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.* 1 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.

(5) (a) W. Klyne, *Biochem. J.*, **47**, xli (1950); (b) T. Reichstein and E. Weiss, *Advan. Carbohydrate Chem.* **17**, 98 (1962).
(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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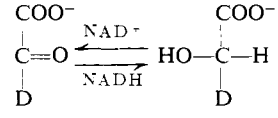
Determination by Neutron and X-Ray Diffraction of the Absolute Configuration of an Enzymatically Formed α-Monodeuterioglycolate[1]

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

(1) Research sponsored in part by the U. S. Atomic Energy Commission under contract with the Union Carbide Corporation, and in part by Grants AM 02884-14 and CA 07818 from the National Institutes of Health, U. S. Public Health Service.
(2) I. A. Rose, *J. Am. Chem. Soc.*, 80, 5835 (1958).
(3) I. A. Rose, *Brookhaven Symp. Biol.*, **15**, 293 (1962).
(4) G. E. Lienhard and I. A. Rose, *Biochemistry*, **3**, 190 (1964).
(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.

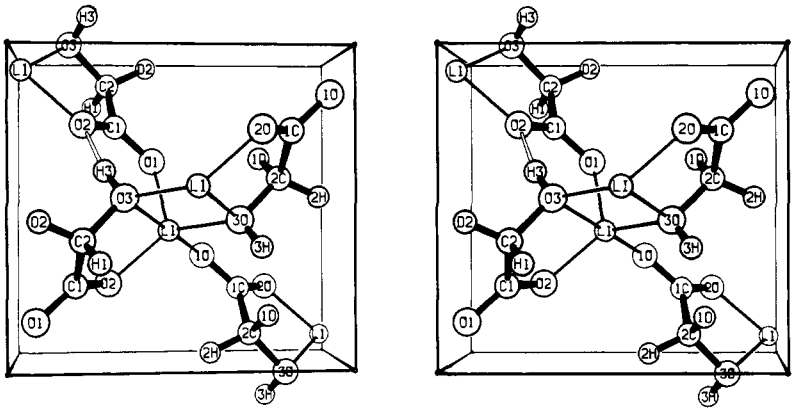


Figure 1. A stereogram showing the unit cell contents of anhydrous lithium (*S*)-glycolate-2-*d*. The unique monoclinic *b* axis points up in the page and the mean *c* axis out from the page. The corners of the cell outline are on symmetry centers of the X-ray space group. The neutron space group has no symmetry centers.

and 0.65×10^{-12} cm. for D) to establish the absolute configuration of the molecule through neutron diffraction measurements of the Bijvoet inequalities.[7]

Glyoxylic-2-*d* acid was prepared in 32% yield by reduction of oxalic acid with magnesium[8] in D_2O. The product was isolated by elution from an anion exchange resin (Dowex-1 acetate) with 4 *N* acetic acid. To prepare glycolic-2-*d* acid, a 100-ml. phosphate-buffered solution (pH 7.3) which contained glyoxylate-2-*d* (20 mmoles), NAD (0.01 mmole), muscle lactic dehydrogenase (1 mg.), ethyl alcohol (200 mmoles), and liver alcohol dehydrogenase (10 mg.) was incubated. The latter two components were required to maintain NAD in the reduced form. The decrease in glyoxylate concentration was followed photometrically.[9] Glycolic-2-*d* acid was isolated in 30% yield by elution from a Dowex-1 acetate resin with 2 *N* acetic acid and subsequent removal of the acetic acid by repeated evaporation to dryness under reduced pressure. The product was then neutralized with ^{6}LiOH.

Lithium α-monodeuterioglycolate crystallizes in two forms, anhydrous and monohydrate. The anhydrous form (monoclinic: $a = 8.139$ Å., $b = 7.607$ Å., $c = 5.174$ Å., $\beta = 93.38°$, and $Z = 4$) was used in the structure determination. An interesting symmetry anomaly arises because the molecule has an isotopically asymmetric carbon atom. The X-ray diffraction experiments, which cannot distinguish between H and D, show the space group as $P2_1/n$. Even with prolonged exposure, (*h*0*l*) photographs showed no indication of reflections with $h + l$ odd. However, the neutron experiments show the space group as $P2_1$ with $Z = 4$. In the X-ray work, block-diagonal least-squares refinement yielded a final discrepancy factor of 0.039 for the three-dimensional Cu Kα data. Figure 1 is a stereoscopic illustration[10] of the structure in which the packing details are readily apparent. The analysis will be reported in detail elsewhere.

Neutron diffraction measurements were made with the Oak Ridge automatic diffractometer using a neutron wave length of 1.078 Å. A crystal of the ^{6}Li salt weighing 1.3 mg. was mounted in such a way that absorption effects (including those arising from the supporting mount) were essentially identical for *hkl* and $\bar{h}\bar{k}\bar{l}$ intensity measurements. Thirty different sets (*hkl*, $\bar{h}k\bar{l}$, $\bar{h}\bar{k}\bar{l}$, $h\bar{k}l$) of reflections were measured using $\theta/2\theta$ step-scanning. Observed and calculated values for the outstanding dispersion observations are shown in Table I as the quantity $100(I_+ - I_-)/(I_+ + I_-)$

Table I. Some Neutron Dispersion Data for Lithium-6 (*S*)-Glycolate-2-*d*. Values of $100(I_+ - I_-)/(I_+ + I_-)$

hkl	Calcd.[a]	Obsd.[b]	*hkl*	Calcd.[a]	Obsd.[b]
(021)	−5.3	− 4.8(0.7)	($\bar{2}$23)	+ 2.6	+ 3.9(0.8)
(022)	+3.9	+ 5.3(1.1)	(331)	+ 4.8	+ 5.9(1.3)
(041)	−9.4	−12.2(1.6)	($\bar{3}$31)	−15.8	−20.2(3.8)
(131)	+7.0	+ 5.8(1.3)	($\bar{4}$22)	+ 4.8	+ 5.4(1.9)
(220)	−2.3	− 3.3(0.6)	(430)	− 4.4	− 6.6(1.6)
($\bar{2}$21)	+8.9	+10.2(1.5)	($\bar{5}$21)	− 2.2	− 3.2(1.1)
($\bar{2}$22)	−4.2	− 4.6(1.5)	(522)	+ 7.0	+11.5(3.1)

[a] The coherent scattering amplitude used for 96% enriched ^{6}Li was $b = (0.16 + 0.024i) \times 10^{-12}$ cm. The glycolate ion was assumed to have 100% deuterium at the position indicated in eq. 1 and in Figure 1. [b] Quantities in brackets are the standard errors based on counting statistics alone.

where I_+ corresponds to the intensity of the *hkl* and $\bar{h}k\bar{l}$ reflections, and I_- refers to the intensity of $\bar{h}\bar{k}\bar{l}$ and its space group equivalent $h\bar{k}l$. In no case was an internally consistent dispersion effect observed which differed in sign from that calculated. As an example of the actual experimental data obtained, the neutron counts for one of two measurements of the (041) group were: (041), 899 ± 45; (04$\bar{1}$), 1241 ± 49; (04$\bar{1}$), 956 ± 46; and (041), 1217 ± 48. The standard errors are based on counting statistics alone. The model on which the calculated values are based can be described[11] as lithium (*S*)-hydroxyacetate-2-*d* or as lithium (*S*)-glycolate-2-*d* and is shown pictorially in Figure 1, and by Fisher convention in eq. 1.

Care was taken to maintain a right-handed coordinate system throughout and to verify that the scattering and reciprocal lattice vectors had the same vector sense.

(7) (a) J. M. Bijvoet, A. F. Peerdeman, and A. J. Van Bommel, *Nature*, **168**, 271 (1951); (b) A. F. Peerdeman and J. M. Bijvoet, *Acta Cryst.*, **9**, 1012 (1956).

(8) K. F. Lewis and S. Weinhouse, *Methods Enzymol.* **3**, 276 (1957).

(9) B. A. McFadden and W. V. Howes, *Anal. Biochem.*, **1**, 240 (1960).

(10) C. K. Johnson, "OR TEP, A Fortran Thermal Ellipsoid Plot Program for Crystal Structure Illustrations," ORNL-3794, in press.

(11) (a) R. S. Cahn and C. K. Ingold, *J. Chem. Soc.*, 612 (1951); (b) R. S. Cahn, C. K. Ingold, and V. Prelog, *Experientia*, **12**, 81 (1956).

To the best of our knowledge, this is the first example of the use of neutron diffraction to establish the absolute configuration of a molecule made asymmetric by isotopic substitution.

Acknowledgment. We are very much indebted to Dr. A. L. Patterson of the Institute for Cancer Research who coordinated the project, and Drs. H. A. Levy and H. G. Smith of the Oak Ridge National Laboratory for their continued interest in and discussion of this work.

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The Parahydrogen Conversion over Synthetic Ruby

Sir:

The purpose of this communication is to report some new information on the chromia and chromia–alumina catalyst systems.

Palladium-diffused hydrogen, equilibrated at −196°, was passed over powdered synthetic ruby containing 1.1 atom % chromium and having a specific surface of about 2 m.², with negligible porosity. Space velocity was 240 (STP)/min., at 1 atm. The temperature was changed at 1°/min. Reactor inlet and outlet were protected by liquid nitrogen traps.

Cooling the ruby from 529° gave the parahydrogen conversion pattern labeled "ruby-cooling" in Figure 1.

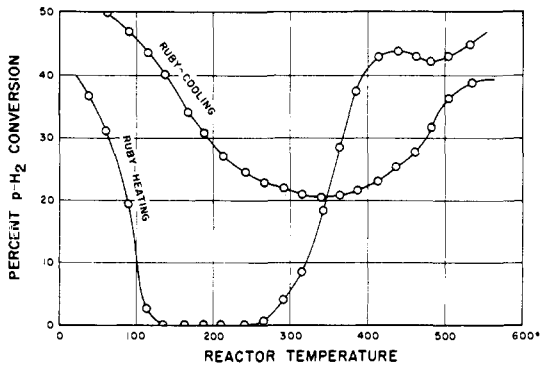

Figure 1. Parahydrogen conversion over powdered synthetic ruby. The cooling curve is reversible, but only if contamination is rigidly prevented.

Prompt subsequent heating showed the "ruby-cooling" curve to be reversible. Hydrogen–deuterium exchange under the same experimental conditions was, as expected, slight at 25°, moderate at 500°.

Impregnated chromia–alumina dehydrogenation catalyst containing 5 atom % chromium was now substituted for the ruby. It was assumed that, in this system, and at the concentration given, about one in twenty of the chromium ions present was on the surface.[1] The space velocity was therefore increased (by decrease of catalyst mass) 1000-fold, so that the number of chromium ions accessible to molecular hydrogen was roughly equal to that in the ruby. The activity of the chromia–alumina catalyst below 200° was about 10-fold *less* than that of the ruby but, starting near 300°, the activity quickly rose to at least 1000-fold *greater* than the ruby.

After the ruby stood at 25° overnight in hydrogen, but not protected by cold traps, the activity pattern was that labeled "ruby heating" in Figure 1. Cooling from 500° produced the "ruby-cooling" pattern, but interruption of heating in the 120 to 280° region produced a system inert at 25° and down to −196°. Chromia–alumina also showed this poisoning effect, but in modified form.

Exposure of an active sample to laboratory air caused complete loss of activity, which was slowly recovered in hydrogen at 500°.

Powdered (chromia-free) synthetic sapphire gave no activity at 25°, and a small, slowly increasing activity above 450°.

In ruby the concentration of chromium ion pairs is almost certainly negligible.[2] In supported chromia–alumina catalysts the situation is quite different, most of the chromia being present in small, widely separated aggregates.[1,3] The results given show that dissociative activity in chromia–alumina occurs with Cr–Cr adjacency, and that chromium ions far from other chromiums are, even though accessible to the reactant, relatively inactive. The small low-temperature magnetic conversion over the supported chromia is possibly related to Cr–Cr exchange (or superexchange) interaction, which is diminished, but still measurable,[1] in these systems.

It is premature to consider in detail the mechanisms for activation, poisoning, and recovery of ruby activity, but the following suggestions may be useful. The high magnetic activity shown at 25° suggests excellent accessibility of molecular hydrogen to the chromium ions. Following current views,[4,5] it is assumed that activated ruby contains surface Cr^{2+} ions, but that these have only three adjacent neighbors (two O^{2-} and one OH^-). Such dissociative activity as exists over ruby is then representable as a reversible, heterolytic chemisorption

$$\begin{array}{ccc} & & H^- \\ O^{2-}Cr^{2+}OH^- + H_2 & \overset{>300^\circ}{\rightleftharpoons} & OH^-Cr^{2+}OH^- \\ O^{2-} & & O^{2-} \\ \text{I } (mag, diss) & & \text{II } (\text{——}, diss) \end{array}$$

The notations (*mag*, *diss*) mean the kind of catalytic activity, magnetic or dissociative or both, expected from the several kinds of sites shown. The mechanism is analogous to a view concerning alkane dehydrogenation over chromia.[6]

If the ruby is exposed to a trace of water vapor (or perhaps merely stands at room temperature) the

(1) R. P. Eischens and P. W. Selwood, *J. Am. Chem. Soc.*, **69**, 1590 (1947).

(2) Private communication from Professor Arthur L. Schawlow.

(3) D. E. O'Reilly, *Advan. Catalysis*, **12**, 31 (1960).

(4) J. Givaudon, E. Nagelstein, and R. Leygonie, *J. chim. phys.*, **47**, 304 (1950).

(5) S. W. Weller and S. E. Voltz, *J. Am. Chem. Soc.*, **76**, 4695, 4701 (1954).

(6) R. L. Burwell, A. B. Littlewood, M. Cardew, G. Pass, and C. T. H. Stoddart, *ibid.*, **82**, 6272 (1960).