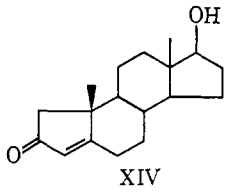
version of the known ketal alcohol XI[7] to the tosylate XII, mp 69° (EtOH), displacement of the tosyl group (NaCN, DMSO, 70°, 2 hr, N_2) to XIII, acid hydrolysis of XIII to the known acid X,[8] and methylation with diazomethane.

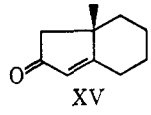
In another degradation scheme (1, O_3–CH_2Cl_2; 2, Zn–AcOH; 3, Ag_2O–NaOH; 4, CH_2N_2), alcohol I, alcohol II, and the hydrocarbon mixture III all yielded the ester VI and methyl levulinate (VII). Additionally, alcohol I yielded the diketo ester V in the scheme (1, O_3–CH_2Cl_2; 2, Zn–AcOH; 3, CrO_3–H^+; 4, CH_2N_2). This evidence, along with spectral measurements (ir, pmr, uv, mass spectrometry) and elemental analyses of alcohol I, its monoacetate, and its octahydro derivative, indicate that this alcohol has structure I. The hydrocarbon fraction, on similar analysis, appears to be a mixture of isomers III resulting from dehydration of alcohols I or II.

The stereochemistry at C_6 and C_7 in alcohols I and II is *trans*, since the ir spectra (neat) of these alcohols show no band in the region 840–670 cm^{-1}, where a *cis*-dialkyl substituted olefin should absorb. Absorption found at 975 cm^{-1} in alcohols I and II is consistent with a *trans* configuration. The chemical shift of the C_3 methyl protons (τ 8.27) superimposed upon broad multiplets in the pmr spectrum of alcohol I indicates that alcohol I is predominantly the 2-*cis* isomer.[9] The configuration around the C_2 double bond of the lipid in the parent antibiotic is still uncertain, however, since isomerization may have occurred during hydrolysis.

Although some racemization of indenone VI may have occurred during its formation by base-catalyzed condensation, it was still possible to obtain an optical rotatory dispersion curve that showed a pattern similar, but opposite in direction, to those of (10*R*)-A-nortestosterone (XIV)[10] and (8*S*)-$\Delta^{3,9}$-8-methylhydrinden-2-one (XV).[11] The ORD curve of VI (*c* 0.09, dioxane) has: $[\phi]_{400}^{27}$ +14°, $[\phi]_{350}$ +117°, $[\phi]_{345}$ +122°, $[\phi]_{340}$ +103°, $[\phi]_{335}$ +89°, $[\phi]_{330}$ +45°, $[\phi]_{326}$ 0°, $[\phi]_{320}$ −45° $[\phi]_{305}$ −167°. The indenone VI, therefore, should have the (*S*) configuration, and the diumycin lipids I, II, and III should have the (13*S*) configurations shown.



XIV



XV

The nonisoprenoid pattern from C_5 to C_{11} in these lipids represents an interesting and unusual biogenesis and suggests the possibility that not all of the carbon atoms in the diumycin lipid are derived from mevalonate.

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Comments on a Proposed Mechanism of Action of B_{12} Coenzyme[1]

Sir:

In this communication, we report experiments which contradict a mechanism of action of diol dehydrase recently proposed by Schrauzer and Sibert.[2] Diol dehydrase requires vitamin B_{12} coenzyme and catalyzes the following reactions. When the substrate

$$CH_3—CHOH—CH_2OH \longrightarrow CH_3CH_2—CHO$$
$$CH_2OH—CH_2OH \longrightarrow CH_3CHO$$

C-1 hydrogen, which appears to migrate during the course of the reaction, is labeled with titrium, both C-5′ hydrogens of the coenzyme are replaced by tritium. When coenzyme containing tritium at the C-5′ position is added to the apoenzyme and nonisotopic substrate, tritium is transferred to the α position of the product aldehyde. Schrauzer and Sibert have proposed[2] that this tritium exchange occurs as follows: 1,2-propanediol-1-^{3}H is converted by a 1,2-hydride shift to propionaldehyde-2-^{3}H.[3] The enzyme-bound propionaldehyde-2-^{3}H then exchanges tritium with an activated form of the enzyme-bound coenzyme. This exchange reaction is the only process which leads to the transfer of tritium between substrate and coenzyme.

To establish whether the coenzyme became labeled by an equilibration with enzyme-bound product, an experiment was carried out with D-1,2-propanediol-1-^{3}H in which the specific activities of the coenzyme, the substrate, and the reaction product were measured during the course of the reaction. The results of this experiment are summarized in Table I. If tritium transfer from propionaldehyde to coenzyme occurs by an equilibration process as proposed by Schrauzer and Sibert, then the maximal specific activity of the coenzyme will approach that of the enzyme-bound propionaldehyde. The specific activity of the enzyme-bound propionaldehyde varies during the course of the reaction but will be greater than that of the accumulated product aldehyde and less than that of the substrate.[4] The data in Table I show that during the course of the reaction the specific activity of the coenzyme is nearly 20 times that of the substrate and 200–700 times that of the product propionaldehyde. Therefore, the specific activity of the coenzyme far exceeds the maximal specific activity which would be obtained through an equilibration pro-

(1) Publication No. 719 from the Graduate Department of Biochemistry, Brandeis University. This work was supported in part by grants from the National Institutes of Health (12633, 5-Fl, GM 20,226, and GM 212).
(2) G. N. Schrauzer and J. W. Sibert, *J. Amer. Chem. Soc.*, **92**, 1022 (1970).
(3) The vitamin B_{12}-coenzyme participates in this reaction. For details of the mechanism, see ref 2.
(4) Since there is a kinetic isotope effect in the catalytic reaction (R. H. Abeles and H. A. Lee, Jr., *Brookhaven Symp. Biol.*, **15**, 310 (1962)), there is discrimination against tritium-labeled substrate molecules, and specific activity of the substrate increases as the reaction proceeds.

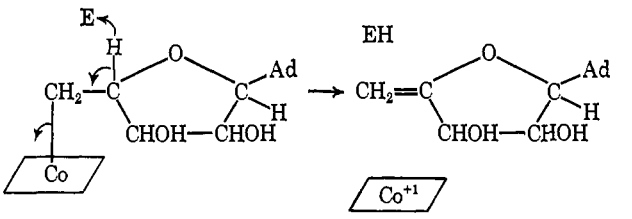
Table I. Specific Activities of Vitamin B_{12} Coenzyme, Residual Substrate, and Product During the Conversion of D-1,2-Propanediol-1-^{3}H to Propionaldehyde-2-^{3}H[a]

Time, sec	Specific activity (cpm/μmol) × 10^{-5}		
	B_{12}-coenzyme	Residual substrate	Product
0	1.8	4.0	
5	75	4.3	0.27
10	88	4.4	0.22
30	130	5.9	0.26
60	160	10.3	0.22

[a] The D-1,2-propanediol was stereospecifically labeled with tritium in the C-1 hydrogen which is transferred to C-2 (B. Zagalak, P. A. Frey, G. L. Karabatsos, and R. H. Abeles, *J. Biol. Chem.*, **241**, 3028 (1966)). The experiment was carried out at 10°. A separate reaction mixture containing 98 units of diol dehydrase, 4 μmol of potassium phosphate buffer, pH 8.0, 3.2×10^{-3} μmol of B_{12} coenzyme, and 29.8 μmol of substrate in a total volume of 0.55 ml was used for each point. The reaction was started by adding the substrate in 0.10-ml volume, and, after the appropriate time, 0.1 ml of 20% TCA was added to stop the reaction. A 0.05-ml aliquot was immediately withdrawn, diluted with water, and assayed colorimetrically for its propionaldehyde content. A separate 0.10-ml aliquot was withdrawn, diluted into 1.0 ml of a solution containing 0.24 *M* propionaldehyde carrier in 0.2 *M* potassium phosphate, pH 8.0, treated with 1–2 mg of charcoal, and centrifuged. Propionaldehyde was isolated from the supernatant fluid as the dimethone derivative, which was assayed radiochemically to determine the specific activity of propionaldehyde. B_{12}-coenzyme was isolated from the remainder of the reaction mixture, and its specific activity was determined according to procedures described previously (P. A. Frey, M. K. Essenberg, and R. H. Abeles, *ibid.*, **242**, 5369 (1967)). The specific activity of residual D-1,2-propanediol-1-^{3}H was calculated from the known initial specific activity and data on the amounts of propionaldehyde and propionaldehyde-2-^{3}H produced at each time.

cess. In an experiment, which has previously been published,[5] it was shown that when propionaldehyde-2-^{3}H and the enzyme-bound coenzyme equilibrate, tritium is not concentrated in the coenzyme. Propionaldehyde-2-^{3}H was added to diol dehydrase–vitamin B_{12} coenzyme complex. Aliquots of the coenzyme were isolated periodically and examined for tritium content. The specific activity of the coenzyme after 1.5 hr of incubation was equal to that of the aldehyde. This shows that no unusual equilibrium isotope effect favoring tritium in B_{12} coenzyme occurs. It must therefore be concluded that the incorporation of tritium into the coenzyme which occurs when 1,2-propanediol-1-^{3}H is converted to propionaldehyde cannot occur through exchange with the enzyme-bound propionaldehyde, and that the mechanism proposed by Schrauzer and Sibert for labeling of the coenzyme is inconsistent with the experimental results obtained with diol dehydrase. Alternative mechanisms for the exchange of tritium between substrate and coenzyme have been proposed.[6,7]

A mechanism for the activation of the enzyme-bound coenzyme was also proposed,[2] in which a basic group on the enzyme abstracts a hydrogen from the C-4′ position of the enzyme-bound coenzyme to produce vitamin $B_{12(s)}$ and dehydroadenosine. According to this mechanism ^{3}H should be introduced into the C-4′ position of the coenzyme when the reaction is carried out in H_2O-^{3}H. To test the exchangeability of the C-4′ hydrogen, DL-1,2-propanediol (2.4 mmol), diol dehydrase (255 units), B_{12} coenzyme (1.2×10^{-2} μmol), and dibasic potassium phosphate (2.5 μmol) were incubated in a total volume of 1.5 ml in H_2O-^{3}H (1.3×10^6 cpm/μg-atom) at 37° for 10 min. At this point about 1400 μmol of diol had reacted so that at least 10^5 turnovers had taken place. The coenzyme was then isolated and purified by procedures previously described.[6] The specific activity of the purified coenzyme was less than 5 cpm/μg. If one carbon-bound hydrogen of B_{12} coenzyme had equilibrated with the solvent during the course of the enzymatic reaction, and if no equilibrium isotope effects had occurred, the specific activity of the coenzyme would be 1.2×10^3 cpm/μg. This experiment therefore does not suport the proposed mechanism of activation of the coenzyme. Since enzyme reactions are known in which proton transfers occur without exchange with the solvent protons,[8] the experiment does not eliminate this activation mechanism. We feel, however, that mechanisms for which experimental support exists are more attractive than those for which no support exists.



The mechanism of action of vitamin B_{12} coenzyme proposed by Schrauzer and Sibert is based on model experiments with cobaloximes and cobalamines. It should be pointed out that no aspect of this mechanism is borne out by experiments with the enzyme, and that some features, as shown by this communication, are directly contradicted by experiments with the enzyme. Although model systems are invaluable in the study of enzyme reactions, it is essential that they be related to the mechanism of the enzymatic reaction. Relevance is established by the ability of the model system to explain a maximum number of experimental facts pertaining to the enzyme system. Coincidental formation of similar reaction products is in itself not sufficient to to guarantee a relevant model system.

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Enzymatic Formation of Chiral Structures in Racemic Form

Sir:

Enzymatic syntheses of chiral molecules from achiral substrates proceed in a stereospecific and asymmetric