-Oleandrose		Oleandomycin also Oleandrin (non-macrolide)
L-Cladinose		Erythromycin A Erythromycin B
L-Mycarose		Magnamycins Leucomycins Erythromycin C Megalomicin A Tylosin Niddamycin
L-Arcanose		Lankamycin Kujimycin
2,3,6-Trideoxy-hexopyranos-4-ulose (cf. L-Cinerulose A)		B58941 also Cinerubine A (non-macrolide)
L-Oliose (L-2-Deoxyfucose)		Azalomycin-B
L-Olivose		*O*-Demethyloleando-mycin

the drawing of a far less complicated overall picture than was portrayed several years ago[17]. As macrolide gross structures first emerged, the vast

array of remaining stereochemical problems was recognized but substantial progress toward their solutions did not start until the beginning of the past decade.

The following outline of specific stereochemical problems closely corresponds to the order in which they were attacked.

1. Macrolide sugars (a) *Liberated state*—Absolute configuration (chirality) at all asymmetric centres except at carbon-one (irrelevant anomeric centre) and conformational habit of separate simple anomeric glycosides; (b) *Macrolide glycosidically bound state*—Chirality at carbon-one (fixed anomeric centre) and preferred conformation.

2. Macrolide aglycones (a) *Various non-epimerized states*—Chirality at all asymmetric centres; (b) *Intact lactone state*—Conformational habit of parent and modified forms.

MACROLIDE SUGAR STEREOCHEMISTRY (Tables 2 and 3)

Liberated sugars and their sources

Most of the macrolide sugars proved to be new pyranosides at the time of their discovery among macrolide hydrolysis products. The neutral sugar we obtained from oleandomycin was exceptional; it was identified as L-oleandrose[3] a known sugar derived much earlier from the steroid glycoside oleandrin[23] and shown to be 3-*O*-methyl-2,6-dideoxy-L-*arabino*-hexose[24]. It now appears that L-oleandrose was a forerunner of macrolide sugars that occur elsewhere in products of natural origin. Thus, the recently disclosed 2,3,6-trideoxyhexopyranos-4-ulose (*Table 3*) from antibiotic B-58941[25] may be identical with L-cinerulose A which was announced shortly afterwards as a component of the non-macrolide cytotoxic antibiotic cinerubine A[26]. This compound has more of the earmarks of a biosynthetic intermediate than a 'finished' sugar; the 4-keto group in L-cinerulose A affords another glimpse of how the L-series can arise biosynthetically from the D-series. Other recent macrolide sugars D-rhodosamine (*Table 2*) from megalomicin A[27], L-oliose from structurally-undefined azalomycin-B[28] and our newly announced L-olivose from *O*-demethyloleandomycin[20] (*Table 3*) all have antipodal counterparts elsewhere, cf. cinerubine A[26] and similar cytotoxic antibiotics rhodomycins[29], olivomycins[30], chromomycins[31], and mithramycin[32]. Even the strange sugar D-aldgarose[33] (*Table 2*) from the structurally uncharacterized macrolide aldgamycin E[34] has a relative (L-tridesoxyoctose) as a constituent of the antibiotics quinocycline A and isoquinocycline A[35]. Grisebach[36] has included the macrolide sugars L-cladinose[37], L-mycarose[37] and L-arcanose[38], as well as D-aldgarose, in a recent review on branched-chain carbohydrates from natural sources.

The neutral sugars D-chalcose[39] and D-mycinose[40] were initially associated with chalcomycin[41], one of the first so-called 'neutral' macrolides, cf. related neutramycin[42]. D-Mycinose also occurs in tylosin[43] which goes along with tylosin's appearance as sort of a hybrid chalcomycin and cirramycin A_1[44]. Among the D-series (*Table 2*), D-forosamine[44], D-rhodosamine and D-mycinose are looked upon as 'optional' sugars located at 'odd' sites, whereas D-mycaminose[45], D-desosamine[46] and D-chalcose are considered 'obligatory' sugars appearing at 'standard' sites. Mycaminose has the added distinction

of having, in most cases, an α-L-pyranosyl substituent (usually α-L-mycarosyl) on its C.4 hydroxyl group. The macrolide L-sugars (*Table 3*) are found in most but not all of the macrolides; where they do occur, there appears a choice between two patterns (a) and (b). In pattern (a), the L site is once removed from the 'obligatory' D-site, cf. L-oleandrose, L-cladinose, L-arcanose, L-olivose in their macrolide origin (*Figure 1*). In pattern (b), the L-site is on C.4 hydroxyl group of D-mycaminose. L-Mycarose is unique in following either pattern (a), cf. erythromycin C[47,48], Megalomicin A[49] origin or pattern (b), cf. Magnamycin[50,51], niddamycin[53], leucomycin[54], (josamycin[55]), tylosin and spiramycins[56,51,57]. The new 2,3,6-trideoxyhexopyranos-4-ulose is expected to follow L-mycarose analogy in pattern (b) and, if so, it is identical with L-cinerulose A. *O*-acyl substituents *may* be found on C.4 hydroxyl group (acetyl, isobutyryl etc., cf. leucomycins) or of L-arcanose, cf. lankamycin[58,59] (acetyl) and kujimycin A[60] (free hydroxyl).

The stereo-structure determination of each new macrolide sugar represents a noteworthy achievement in its own right. With the advent of n.m.r. spectral analysis, rapid progress was made in determining relative configurations in stable chair conformations. The chirality of most macrolide sugars has been established by stereospecific synthesis; overall available information permits the generalized representations shown in *Tables 2* and *3*. The extent to which individual sugars may depart from the idealized chair forms is a subject of growing interest because of the important contributions these sugars make to the biological activity of their macrolide of origin.

The newly recognized macrolide sugars continue to appear as consistent 6-deoxy D- or 2,6-dideoxy L-forms but there is no striking overall configurational pattern among non-anomeric asymmetric centres. However, it so happens that existing substitution situations are such that sugars of the D- and L-series favour C1 and 1C (Reeves designation)[61] chair forms, respectively. Thus, there is, after all, sufficient configurational order among all macrolide sugars as to dictate a conformational order, i.e. D-(C1) and L-(1C), among their simple α- and β-glycosides.

Macrolide-bound sugars

After determining the β-D- and α-L-anomeric nature of desosamine and oleandrose, respectively, in oleandomycin[11] we noticed that this same sort of pattern (Klyne's Rule)[62] was previously observed among steroid glycosides[14]. Exceptions to Klyne's Rule had been pointed out[63] but we further observed that the rule held well in the steroid series if its application was limited to glycosides containing 6-deoxy sugars. Thought that the rule might generally apply to the macrolide glycosides was confronted by an earlier publication[64] claiming a β-L-cladinoside situation in erythromycin A. Since a β-D-, α-L-pattern has a rational biogenetic basis[12] we decided to explore the anomeric nature of other macrolide glycosides and to reinvestigate the non-conforming case using n.m.r. and/or molecular rotation difference methods. Our results showed that a β-D-, α-L-pattern evidently existed in all the macrolides studied, including erythromycin A and erythromycin B[14,16]. Our revision of β-L to α-L in erythromycin was later corroborated by an x-ray structural analysis. To date, there are no known exceptions to the β-D, α-L rule; experimental support is lacking in only the few instances

indicated in *Tables 2* and *3*. Similarly, all evidence supports the same gross conformations for macrolide-bound sugars as are seen in their simple glycosides.

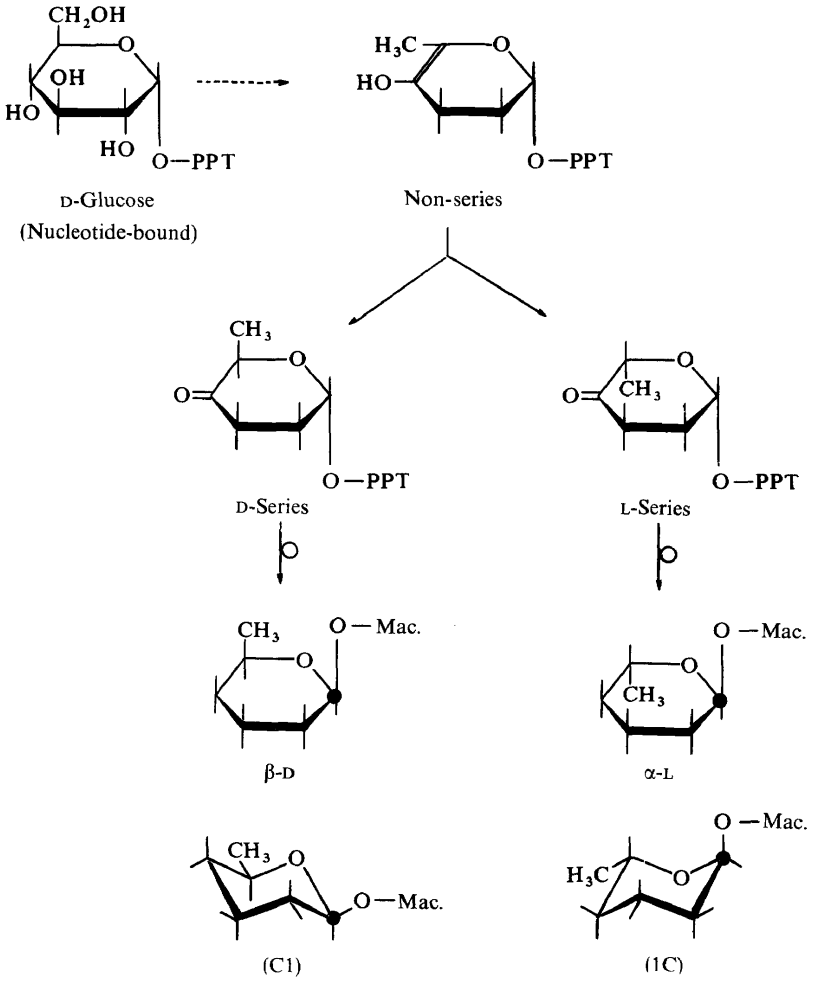


Figure 2. Plausible biosynthetic basis for the β-D, α-L rule

The plausible biochemical basis for the β-D, α-L rule outlined in *Figure 2* takes the following notes into account:

(a) Evidence to date points to D-glucose as the precursor in the biosynthesis of all macrolide sugars[65].

(b) Examples of unusual branching are the result of additions, e.g. *C*-methyl from methionine[36] and *C*-hydroxyethyl from pyruvate[33].

(c) D-Glucose is bound to a nucleotide (e.g. thymidine diphosphate) in conjunction with its enzymic conversion to 6-deoxy sugars and ultimate transfer to a macrolide site[66–68].

In *Figure 2*, D-glucose is shown bound to a nucleotide (PPT) in an arbitrary attractive α-D-manner. In any event, the anomeric centre remains fixed following a series of transformations elsewhere in the molecule involving a 'non-series' common intermediate for either the D- or L-series. Assuming a common stereospecific transferase mechanism (in this case involving inversion at the anomeric centre), the macrolide (Mac.) receives D- or L-sugar in identical manner, i.e. the chirality at carbon-one is the same in β-D and α-L. As previously discussed, the nature of the overall configuration involving the remaining substituents (not specified for simplicity) dictates the conformations shown, (C1)β-D and (1C)α-L.

MACROLIDE AGLYCONE STRUCTURAL STEREOCHEMISTRY

Chirality at asymmetric centres

The growing extent to which the numerous macrolide aglycone asymmetric centres have been experimentally defined, during the past seven years, is exemplified by *Tables 4* and *5*. Although the output of the pioneering effort during the earlier (1956–1963) period was sparse, the available data and their interpretations provided powerful stimuli for thought. The centres

Table 4. Reported configurational data (1956–1963)

Macrolide	Absolute	Relative	References
Methymycin	4S:6R	C.2, C.3, C.4, C.6 same[a]	76
Neomethymycin			77
Narbomycin	6S:8R	C.4, C.5, C.6, C.8	71
Erythromycin	2S*3R*4R* 8R:10R:13R	*cis*-subst. at C.2, C.4	82, 91
Oleandomycin	6S	*cis*-subst. at C.2, C.4	5
Magnamycin	9S*	*cis*-subst. at C.13, C.14	22a

[a] Includes pikromycin, then of unsettled structure.
* Subsequently revised.

noted in *Table 4* were all deciphered from configurational analysis of aglycone degradation products. One of these, the Prelog–Djerassi lactone (*Table 6*), was derived from methymycin[69], neomethymycin[70], narbomycin[71] and pikromycin[72–75] but only two of its four centres were defined at the time[76,77]. This compound was the object of numerous additional studies (1964)[11], (1966)[78] and (1970)[79] before its stereochemistry was finally settled. The approach to aglycone configurations through chemical degradation products has proven fruitful in the cases of other antibiotics as well, e.g. erythromycin A[80], erythromycin B[81], 9-dihydroerythromycin[82], oleandomycin[5,13], Magnamycin[18,22a], chalcomycin[41] and, in retrospect[18], the leucomycins and spiramycins.

A comprehensive list of key macrolide fragments is outlined in *Tables 6a, b, c, d, e, f*. These are portrayed as Fischer projections and, where appropriate, as the conformation evident from n.m.r. analysis. The sources of the

fragments are indicated along with the structure of useful model compounds of known absolute configuration. Many of the fragments have a striking resemblance to certain natural carbohydrates in their appearance, conformational behaviour and optical rotatory power. We were particularly impressed

Table 5. Macrocyclic lactone configurational analyses (1956–1970)

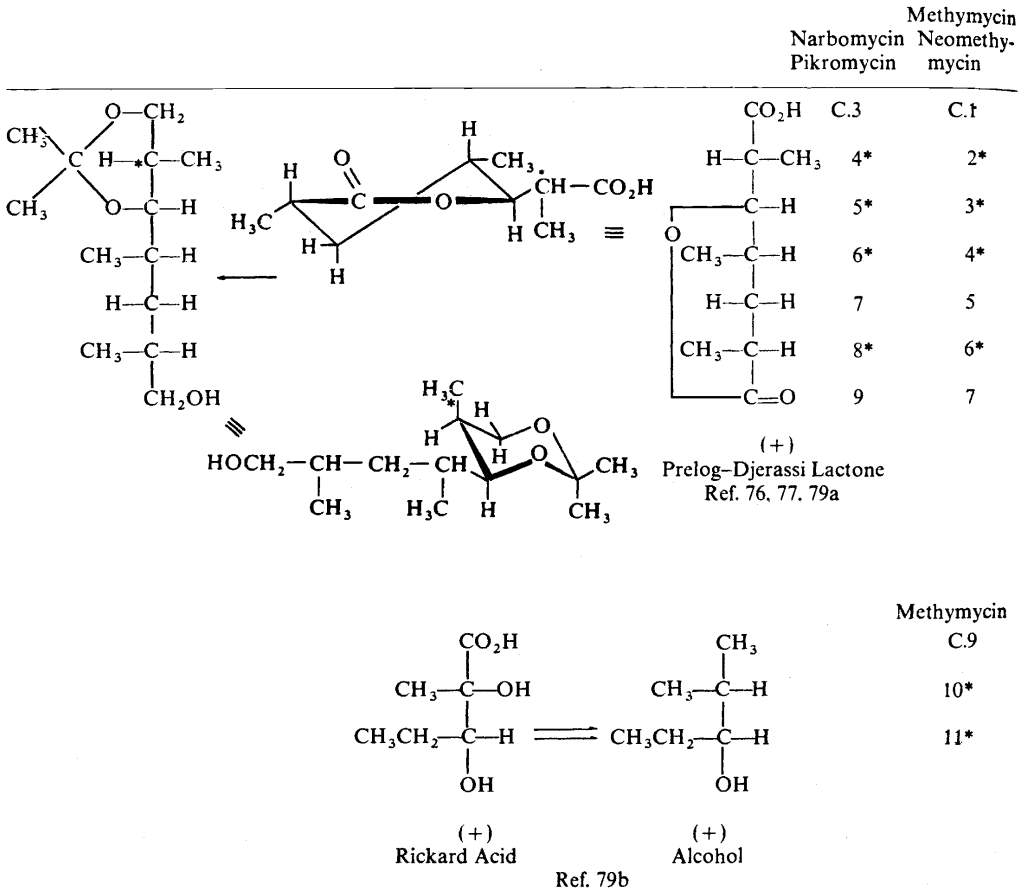
Distinctive macrolide	Ring size atoms	Number chiral centres	Evident chirality at carbon atom No. (*)																References
			2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
Oleandomycin	14	10	R	S	S	S	S	A	R	A	R	S	R	R	A	—	—	—	5, 12, 13
Erythromycin A	14	10	R	S	S	R	R	A	R	A	R	R	S	R	A	A	—	—	14, 82, 83, 91
Erythromycin B	14	10	R	S	S	R	R	A	R	A	R	S	R	R	A	A	—	—	110
Lankamycin	14	12	(R)	(S)	(S)	(S)	(S)	A	(S)	(A)	(R)	(S)	(R)	(R)	(?)	(?)	—	—	59
Pikromycin	14	7	R	A	R	S	S	A	R	A	A	A	S	R	A	A	—	—	76, 77, 79, 89
Narbomycin	14	7	?	A	R	S	S	A	R	A	A	A	?	?	A	A	—	—	76, 77, 79
Methymycin	12	6	R	S	S	A	R	A	A	A	S	R	A	A	—	—	—	—	76, 77, 79
Neomethymycin	12	7	R	S	S	A	R	A	A	A	?	?	?	A	—	—	—	—	76, 77, 79
Chalcomycin	16	8	A	A	S	?	S	A	?	A	A	A	?	?	?	?	A	—	41
Neutramycin	16	8	A	A	A	?	?	A	?	A	A	A	?	?	?	?	A	—	—
Tylosin	16	7	A	?	?	?	?	A	?	A	A	A	A	A	?	?	A	A	—
Cirramycin A_1	16	9	A	?	?	?	?	A	?	A	A	A	?	?	?	?	A	A	—
Magnamycin A	16	8	A	R	R	S	R	A	R	A	A	A	R	S	A	R	A	—	18, 22a, 52
Magnamycin B	16	6	A	R	R	S	R	A	R	A	A	A	A	A	A	R	A	—	18, 22a, 52
Leucomycin A_3	16	7	A	R	R	S	R	A	R	R	A	A	A	A	A	R	A	—	85, 87
Spiramycin II	16	7	A	R	R	S	R	A	R	R	A	A	A	A	A	R	A	—	18, 85, 87

* R, S = Absolute sense; (R), (S) = Relative sense; A = Archiral; (?) = Chirality untested experimentally

with the similarity to D-galactose of the lactone terminus fragment from oleandomycin (*Table 6b*); this provided the first clue to its proven overall D-galacto-configuration. Since such fragments obey the laws of carbohydrate chemistry they are rightfully termed 'semisynthetic' sugars and should be investigated further, in their own right, for interesting sugar chemistry. As a case in point, our 2,4,6-trideoxy-2,4-di(*C*)methyl-D-galactose[12, 13], 'zerviose' (*Table 6c*), crystallizes as the pure β-anomer which, unlike β-D-galactose, shows little or no tendency to mutarotate in water. The pure α-methyl glycoside is uniquely formed via rapid, but controllable, stereospecific inversion during reaction of the β-sugar with methanol (hydrochloric acid catalyst), i.e. the overall inversion followed by equilibrium in glycoside formation is often too rapid to allow direct preparation of a pure anomer. It would be interesting to know whether we are witnessing a specific 2-*C*-methyl effect and, if so, whether it is general among all pyranosides.

On a related tack, many of the linear macrolide fragments resemble the

Table 6a. Macrolide aglycone fragments and model compounds

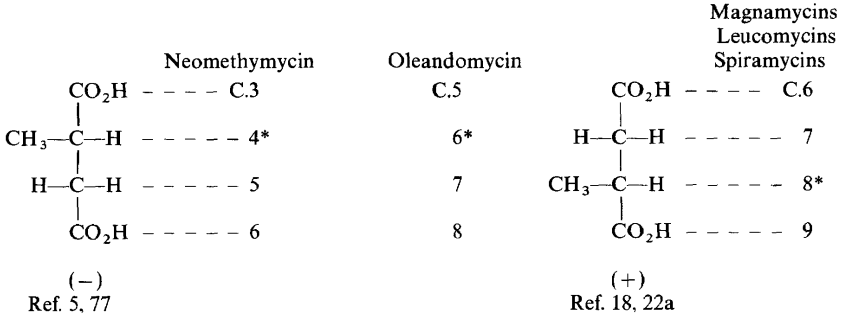


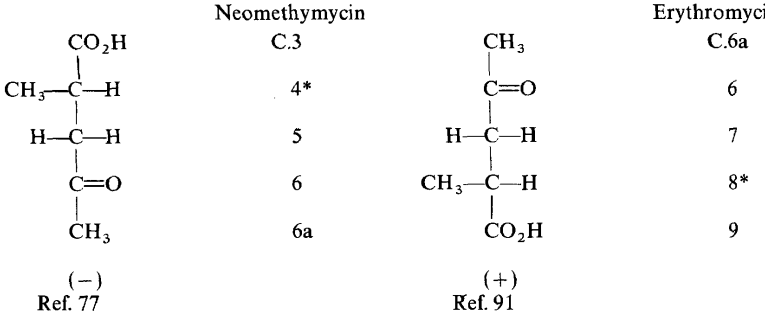
classical carbohydrate polyols which prompted us to treat a C_{10} fragment from oleandomycin as a (–)2,4,6-trideoxy-2,4,6-tri(*C*)methyl-heptitol[12,13], 'jeffitol' (*Table 6d*). This led us to assign an L-*glycero*-L-*ido* configuration using classical heptitols as model compounds. The same C_{10} fragment should be obtainable from lankamycin; such realization would automatically fix the corresponding relative configuration recently observed by Egan and Martin[59] in lankamycin on an absolute basis.

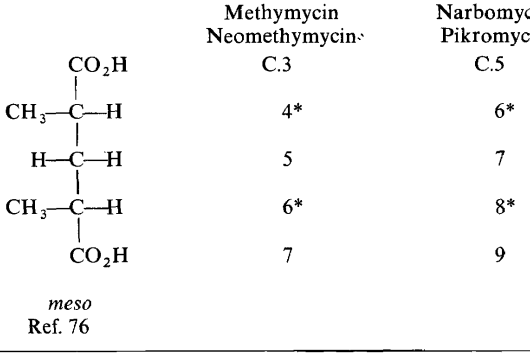
Further comments on many of the fragments outlined in *Table 6* are better postponed for dovetailing with discussion of specific issues later in this report. Otherwise, it is felt that all of the fragments as portrayed speak highly for themselves.

Powerful x-ray crystallographic analysis first entered the macrolide scene in 1965 with the determination of the structure and stereochemistry

Table 6b.



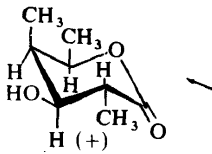




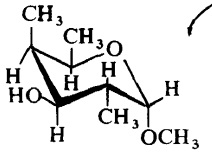
of erythromycin by Harris, McGeachin and Mills[83]. This work confirmed the gross structure announced in 1957 by Wiley and co-workers[80] and placed all reported[14,82] stereochemical studies on erythromycin in an unequivocal absolute perspective (*Figure 3a*).

The second x-ray example, announced briefly in 1967[84] and expanded in 1970[85] by Hiramatsu *et al.*, has provided deep insight into a 16-membered ring aglycone (*Figure 3b*). The authors describe their object of study as demycarosyl leucomycin A_3, but Omura *et al.* have called attention[86,87] to

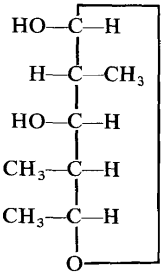
Table 6c



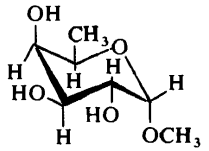
(+)



M_D + 380°

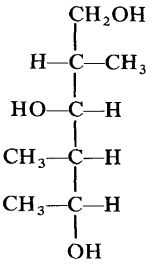


(+)
'Zerviose'
Ref. 13

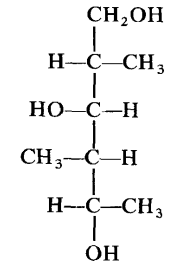


M_D + 380°

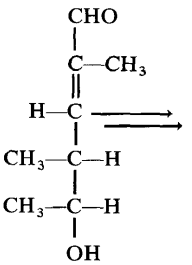
Methyl α-D-Galactoside
Model



(+)
'Zervitol'
Ref. 100

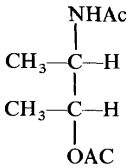


(+)
Cope Triol.
Ref. 100



(+)

Ref. 13

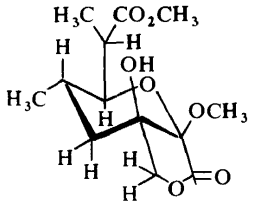


(−)

Ref. 13

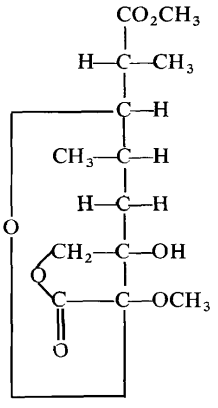
Oleandomycin	
C. 9	
10*	
11*	
12*	
13*	

Amphotericin B	
C.33	
34*	
35*	
36*	
37*′	



β-D (R) (+)
Ref. 5, 13

≡



Oleandomycin

3

4(*)

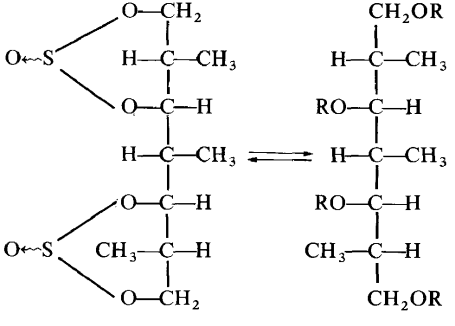
5*

6*

7

8*

10, 9



Four
epimers
Ref. 13

R = H, Ac
H → Ac (−)
'Jeffitol'
Ref. 13

Oleandomycin

1

2*

3*

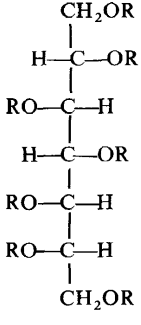
4*

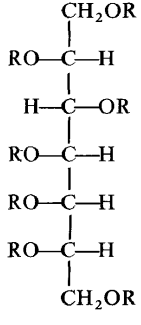
5*

6*

7

Model heptitols





R = H, Ac
H → Ac (−)

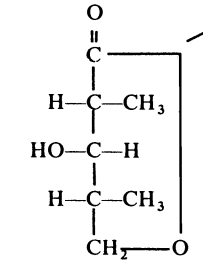
R = H, Ac
H → Ac (+)

Refs. 21, 94

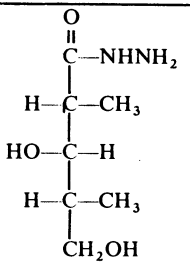
Table 6e

Erythromycin
Oleandomycin

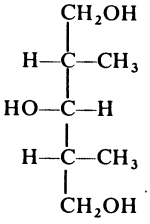
C.1	
2*	
3*	
4*	
5	



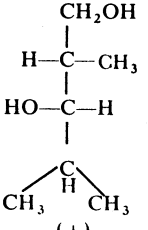
(M_D − 7.2°)
Lactone A
Ref. 14, 82



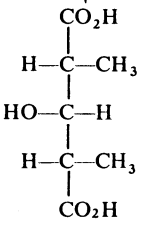
(M_D − 74°)



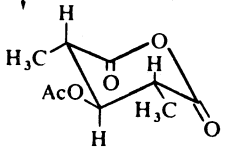
meso



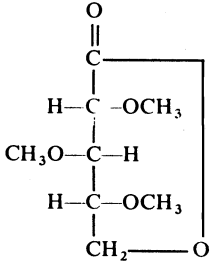
(±)
Batrokov–Bergel'son
Diol Ref. 93



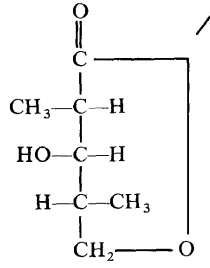
meso



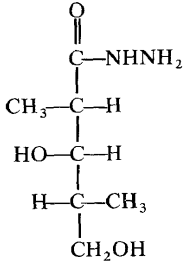
Xylo
Ref. 14



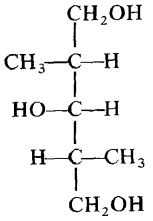
(M_D − 7.6°)
Model
Ref. 14



$(M_D + 81°)$
Lactone B
Ref. 14, 82



$(M_D + 12°)$

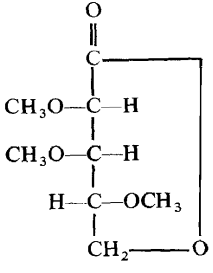


$(M_D - 21°)$
Gerzon triol

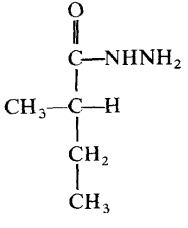
9-Dihydro-erythromycin

C.11
10*
9*
8*
7

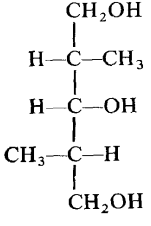
Ref. 14, 82



$(M_D + 68°)$
Model
Ref. 14



$(M_D + 44°)$
Model
Ref. 14, 82

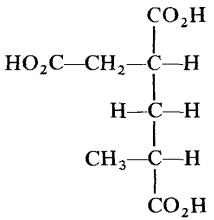
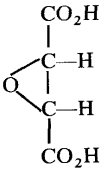
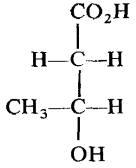
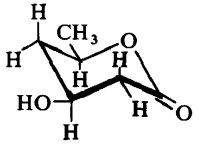
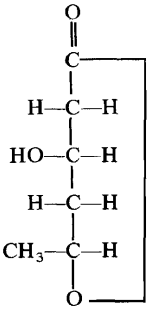
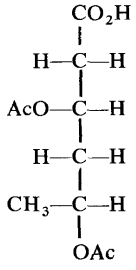


$(M_D + 21°)$
Woo triol
Ref. 41

Chalcomycin

C.3	or	C.7
4		6
5		5
6		4
7		3

Table 6f

	Magnamycins Leucomycins Spiramycins				Magnamycins Leucomycins Spiramycins
	C.5		Magnamycin A C.11		C.13
	6*		12*		14
	7		13*		15*
	8*		14		
	9				
(+) Woodward triacid Ref. 18, 22a, 52		*meso* Ref. 18, 22a		(−) Ref. 18	
					Magnamycin A
					C.11
					12
					13*
					14
					15*
		(+) (Ref. 18)		(−) Ref. 18	

chemical evidence that the compound studied by x-ray was in fact an allylic rearrangement product, demycarosyl-*iso*-leucomycin A_3. Accepting this, the x-ray determination nevertheless presents considerable data regarding the chirality of the β-D-sugar and C.3, C.4, C.5, C.6, C.8, C.15 that are applicable to 'normal' demycarosyl leucomycin A_3. These data favourably take into account fixed β-D-mycaminose in leucomycin, specifications evident at C.6, C.8 and C.15 in Magnamycin B[18] that are applicable to leucomycin A_3 predictions at C.3 and C.5 and reveal for the first time experimental evidence for chirality at C.3, C.4, and C.5. Since Omura *et al.*[87] have assigned the chirality at C.9 in leucomycin A_3 (using optical rotatory rules that appear to be applicable in their case)[88], all of the centres in leucomycin are now defined.

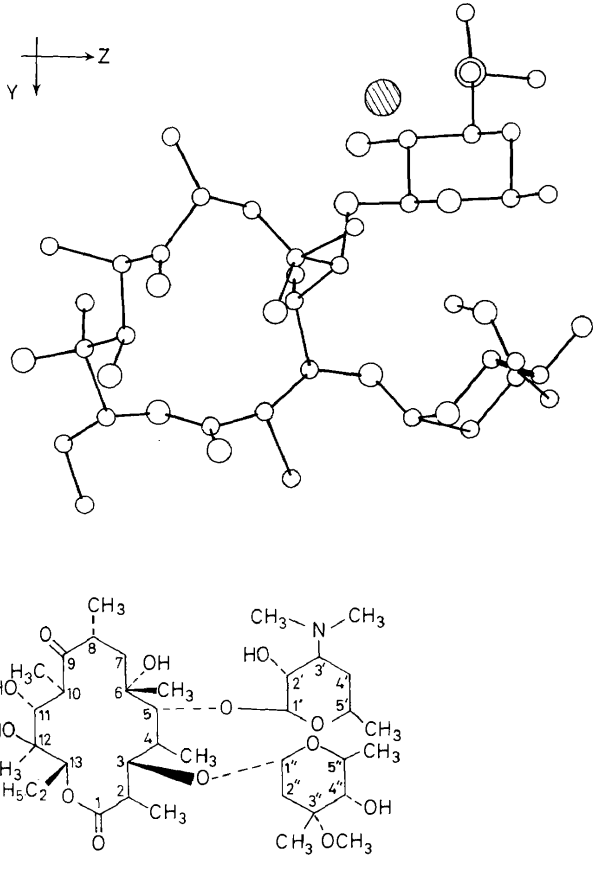


Figure 3. Macrolide x-ray crystal analyses
Figure 3a. Erythromycin A · hydroiodide · dihydrate (Harris *et al.*, 1965)

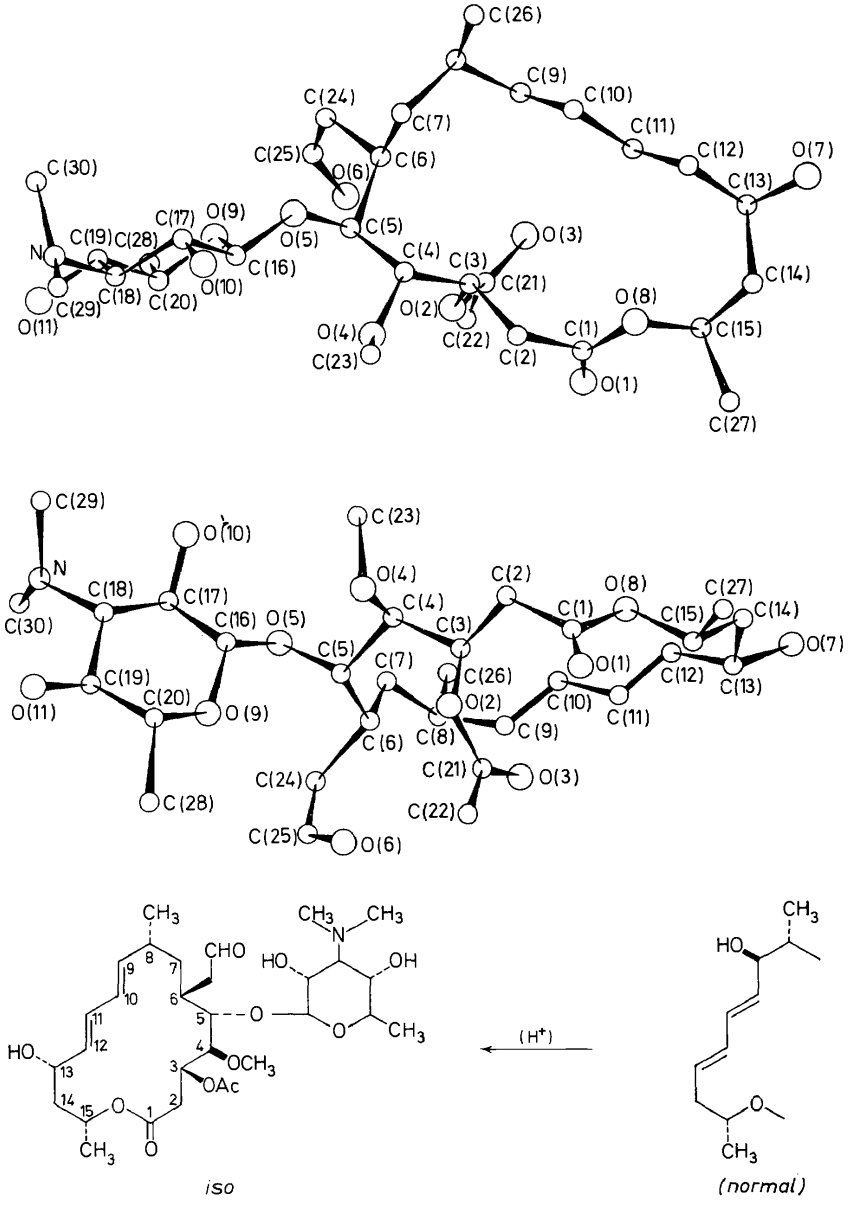


Figure 3b. Demycarosyl *iso*-leucomycin A_3 (Hiramatsu *et al.*, 1967, 1970) (cf. Omura *et al.*, 1968, 1970)

The third x-ray example, Hughes *et al.* (1970)[89], involves kromycin, i.e. $\Delta^{4,5}$ anhydro pikronolide (*Figure 3c*). Since Muxfeldt *et al.*[75] had reported that their attempts to exchange the proton at C.2 in both pikromycin and kromycin with D_2O were unsuccessful, it is a reasonable assumption that

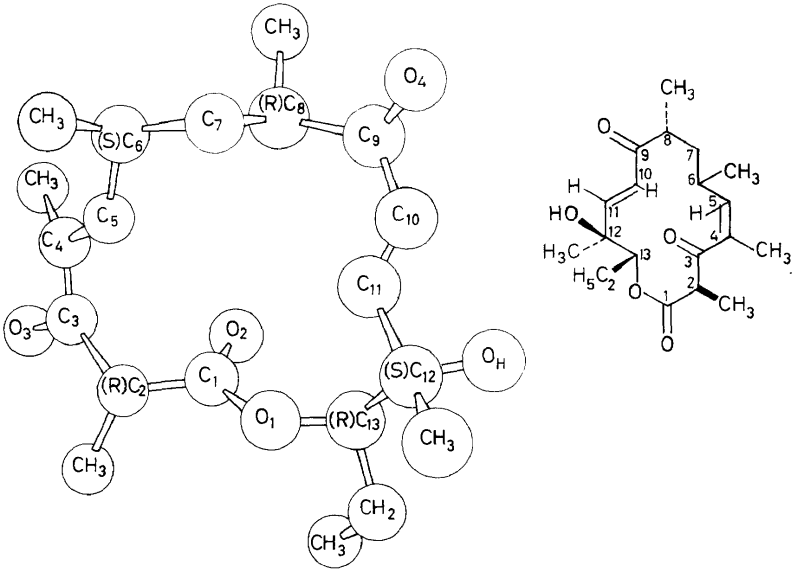


Figure 3c. Kromycin (Hughes *et al.*, 1970)

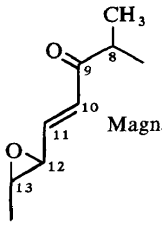
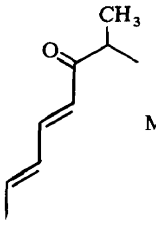
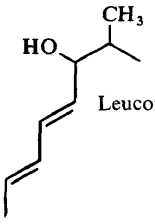
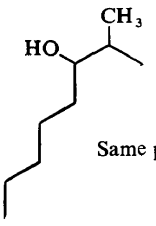
the chirality at C.2 revealed by x-ray is applicable to the parent antibiotic. Other x-ray assignments at C.12 and C.13 unequivocally establish their chirality for the first time considering their orientations relative to previously fixed centres at C.6 and C.8[76,77].

Rickards and Smith[79a] have recently provided chirality specifications applicable to C.4 and C.5 in pikromycin. Accordingly, all asymmetric centres in pikromycin are now defined in an absolute sense. Moreover, Manwaring, Rickards and Smith[79b] have specified C.10 and C.11 in methymycin thereby completing its total absolute configuration.

The few instances where macrolide antibiotics have been chemically interrelated directly or indirectly are listed in *Table 7*. It follows that all asymmetric centres common to Magnamycin A, Magnamycin B, leucomycin A_3 and spiramycin II are identical. Moreover, since the various leucomycins (A_1, A_3, A_4, A_5, A_6, A_7, A_8, A_9) and spiramycins (I, II, III) are distinguished only by different *O*-acylation patterns not involving an asymmetric centre *per se*, all of their configurations are the same.

The above discussion and relationships shown in *Tables 6* and *7* and *Figure 3* have, by and large, accounted for the specifications summarized in *Table 5*.

Table 7. Chemical interrelationships of macrolide antibiotics

			Reference
 Magnamycin A	$\xrightarrow{KI/HOAc}$	 Magnamycin B	22a
 Leucomycin A_3	$\xrightarrow{MnO_2}$	Magnamycin B	57
Leucomycin A_3	$\xrightarrow{2H_2}$ $\xrightarrow[-\text{mycarosyl}]{H_3^+O}$		
Spiramycin II	$\xrightarrow{2H_2}$ $\xrightarrow[-\text{mycarosyl}\ -\text{forosamyl}]{2H_3^+O}$	Same product	57

CONFIGURATIONAL MODEL

Attention is next called to the development of a configurational model for macrolide antibiotics and its application in assigning 'biosynthetically-expected' chirality to aglycone centres. The endocyclic constitutional framework of the present model notably follows Gerzon's *Propionate Rule* (1955)[82,90] which points out the perfect branching pattern in the aglycone of erythromycin. In the earliest effort to decipher the 'configurational code' of a macrolide aglycone, Gerzon *et al.* proposed[82] a configurational model, of 9-dihydroerythronolide, cf. (*1956*) in *Figure 4a.* This (*1956*) model involved tentatively determined chirality at centres C.2, C.3, C.4, C.8, (C.9), C.10, and C.13 and 'arbitrary' chirality at C.5, C.6, C.11, and C.12 (?) given in a manner that the molecule as a whole acquired a *meso* nature (to account

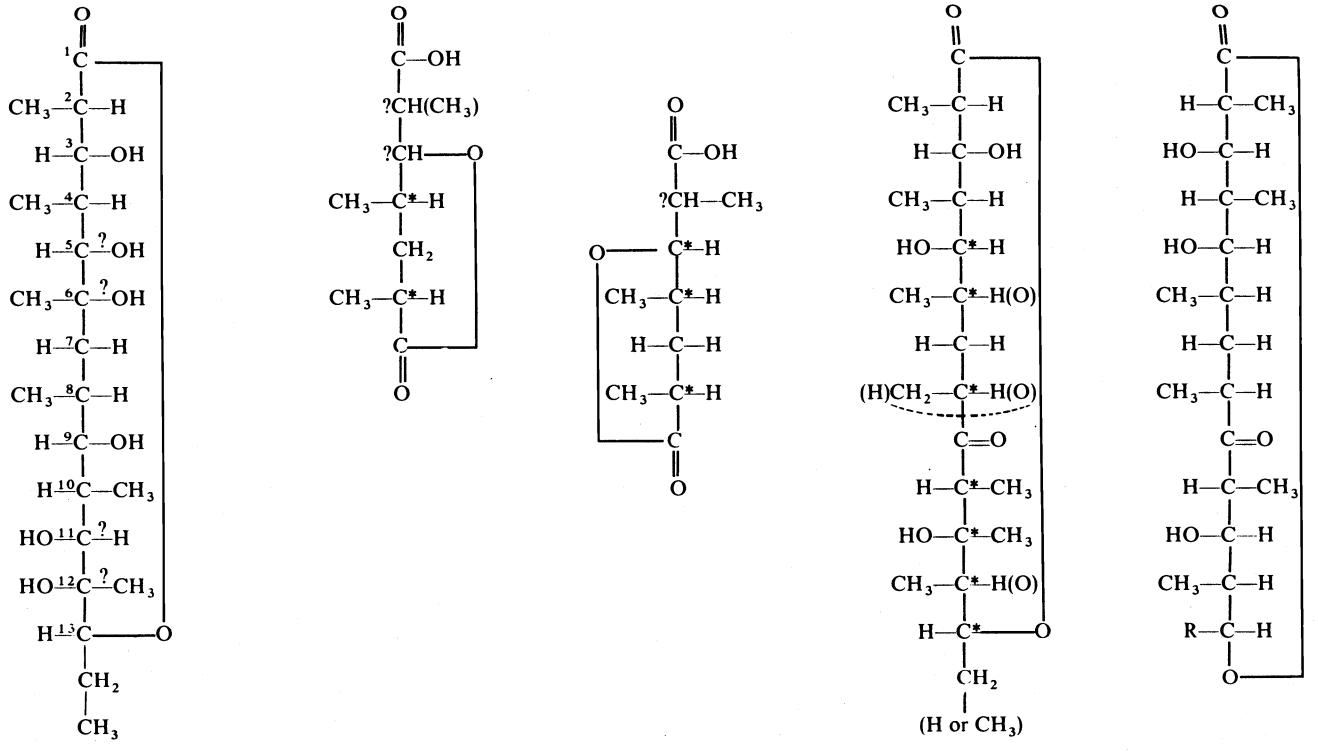


Figure 4. Configurational model for macrolide antibiotics

Figure 4a. Development of the configurational model.

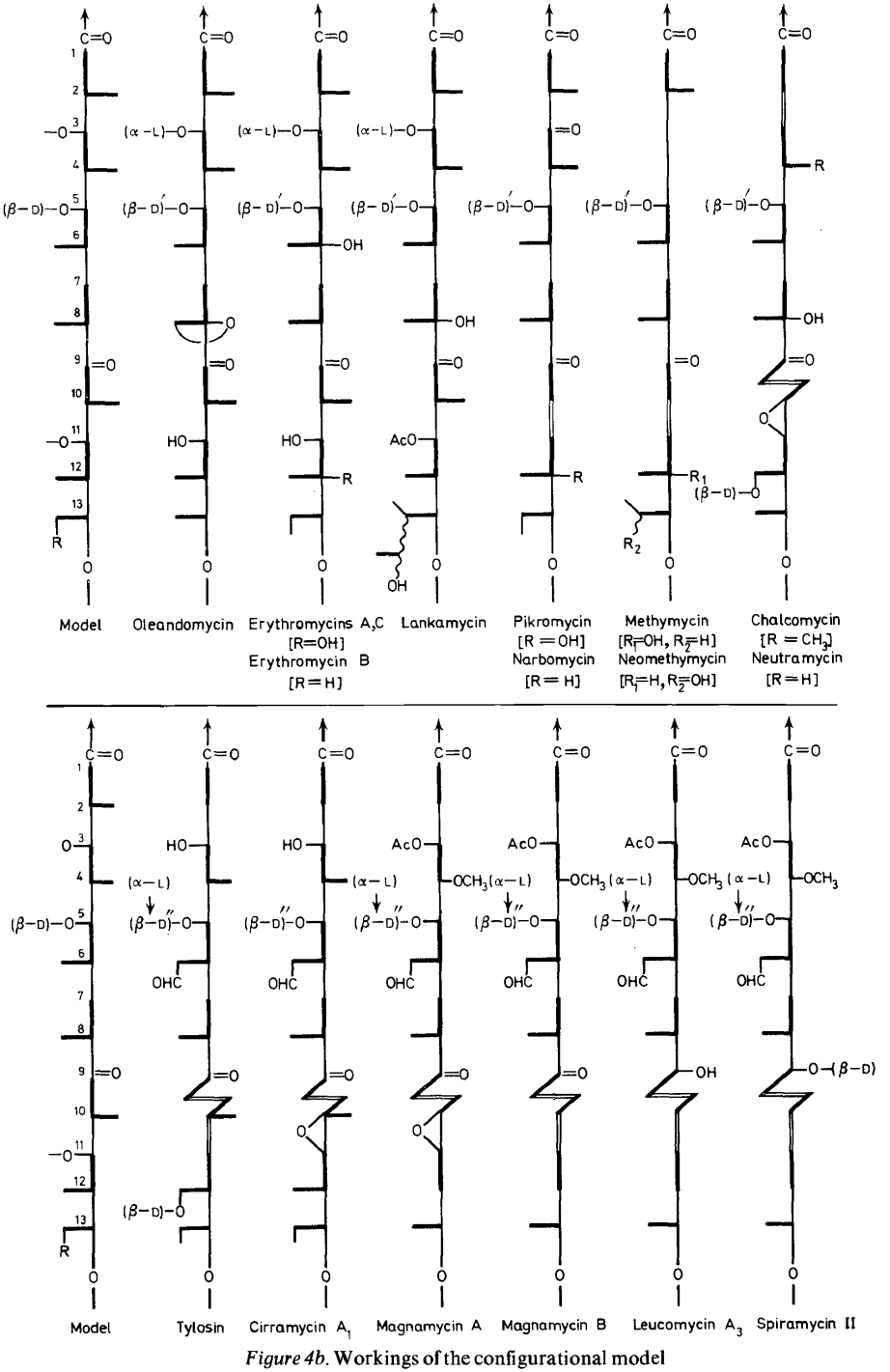


Figure 4b. Workings of the configurational model

for its low optical rotatory value). In a subsequent report on erythromycin, Djerassi *et al.* (1958)[91] unequivocally established the chirality of C.8 and C.10 and reasoned that the chirality at C.2 and C.4 should stand as originally proposed[82] (but the latter was reversed in 1965[14,83]).

Earlier, Djerassi and Halpern (1957)[77] called attention to the Prelog-Djerassi lactone as 'a standard of absolute configuration among macrolide antibiotics'. This model, cf. (*1957*) in *Figure 4a*, was discussed as representing C.1 through C.7 in methymycin and neomethymycin and possibly the same in the then unsettled structures of pikromycin and narbomycin 'provided these antibiotics possessed a hydroxyl group at C.3'. The latter condition was not fulfilled since the completed gross structures of narbomycin (1962)[71] and pikromycin (1968)[74,75] show the lactone to originate from C.3 through C.9 in these macrolides. Subsequently, we discussed in Prague (1964)[11] the possible general applicability of the (*1957*) model viewed according to its narbomycin (*1962*) origin, cf. in *Figure 4a* matching the Prelog–Djerassi lactone with a constitutionally comparable section of erythromycin. We noted further that the (*1962*) model matched C.6 and C.8 configurational data from a key oleandomycin degradation product (cf. Hochstein–Els lactone in *Table 6d*). With additional unequivocal assignments at C.10, C.11, C.12 and C.13 in oleandomycin via another key fragment (cf. 'zerviose' in *Table 6c*), we extended the model completely in the lactone terminus direction. We further noted that the Prelog–Djerassi model was dextrorotatory[11] which indicated a D-configuration according to Hudson's lactone rule, cf. translation in the model (*1964a*), *Figure 4a*. [Observation of dextrorotation for the similarly branched D-lactone (from 'zerviose', *Table 6c*) strongly suggested that Hudson's rule was valid in these cases[12]. In any event, later n.m.r. studies corroborated this assignment in the Prelog–Djerassi lactone]. Relevant to building the model further toward the lactone head, we localized centres C.2, C.3, C.4, C.5 and C.6 from oleandomycin in the optically active, constitutionally symmetrical C_{10}-tetraol 'jeffitol' (*Table 6d*). Further concrete knowledge relevant to the stereochemistry of 'jeffitol' (L-*erythro* C.4, C.5 and either *xylo* or *ribo* C.2, C.3, C.4) greatly limited the possibilities of its overall configuration. At this stage we adopted the tentative L-*xylo* C.2, C.3, C.4, assigned earlier to erythromycin, as possibly applicable to oleandomycin, considering that the same *meso*-2,4-dimethyl-3-hydroxyglutaric acid is derived from comparable segments of both antibiotics. This led to the tentative (*1964b*) model, *Figure 4a*, that notably also included a proviso for configurational assignments 'extra' oxygen at branched sites, cf. C.6, C.12 in erythromycin and C.8 in oleandomycin. It was reasoned that since the mechanism of oxygenases[92] involves retention of configuration and the 'extra' oxygen most likely reflects a 'late' biosynthetic event, the fundamental pattern for branched (*Propionate Rule*) centres would be retained in the event of oxygen insertion. Accordingly, earlier arbitrary assignments at C.6 and C.12 applicable to erythromycin, (*1956*) model, were refined by 'biosynthetic' estimates in the (*1964b*) model.

While we believed that the (*1964b*) model demonstrated that 'a standard of absolute configuration among *all* macrolide antibiotics' was likely *in principle*, we were still concerned about certain details, i.e. need for better evidence of absolute configuration at C.2, C.3, C.4. Towards this end, the

earlier reasonable *xylo* assignment was corroborated by Batrakov and Bergel'son (1964)[93] and by ourselves (1965)[13,14], using independent approaches to the problem (*Table 6e*). With the *xylo* question dismissed, the overall configuration of 'jeffitol' from oleandomycin was narrowed down to two possibilities. Of these, (a) L-*glycero*, L-*ido*- was favoured over (b) L-*glycero*, L-*gluco*-, particularly on the basis of consistency in the sign of the optical rotatory shift following acetylation which is diagnostic of the various chiral heptitols, i.e. (a) negative shift, (b) positive shift (*Table 6d*). As a rule, the polyacetates of diastereoisomeric polyols exhibit rich distinctive n.m.r. spectra indicative of configurationally-imposed preferred conformational populations. It was reasoned that *C*-methyl groups replacing spatially similar *C-O*-acetyl groups at C.2, C.4, C.6 in a given chiral heptitol heptaacetate would not drastically alter its particular conformational habit and hence the net helicity responsible for its characteristic optical rotation[94]. This line of reasoning was also applied to erythromycin-derived lactones A and B and corresponding linear hydrazides and triols (*Table 6e*). It was noted that in arriving at L-xylo at C.2, C.3, C.4 in erythromycin, using the hydrazide rule the earlier workers had fundamentally misapplied the rule by attempting a correlation between a conformationally rigid cyclic ester (Lactones A or B) and its flexible linear hydrazide derivative. We then re-interpreted the available optical rotational data with the help of model systems and came to the conclusion, on several counts, that the original assignments, cf. model (*1956*), at C.8, C.9, and C.10, were fortuitously correct but that specifications at C.2, C.3, C.4 had to be reversed, i.e. to D-*xylo*. These data collectively led to completion of the absolute configuration of oleandomycin and proposal of the general (*1965*) model. This refined model contained broad provisos for 'extra' oxygen (comparable to that for the *1964b* model) and for possible extension of its 14-membered ring framework to smaller and larger rings.

The proposed workings of the model were in complete accord with the x-ray crystallographic analysis of erythromycin A by Harris *et al.* (1965)[83]. However, shortly afterwards Bergel'son and Batrakov (1966)[78] claimed that our hypothesis was in conflict with their assignment at C.2, C.3 in methymycin and neomethymycin, as well as at C.4, C.5 in narbomycin. The arguments put forth by Bergel'son and Batrakov, which involved relationships among synthetic racemic Prelog–Djerassi lactone and its diastereomers, were not readily assailable. Suffice to say, this raised doubts in some quarters as to general applicability of the model, particularly as we expanded on its possible coverage of other macrolide structures as they prevailed in late 1965, including Magnamycin, the classical outlier among the macrolides, which at the time confronted the model with two 'non-conforming' specifications.

There have been many speculations[95–97] regarding the biogenesis of some of the unique features of Magnamycin (before and after revision of its gross structure) that, in effect, argued against its being expected to follow the *1965* configurational model. Thus, it had been pointed out that Magnamycin primarily follows an *Acetate Rule* (as opposed to the *Propionate Rule* followed by the model) and that Magnamycin's peculiar four carbon aldehyde fragment (still of unknown biosynthetic origin)[98] is not found in smaller ring macrolides from which the model was fashioned. Nevertheless, our configurational analysis of Magnamycin[18] led to the fixing of five centres in

an absolute sense, including reversal of the two 'non-conforming' centres. This turn of events suggested that Magnamycin was amenable to the workings of the *1965* model. The studies of Omura *et al.* (1967–1970) have brought final order among the Magnamycins, spiramycins and leucomycins (*Table 7*)[57]. The contribution by Hiramatsu *et al.* (1967–1970)[85] on the x-ray structure of demycarosyl-*iso*-leucomycin A_3 brought forth the final proof of the general applicability of the *1965* model to Magnamycin and related macrolides.

Finally, Rickards and Smith (1970)[79] have shown that the *1965* model specifications (2R, 3S) apply to Prelog–Djerassi lactone and that the 2S, 3R specifications proposed by Bergel'son and Batrakov in 1966[78] must be reversed. At present, the model has no remaining confrontation.

In many respects, the model continues to develop to handle special situations. Notable examples include: (a) Omura *et al.*[87] have added a specification for the special chiral secondary hydroxyl group at C.9 that is applicable to the leucomycins and spiramycins. (b) Hiramatsu *et al.*[85] have specified the unique C.4 centre applicable to the leucomycins/spiramycins/Magnamycins that represent 'extra' oxygen at an unbranched site (an earlier effort to rationalize the orientation at this point was unsuccessful[18]). (c) In connection with their revision of the gross structure of lankamycin, Egan and Martin (1970)[59] have called attention to regularity of a C.3-*O*-α-L and C.5-*O*-β-D glycoside-substitution pattern among all of the macrolides in this subgroup. (d) In building further on the Egan–Martin observation, if one regards D-chalcose, D-desosamine and D-mycaminose as comparable D-sugars, then there is a remarkable conformity as to such β-D-substitution on the macrolide aglycone. Methymycin and neomethymycin are brought 'into line' by viewing the C.3, C.4 unit of the model as 'missing' in these antibiotics.

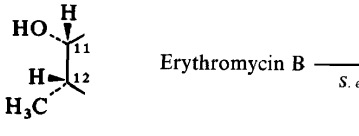
The overall workings of the configurational model for macrolide antibiotics are demonstrated in *Figure 4b*. The aglycone model in *Figure 4b* is drawn in a manner that stresses the biosynthetic units involved. The absolute configuration at each asymmetric centre is understood from Fischer projection, cf. (*1965*) model in *Figure 4a*. Various macrolide aglycones whose constitutions are known, are matched with the model as indicated, i.e. the lactone is 'opened' and the 14-membered rings are compared directly, 12-membered rings are envisaged to have the model's C.3, C.4-unit 'missing', and 16-membered rings are treated as having an 'extra' unit which is off-set with respect to the C.9, C.10- and C.11, C.12,-units of the model. The position in space of a carbon branch in the model is not altered by 'extra' oxygen.

The model has no provision for predicting in certain macrolides the chirality of asymmetric centres at exocyclic sites (cf. lankamycin and neomethymycin) or at an unbranched site containing 'extra' oxygen (cf. C.4 in Magnamycins/spiramycins/leucomycins), or at a 'newly created' asymmetric centre with respect to the model (cf. C.9 in spiramycins/leucomycins). The fundamental carbohydrate corollary states that all 6-deoxypyranoside substituents in the macrolides have identical chirality (β-D- or α-L-sense) at the anomeric centre. It is further noted that all macrolide antibiotics have a special β-D-glycosidic substituent at a position identical with or equivalent to C.5 in the model; such special glycosides are indicated and correspond to

β-D″-6-deoxy-D-*gluco*(mycaminose) and 4,6-dideoxy-D′-*gluco*(desosamine, chalcose) configurations. There is no other generally predictable location for additional glycosidic substituents although there are patterns that characterize certain macrolide subgroups.

The model, in keeping with the ground rules outlined above, is in complete accord with all experimentally defined centres listed in *Table 5*. Application of the model, to as yet 'experimentally untested' chiral centres (*Table 5*) affords the following likely insights: (a) *spiramycins*: β-D-forosaminide at C.9; (b) *lankamycin*: all centres expressed in a relative sense follow in an absolute sense; (c) *narbomycin*: 2R:12R:13R; (d) neomethymycin; 10R:11S; (e) chalcomycin; 5S:8S:12R:13S:14R:15R; (f) neutramycin; 5R:6S:8S:12R:13S:14R:15R; (g) tylosin; 3R:4S:5R:6S:8R:14S:15R; and (h) cirramycin A_1: 3R:4S:5R:6S:8R:12R:13S:14R:15R. The above assignments (which follow from the Fischer projections in *Figure 4b*) are regarded as 'biogenetically expected' specifications. Similarly, mycaminose in tylosin is expected at position C.5.

Table 8. Biochemical interrelationships of macrolide antibiotics



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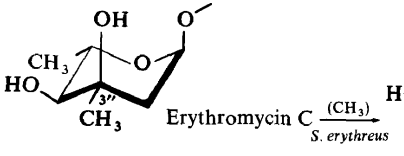


99

Other co-produced macrolides (presumed pathway)

Narbomycin (B-62159B)	(O) C.12	Pikromycin (B-62159A)	118
Intermediate	(O) C.10	Methymycin	69
Intermediate	(O) C.14	Neomethymycin	70
O-Demethyloleandomycin	CH_3 C.3″	Oleandomycin	20

The reliability index of such specifications can be judged after taking into account the following considerations. Martin[99] has demonstrated in the case of the co-produced erythromycins that erythromycin B as well as erythromycin C are progenitors of erythromycin A in a *Streptomyces erythreus* fermentation (*Table 8*). With the absolute configurations of erythromycin B and erythromycin C now known to follow that of erythromycin A, a precedent has been firmly established that different macrolides co-produced in a given fermentation are configurationally identical. Accordingly, we fully expected the relationship we found between oleandomycin and *O*-demethyloleandomycin. Moreover, we would be very surprised if other reported co-produced macrolides (methymycin-neomethymycin and pikromycin-narbomycin) are not in complete configurational concert (*Table 8*). Most of the remaining untested centres fall into a 'fully expected' category. However. C.12 in cirramycin A_1 is a very special case: here there is a branched methyl group and an 'extra' oxygen but since the latter is part of an epoxide, it can also be viewed as 'normal' oxygen through its ligancy at C.13.

Note on polyene antifungal macrolides

In looking farther afield into the giant-ring polyene antifungal macrolides, we noticed[15,16] that certain *28-membered ring* polyenes (Rimocidin, filipin, fungichromin) were reported to have a D-configuration at the lactone juncture. It was further noted[17] that *38-membered ring* polyenes (nystatin, amphotericin B) contained a lactone terminus segment that was constitutionally the same as that at C.10, C.11, C.12, C.13 in oleandomycin. Interestingly, Cope *et al.*[100] found that these comparable segments are diastereoisomeric through first hand comparisons of our (+)2,4,6-trideoxy-2,4-di(C)methyl-D-galactitol ('zervitol') from oleandomycin and a grossly comparable but not identical (+)triol from amphotericin B (*Table 6c*). These compounds were readily distinguished through the ORD spectra of their tri-*O*-acetyl derivatives, i.e. Cotton effect was strongly positive for the oleandomycin derived ester and weakly negative for the amphotericin B derived ester.

The recently published x-ray crystal stereo-structure of *N*-iodoacetyl-amphotericin B[101] revealed that the segments examined by Cope are epimeric at one centre (corresponding to that of the lactone juncture). Hence, the Cope triol from amphotericin B must be 2,4,6-trideoxy-2,4-di(C)methyl-L-altritol (*Table 6c*), which demonstrates that the polyene macrolides avail themselves to either D- or L-lactone configurations depending on ring size.

CONFORMATIONAL ANALYSIS OF MACROLIDE ALGYCONES

As our determination of the total absolute configuration of oleandomycin neared completion, we began to wonder about the feasibility of conformational analysis in the aglycone large ring. Molecular rotation difference data (*Table 9*) clearly showed that removal of the large sugar substituents did not drastically alter the aglycone's conformation. Moreover. early n.m.r. studies on macrolides by Shapiro (1960)[5,102] gave us encouragement that the 14-membered ring in oleandomycin had definite geometry. In our search for a suitable model, we noticed that the classical thermochemical

Table 9. Molecular rotation differences

		M_D^*	Conclusion
Oleandomycin (D-desosaminyl-L-oleandrosyl-oleandolide)		−447°	
D-Desosaminyl-oleandolide		−190°	
	Δ	−257°	α-L
O-Demethyloleandomycin (D-Desosaminyl-L-olivosyloleandolide)		−426°	
D-Desosaminyl-oleandolide		−190°	
	Δ	−226°	α-L
Anhydroöleandomycin (D-desosaminyl-L-oleandrosyl-$\Delta^{10,11}$-anhydroöleandolide)		+435°	
D-Desosaminyl-$\Delta^{10,11}$-anhydroöleandolide		+610°	
	Δ	−175°	α-L
D-Desosaminyl-$\Delta^{10,11}$-anhydroöleandolide		+610°	
$\Delta^{10,11}$-anhydroöleandolide		+690°	
	Δ	− 80°	β-D

M_D^*'s of simple glycosides

Methyl oleandrosides	Methyl olivosides	*n*-Butyl desosaminides
α-L −221	α-L −212	β-D −73.6°
β-L +125	β-L +138	α-D +330°

strain plot (*Figure 5*) shows a 14-membered cyclic hydrocarbon to be relatively strain-free. In 1963, Dale[103] proposed a model of cyclotetradecane (*Figure 6*) on the basis of theoretical considerations which took into account this ring's low energy requirements. Subsequent x-ray studies on 1,8-diazacyclotetradecane[104] and further calculations[105] have supported Dale's proposed conformation of $(CH_2)_{14}$.

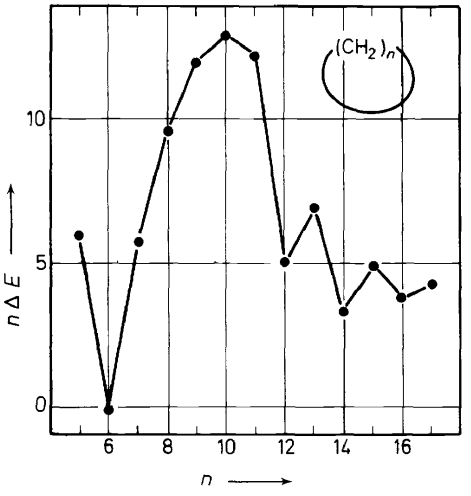


Figure 5. Relative energies of cyclic hydrocarbons according to size

After completing the configuration of oleandomycin, we proceeded to fit the structure of our antibiotic into the $(CH_2)_{14}$ model using Dale's 'too close' neighbours instability index (*Figure 6*) as a guide. In the final analysis, the lactone system was placed in a low energy *trans* (*anti*) form and arrangements were found whereby no large groups were 'too close' to each other. The result was regarded as a plausible conformation of oleandomycin (*Figure 6*) as it exists in solution with thoughts that erythromycin might follow similar geometric lines.

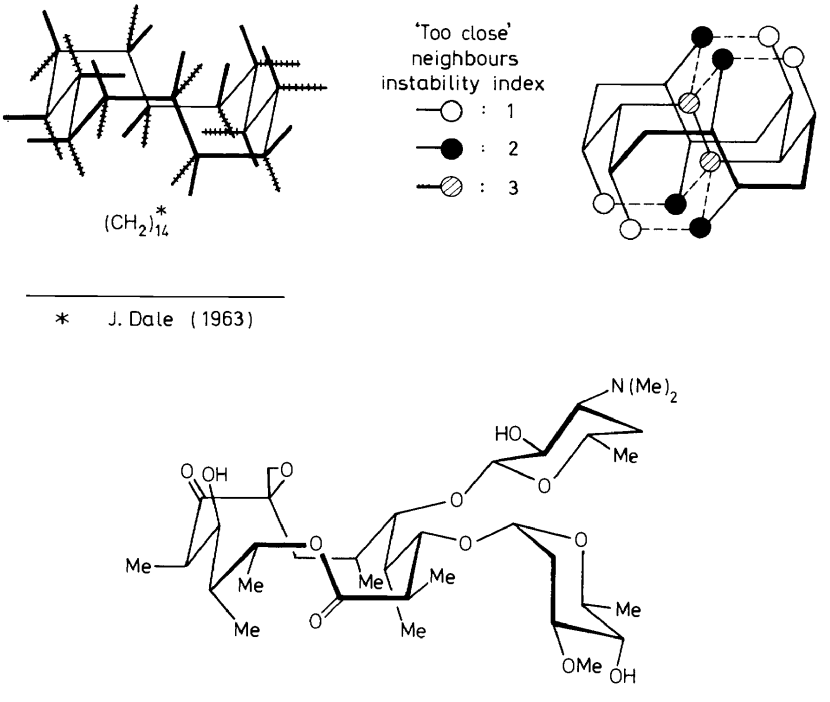
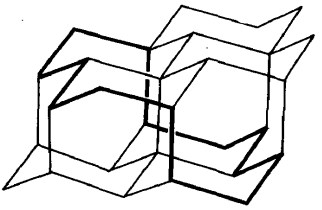
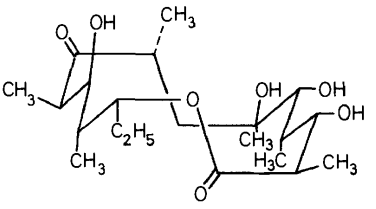


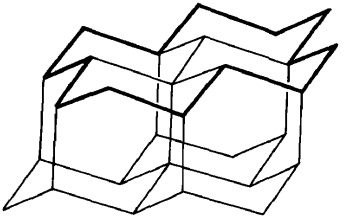
Figure 6. Derivation of a plausible conformational model for oleandamycin (Celmer model)

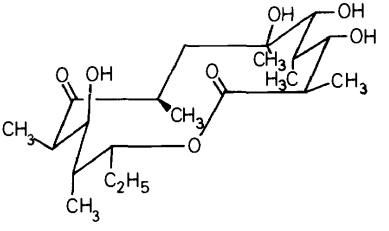
Recent studies on erythromycin aglycones in solution using 100 MHz/220 MHz nuclear magnetic resonance techniques (Perun *et al.*[106, 107]) have led to definition of a single stable conformation in different solvents at variable temperatures. As a result of this work, the diamond lattice conformational model has been refined (*Figure 7*). Perun has proposed that erythronolide B in solution (*Figure 8*) departs from the alternate diamond lattice by a simple 'upward' rotation of the C.6 atom to relieve interactions between methyls on C-4 and C-6. Demarco[108, 109], after studying the n.m.r. spectra of several 9-hydroerythronolides using an aromatic solvent induced shift method, proposed a somewhat related erythromycin aglycone conformation





Celmer–Dale diamond lattice conformation





Alternate diamond lattice conformational model

Figure 7. Refinement of the diamond lattice conformation for erythronolide B

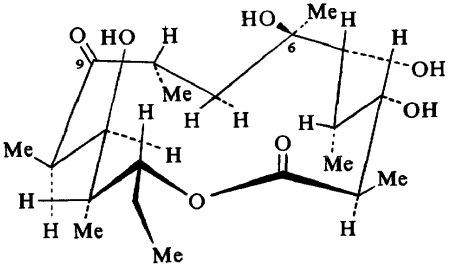


Figure 8. Proposed preferred conformation of erythrolide B in solution (Perun model)

(*Figure 9*). Although the Demarco and Perun models are similar overall, they notably differ in the C.9–C.11 and lactone regions. One important point of difference involves the relative orientation in space of the hydroxyl groups at C.9 and C.11 in the epimeric 9-dihydroerythronolides. In the

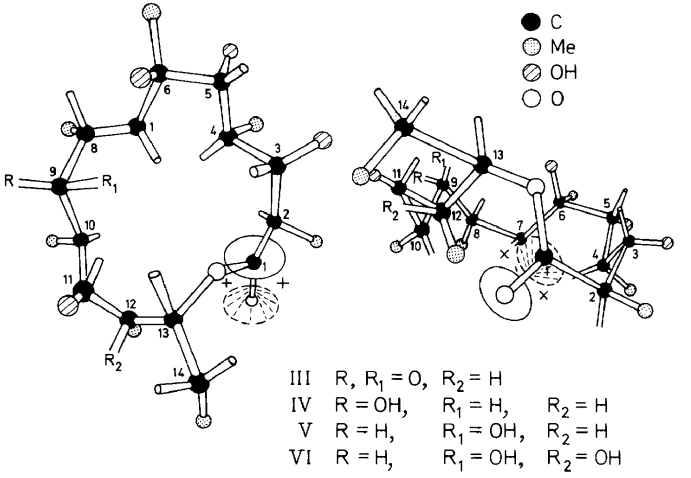


Figure 9. Proposed conformation of some erythromycin aglycones (Demarco model)

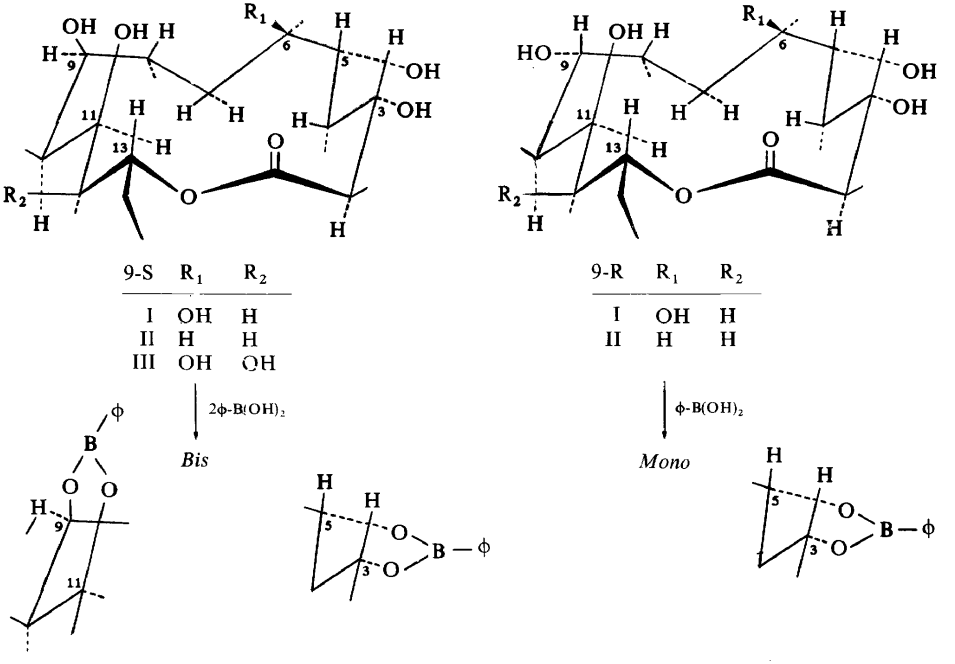


Figure 10. Stereospecific cyclic phenylboronate ester formation

Demarco model of 9S dihydroerythronolide, the hydroxyl groups at C.9 and C.11 are at a relative angle of about 90 degrees whereas in the Perun model (and Celmer model) they are virtually eclipsed and would be expected to form a cyclic ester. This was proven to be the case; cyclic phenyl boronate ester involving the 9,11-hydroxyls takes place with 9S but not with 9R[107] (*Figure 10*); this stereospecific reaction discounts the C.9–C.11 region of the Demarco model.

Strong support for the Perun model was advanced by Mitscher *et al.*[110,111] using arguments based on circular dichroism data and theory (*Figure 11*).

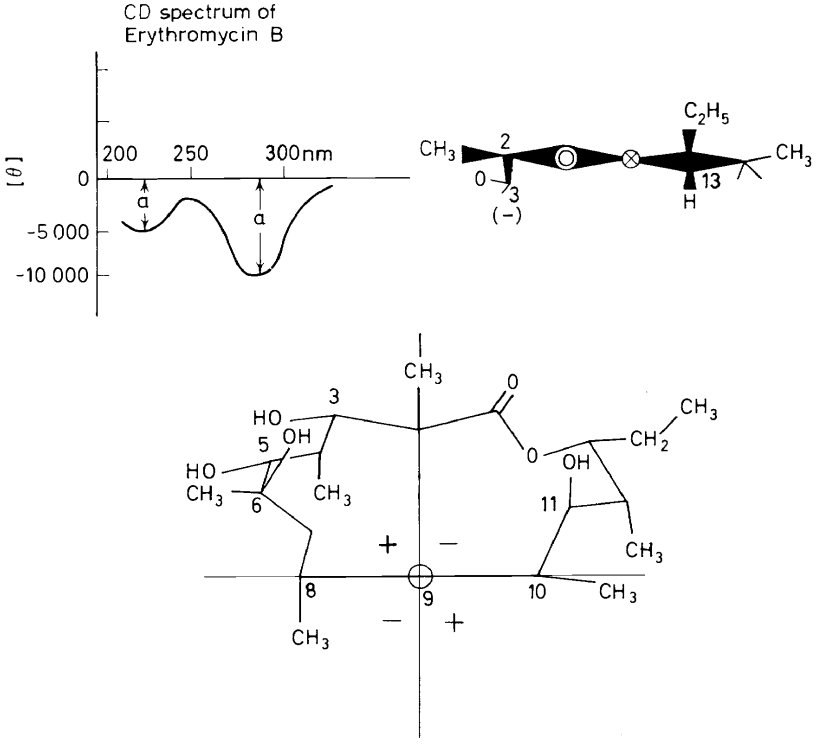


Figure 11. Circular dichroism of macrolide lactone and ketone chromophores (Mitscher analysis)

Focusing on the ketone chromophore, using the well known octant rule as modified for moderately twisted systems, Mitscher pointed out that the Perun model leads to the correct prediction of negative ketone peaks whereas both the Celmer model and the Demarco model are inconsistent with certain spectra. Likewise, for the lactone chromophore, the Perun model leads to correct predictions of a negative trough based either on chirality rules or modified lactone sector rule. On the other hand, the Celmer model would lead to positive peak prediction; the Demarco model's involvement of the lactone region is similar to the Celmer model although it is seriously twisted.

Mitscher has emphasized that no previous CD spectra have been recorded involving lactone rings large enough to exist in the energetically favoured S-*anti* geometry.

While all evidence supports very *similar* aglycone conformations in erythronolide and in its *mono*- and *bis*-glycosides, there are clear indications that the sugar substituents bestow somewhat greater conformational stability to the aglycone. One manifestation is a much higher degree of stereoselectivity during borohydride reduction of the 9-keto groups in fully glycosidic erythromycin A and B compared to 'sugarless' erythronolide B and 6-deoxyerythronolide B (*Table 10*).

Table 10. Stereoselective reduction of the 9-keto group in erythromycin A and related compounds with borohydride.

Starting compound	9-Dihydro products 9S	9R	References
Erythromycin A	Specific	Trace	14, 82, 120
Erythromycin B	Specific	Trace	120
Erythronolide B	Major	Minor	120
6-Deoxyerythronolide B	Major	Minor	120

Further comparative 100 MHz/220 MHz n.m.r.[112] and circular dichroism studies[113] on lankamycin, oleandomycin, oleandomycin derivatives, erythromycin and erythromycin derivatives indicate that the 14-membered macrolide rings in these cases all have *approximately* the same conformation. The extent to which various substituents, especially the exocyclic epoxide grouping in oleandomycin, influence the *precise* shape of the aglycone is currently under investigation. Moreover, exploratory work has started on the application of ^{13}C n.m.r. to conformational analysis of oleandomycin and related macrolides[114].

It seems timely to review and update our earlier views involving the stereochemistry and antibacterial activity of certain oleandomycin *epoxide* derivatives using the presently available experimentally-based conformational models and new chemical[21] and biological data[19]. One problem involves reductive deoxygenation of the epoxide with Raney nickel which directly affects the asymmetric centre at C.8. With oleandomycin, we obtained the expected 8,8a-dihydro-8,8a-deoxy (8-methyl) product as an unequal mixture of C.8-diastereomers, i.e. Isomer A (major) and Isomer B (minor). In contrast, triacetyloleandomycin afforded virtually homogeneous triacetyl-Isomer B, verified by comparison with the separate triacetylated forms of Isomers A and B from oleandomycin. With relatively pure samples in hand, it was established that Isomer A and triacetyl-Isomer A were closely comparable to oleandomycin and triacetyloleandomycin, respectively, as antibacterial agents while the Isomer B series exhibited very little potency (*Table 11*). Under the circumstances and using both configurational and conformational models as guides, it seems a safe assumption that Isomer A has the 'natural' configuration of C.8 (R) whereas Isomer B is C.8.epimer

Table 11. Stereo-structure dependence of activity and cross-resistance

Compound	M.I.C.'s (μg/ml) versus strains of *Staphylococcus aureus* Wild-strain (SA-5)	E-resistant strain (SA-400)
Oleandomycin	0.4–0.8	0.8–1.5
8R-methyl oleandomycin (Isomer A)	0.4	6.24
8S-methyl oleandomycin (Isomer B)	800	1000
Oleandomycin-8a-acetylmercapto-8-hydrin	>1000	>1000
8-methyl-8-hydroxy-oleandomycin	0.6	>1000
Erythromycin B	0.1–0.2	>1000
Erythromycin A	0.05–0.1	>1000

(S). Thus, either the Celmer (*Figure 6*) or the Perun (*Figure 8*) model helps to envision the different stereoselective hydrogenolyses involved; compare relative steric hindrance of C.11—OH and C.11—O—CO—CH_3 in *Figures 12a* and *12b* as determinants for the course of the catalytic action.

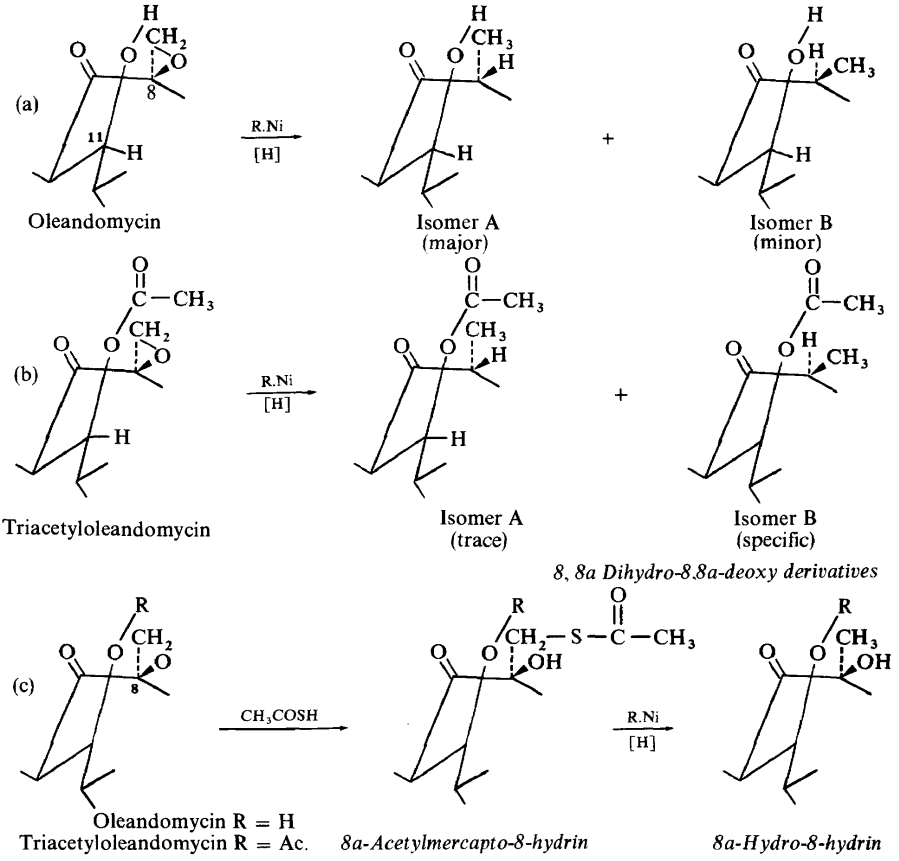


Figure 12. Stereoselective and stereospecific reductions at C.8 in oleandomycin and triacetyloleandomycin

Preparation of another related epoxide derivative of oleandomycin or triacetyloleandomycin involved selective nucleophilic attack with thiolacetic acid at C.8a (forming exclusively 8a-acetylmercapto-8-hydrin) followed by reductive desulphurization with Raney nickel to afford the 8a-hydro-8-hydrin (8-methyl-8-hydroxy) product. Since the reaction sequence does not involve the asymmetric centre, the configuration at C.8 in oleandomycin must be retained. While the sulphur-containing intermediate is microbiologically inactive, the 8-methyl-8-hydroxy product is comparable in potency to oleandomycin but with a notable exception. The 8-methyl-8-hydroxy compound is inactive against a certain strain of *Staphylococcus aureus* (SA 400) that is resistant to erythromycin A and B but notably sensitive to oleandomycin and its 8-R-methyl product. A wild strain of *Staphylococcus* (SA-5) was sensitive to the erythromycins as well as to oleandomycin and its derivatives (*Table 11*). The question is simply stated now, as follows:
How does the resistance mechanism in SA-400 'recognize' 8-methyl-8-hydroxy oleandomycin as an 'erythromycin-like' antibiotic while failing to do so with oleandomycin and its 8R methyl product?

We had previously speculated on this question and would now like to abandon our earlier enzymic 'forbidden space' concept in favour of a 'specific binding space' view, outlined in *Figure 13*. The idea is supported by the Perun

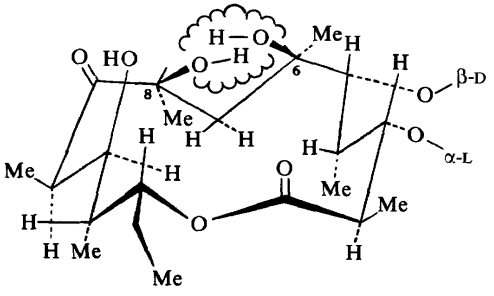


Figure 13. The isosteric nature of hydroxyls at C.6 in the erythromycins and at C.8 in methyl-hydroxyoleandomycin

model which uniquely positions the C.8 hydroxyl group of the oleandomycin derivative into the *same space occupied by the C.6 hydroxyl group of erythromycin A or B.* It follows that oleandomycin and 8-R methyl oleandomycin escape 'recognition' through lack of a free hydroxyl group that could mimic the binding action of the C.6 hydroxyl group in the erythromycins.

OLEFINIC CONSIDERATIONS

The aglycones of many macrolides, as well as chemically modified macrolides, contain one or two carbon–carbon double bonds often as α, β- or α, β, γ, δ-conjugated carbonyl systems. *Trans*-olefinic bonds are evident in all monocyclic cases where the geometry has been studied, e.g. pikromycin[75], kromycin[89], Magnamycin A[22a], Magnamycin B/leucomycin A_3/spiramycin

II[57], Δ(10,11)-anhydroöleandomycin[13], Δ(10,11)-anhydroerythronolide B[115], and Δ(6,7), (10,11)-*bis*-anhydroerythronolide B[115], and Δ(6,6a) (10,11)-*bis*-anhydro-8-epi-erythronolide B[115]. It so happens in the cases of the semi-synthetic Δ-anhydro-aglycones derived from oleandomycin or erythromycin, that the leaving groups have a *trans*-relationship in all of the proposed 14-membered macrolide ring conformational models (*Figures 6, 8* and *9*). Available comparative n.m.r. data support the argument that there is little difference in the overall conformation of saturated and corresponding Δ-*trans*-anhydro macrolide aglycones except that the latter are more stable. Since all of the olefinic groupings encountered in macrolide aglycones are inherently dissymmetric chromophores, it follows that each case poses special problems in the interpretation of its optical rotatory spectra[116]. Mitscher *et al.*[113] have shown that Δ(10,11)-anhydroöleandomycin diacetate containing a C.8,8a-epoxide grouping exhibits: $[\theta]_{334} - 1222$, $[\theta]_{296} + 1775$, $[\theta]_{236} + 33000$; this pattern correlates well with the 8α-(Δ10,11) anhydro-erythrolides rather than with the 8-β-epimers.

We have been intrigued for some time with the possibility of an additional element of chirality imposed by restricted rotation of the sp_2-hybridized carbon bonds in a highly substituted, *trans*-olefinic macrolide ring, see stable enantiomeric *trans* cyclo-octenes as an example of 'axial' chirality[117]. At this point, the question still appears to be open with regard to intact methymycin/neomethymycin, narbomycin/pikromycin[118] and the 16-membered ring macrolides.

It is of particular interest that in kromycin[89] the dihedral angles C_3-C_4-C_5-C_6 and C_9-C_{10}-C_{11}-C_{12} are 176.6 degrees and −179.6 degrees respectively; since there are not strong intermolecular interactions evident in the crystal structure, it is believed that this essentially planar conformation also persists in solution[119].

CONCLUSION

Although the configurational and conformation models for macrolide antibiotics are still undergoing further refinements, they have proven useful during the entire course of their development in testing certain biogenetic and physicochemical theories. In the future, they should find increasing applications as aids in explaining and/or predicting a wide variety of macrolide phenomenology involving stereospecificity in biosynthesis[99], chemical modifications[120], mode of action[121] and mechanism of drug resistance.

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