This conclusion is substantiated by MD correlations involving lactones A:B and models[30] (*cf.* V with I and VII with III in Chart I). The above specifications then follow.[26]

tion become disqualified due to their untenable arithmetic. Now, knowing only the *sign* of RC at all centers, it is possible to check the magnitude of apparent RC from the α center, *e.g.*, since $-\alpha - \beta - \gamma = -74$ then $+\alpha - \beta - \gamma = +12$, *i.e.*, $\alpha = 43°$ (*cf.* 44° for IX).

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Macrolide Stereochemistry. III. A Configurational Model for Macrolide Antibiotics[1]

Sir:

A model (*cf.* **1** and **2**) is proposed as a vehicle of configurational thought in macrolide antibiotic problems[2] dealing with molecular structures, biogenesis, and mode of action. The model's nucleus can be specified as: 2-D, 3-L, 4-D, 5-L, 6-L, 8-L, 10-D, 11-L, 12-L, 13-D, or D-*threo*-L-*gulo*-L-*ido*-, or (2*R*:3*S*:4*R*:5*S*:6*S*:8*R*:10*R*:11*S*:12*R*:13*R*)-2,4,6,8,10,12-hexamethyl-3,5,11,13-tetrahydroxy-9-ketotridecanoic acid 1,13-lactone. A carbohydrate corollary states that any L- or D-6-deoxypyranoside substituent bears an α-L- or β-D-specification at the anomeric center (*cf.* **2**). There are no provisions for predicting the configuration at other carbohydrate or at *exo*-macrocyclic asymmetric centers or the precise position of sugar substitution. The model operates on the premise that the absolute configuration ascribed to a given center is general and is not altered by "extra" oxygen substitution involving replacement of hydrogen at an asymmetric center in certain macrolide antibotics. This

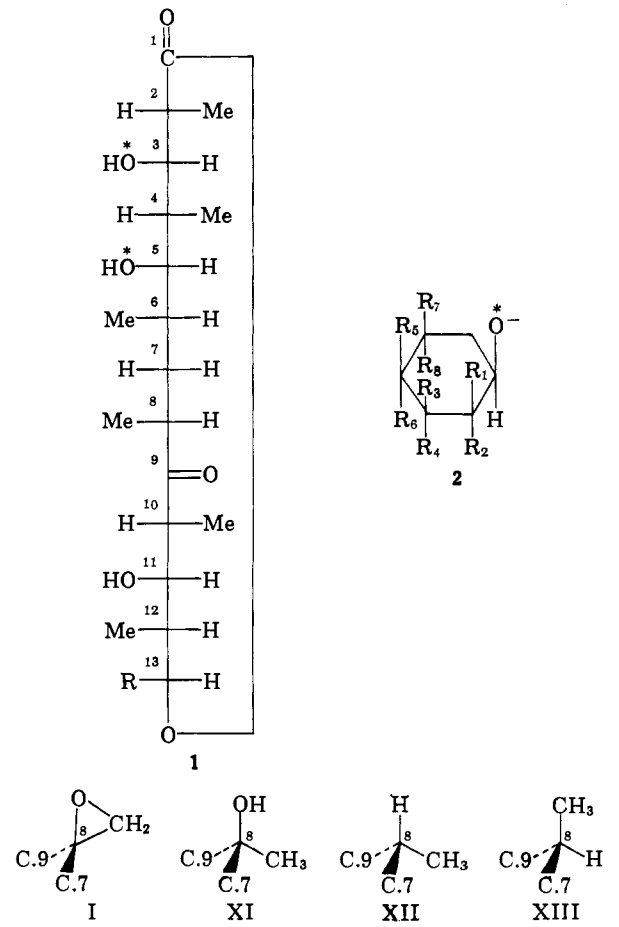

Figure 1

of earlier[1c,3] evidence for and concepts of a principle of configurational standardization among macrolide antibiotics. The model is tied with Gerzon's propionate rule,[4] Klyne's glycoside rule,[1b,5] and the total absolute

Table I. Macrolide Specifications

Macrolide[o]	Aglycone centers: Evident	Aglycone centers: Predicted	Anomeric centers[c]: Evident	Anomeric centers[c]: Predicted
Oleandomycin (I)	(2*R*:3*S*:4*S*:5*S*:6*S*:8*R*:10*R*:11*S*:12*R*:13*R*)[a]	Same	(β-D:α-L)[b]	Same
Erythromycin-A (II)	(2*R*:3*S*:4*S*)[c] (8*R*:10*R*)[d] (13R)[e]	Same and (5*R*:6*R*:11*R*:12*S*)	(β-D)[e,f](α-L)[c]	Same
Erythromycin-B (III)	(*xylo*-C-2,3,4)[e,g]	(2*R*:3*S*:4*S*:5*R*:6*R*:8*R*:10*R*:11*S*:12*R*:13*R*) (*xylo*-C-2,3,4)[h]	(β-D:α-L)[c]	Same
Erythromycin-C (IV)	*Cf.* II	*Cf.* II		(β-D, α-L)
Lankamycin (V)	(*galacto*-C-10,11,12,13)[i]	(2*R*:3*S*:4*R*:5*S*:6*S*:8*S*:10*R*:11*S*:12*R*:13*R*) (*galacto*-C-10,11,12,13)[h]	(β-D, α-L)[c]	Same
Narbomycin (VI)	(6*S*:8*R*)[j]	Same and (2*R*:4*R*:5*S*:12*R*:13*R*)	(β-D)[c]	Same
Methymycin (VII)	(4*S*:6*R*)[k]	Same and (2*R*:3*S*:10*S*:11*R*)		(β-D)
Neomethymycin (VIII)	(4*S*:6*R*)[l]	Same and (2*R*:3*S*:10*R*:11*S*)		(β-D)
Chalcomycin (IX)	(4*S*:6*S*)[m]	Same and (5*S*:8*S*)	(β-D, β-D)[m]	Same
Picromycin (Xa)	"(4*S*:6*R*)[n]"	(2*R*:3*S*:4*S*:6*R*:10*S*:11*R*)		(β-D)
Picromycin (Xb)	"(2*R*:4*S*)[n]"	(2*R*:4*S*:5*S*: *R*:1*S*:11*R*)		(β-D)

[a] *Cf.* ref. 1a. [b] *Cf.* footnote 8 in ref. 1b. [c] Ref. 1b. [d] *Cf.* ref. 3e. [e] Ref. 4. [f] *Cf.* footnote 4 in ref. 1b. [g] *Cf.* footnotes 6, 11, and 27 in ref. 1b. [h] *Cf.* relative configuration. [i] W. Keller-Schierlein (private communication); *cf. Helv. Chim. Acta*, **47**, 78 (1964). [j] *Cf.* ref. 3f. [k] *Cf.* ref. 3c. [l] *Cf.* ref. 3b. [m] *Cf.* footnote 18 in ref. 1b. [n] This follows from ref. 3a,b according to the assigned structures in each case (Xa, ref. 6 and Xb, ref. 7). [o] *Cf.* ref. 2.

extends the applicability range of the model in its otherwise self-evident constitution–configuration matching workings.

Derivation. The current model represents fruition

(1) (a) Part I: *J. Am. Chem. Soc.*, **87**, 1797 (1965); (b) part II: *ibid.*, **87**, 1799 (1965); (c) footnote 1c in ref. 1a.

(2) For reviews see: (a) M. Berry, *Quart. Rev.* (London), 17, 343 (1963); (b) H. Grisebach and W. Hofheinz, *J. Roy. Inst. Chem.*, 332 (1964)

(3) (a) R. Anliker, D. Dvornik, K. Gubler, H. Keusser, and V. Prelog, *Helv. Chim. Acta*, **39**, 1758 (1956); (b) C. Djerassi and O. Halpern, *J. Am. Chem. Soc.* **79**, 2022 (1957); *Tetrahedron*, **3**, 255 (1958); (c) C. Djerassi and J. A. Zderic, *J. Am. Chem. Soc.*, **78**, 6390 (1956); (d) C. Djerassi and O. Halpern, *ibid.*, **79**, 3626 (1957); (e) C. Djerassi, O. Halpern, D. I. Wilkinson, and E. J. Eisenbraun, *Tetrahedron*, **4**, 369 (1958); (f) V. Prelog, A. M. Gold, G. Talbot, and Z. Zomojski, *Helv. Chim. Acta*, **45**, 4 (1962).

(4) K. Gerzon, E. H. Flynn, M. V. Sigal, Jr., P. F. Wiley, R. Monohan, and U. C. Quarck, *J. Am. Chem. Soc.*, **78**, 6396 (1956).

configuration of oleandomycin (I)[1a] in accord with aforementioned provisos.

Applications. Molecular Structure. The relationship of the model with known and predicted specifications in ten representative macrolides is shown in Table I. The model has already served a useful role in confronting reported "nonconforming" configurational conclusions which resulted in experimentally based revisions in accord with prediction.[1b] Attention is called to possible further utility in helping to decide between two proposed structures for picromycin (Xa[6] and Xb).[7]

Biogenesis. While the model is not inconsistent with current views relating macrolide aglycone and classical fatty acid biosyntheses,[2,4,8] it provokes additional thoughts regarding configurational aspects now incorporated in Gerzon's rule which reach into consideration of D- and L-2-methylmalonyl-CoA.[1c,9] The "extra" oxygen proviso is notably consistent with the nature of C-8 observed[1a] for I and the configurational retention feature known for oxygenase systems.[10] A biogenetic basis for the model's feature covered by Klyne's rule has been mentioned.[1b,11]

Mode of Action. With the total absolute configuration of I known[1a] and that of II and III reasonably estimated (*cf.* Table I), the phenomenon[12] of nonpredictable bacterial sensitivity involving I:II (and III) can now be reasonably explained on the basis of conformational difference in I arising from its unique spiro[3.14] ring system. This view is in keeping with current molecular biological concepts of complementariness involving permitted and forbidden space at the active site of an enzyme[13] which, in turn, is equated here with an antibiotic-susceptible site in a bacterium. Experimental substantiation of the present theory was gained through a series of chemically prepared[14] C-8 variants of I, *i.e.*, XI, XII, and XIII (*cf.* I in ref. 1a and detailed discussion in ref. 1c). In the following comparisons it should be recalled that the expression XII also reflects C-8 in II and III. Sensitivity tests revealed I, II, III, XI, XII, and XIII as all fully active (M.I.C.'s μg. level)[15] against a wild strain of *Staphylococcus aureus* (SA-5), while only I and XIII exhibited comparable potency against an erythromycin-resistant strain (SA-M400); all others failed to inhibit at >1000 μg./ml.[15] These findings are therefore in full accord with expectation based on space-filling[13] considerations.

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(6) R. Anliker and K. Gubler, *Helv. Chim. Acta*, **40**, 119, 1768 (1957).
(7) H. Brockmann and R. Oster, *Ber.*, **90**, 605 (1957).
(8) T. Kaneda, J. C. Butte, S. B. Tambman, and J. W. Corcoran, *J. Biol. Chem.*, 237, 322 (1962), and cited references.
(9) (a) R. Mazumder, T. Sasakawa, Y. Kaziro, and S. Ochoa, *ibid.*, 237, 3065 (1962); (b) S. H. G. Allen, R. Kellermeyer, R. Stjernholm, B. Jacobson, and H. G. Wood, *ibid.*, **238**, 1637 (1963).
(10) M. Hayano in "Oxygenases," O. Hayaishi, Ed., Academic Press Inc., New York, N. Y., 1962, pp. 182–240.
(11) The α-L:β-D-anomeric center and D-lactone terminus center features of the subject model are viewed as primitive phylogenetic markers and, as such, evidently and predictively apply to comparable centers in macrolides not otherwise covered (*cf.* ref. 1b and D-C-27 in fungichromin: A. Cope, *et al.*, *J. Am. Chem. Soc.*, **84**, 2170 (1962)).
(12) (a) A. R. English, *Antibiotics Annual*, 756 (1958); (b) A. R. English and F. C. Fink, *Antibiot. Chemotherapy*, **8**, 420 (1958); (c) J. R. Fowler, J. L. Watters, and J. W. Levy, *Clin. Med.*, **70**, No. 3 (1963); (d) H. Isenberg, *Health Lab. Sci.*, 1, 185 (1964).
(13) I. B. Wilson and B. F. Erlanger, *J. Am. Chem. Soc.*, 82, 6422 (1960).
(14) Reaction of I with AcSH gave the specific 8a-AcS-8-OH derivative, $C_{37}H_{65}NO_{13}S$, which was reduced (Raney nickel) to XI, $C_{35}H_{63}NO_{12}$; direct reduction (Raney nickel) of I gave XII and XIII, $C_{35}H_{63}NO_{11}$, which were then separated (W. D. Celmer, forthcoming publication). The method of preparation and microbiological correlations described here were invoked earlier as circumstantial evidence for 8*R* in I (*cf.* ref. 1c).
(15) A. R. English, private communication; the author thanks Dr. English for the microbiological tests involving determinations of minimum inhibitory concentrations (M.I.C.).

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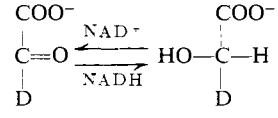
Determination by Neutron and X-Ray Diffraction of the Absolute Configuration of an Enzymatically Formed α-Monodeuterioglycolate[1]

Sir:

The muscle enzyme lactic dehydrogenase which catalyzes the conversion of L-lactate to pyruvate will also act on the next shorter α-hydroxy acid, glycolic acid (hydroxyacetic acid), to produce glyoxylate. The logical assumption[2] is that the sterically corresponding hydrogen atoms of L-lactate and glycolate are removed by the enzyme, and this assumption has been made the basis for several discussions of stereochemical specificity.[3,4]

The work reported in this note provides conclusive evidence that this assumption is indeed correct by establishing the absolute configuration of the α-monodeuterioglycolate ion produced by the action of muscle lactic dehydrogenase on deuterioglyoxylate ion as shown in eq. 1, where NAD^+ and NADH represent



(1)

the oxidized and reduced forms of the coenzyme nicotinamide–adenine dinucleotide (NAD).

The determination was performed through the collaboration of three groups. First, one of us (I. A. R.) prepared a 0.5-g. quantity of the enzymatically formed α-monodeuterioglycolic acid. Second, E. J. G. and M. R. T. grew crystals of the ^{6}Li salt and determined the structure by X-ray diffraction methods. However, since hydrogen and deuterium have the same atomic number, they could not be distinguished by the X-ray analysis. Finally, C. K. J. utilized the anomalous neutron scattering amplitude[5] of ^{6}Li ($[0.18 + 0.025i] \times 10^{-12}$ cm.), and the markedly different neutron scattering amplitudes[6] of H and D (-0.378×10^{-12} cm. for H

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(5) S. W. Peterson and H. G. Smith, *J. Phys. Soc. Japan*, 17, B-II, 335 (1962).
(6) G. E. Bacon, "Neutron Diffraction," Oxford University Press, London, England, 1962.