If spin density in the π framework were the only mechanism causing the shifts, the following relationships are expected to apply, which relate the carbon-13 and proton isotropic shifts to the appropriate a^C and a^H value for the ring positions of the molecular fragments **2** and **3**, where ρ_{Ci}^{π} is the spin density in the p_π orbital centered at C_i.[5]

$$\sigma_{con}^{C_i}/\sigma_{con}^{H_i} = (\gamma_H/\gamma_C)(35.5\rho_{C_i}^{\pi} - 14.0[\rho_{C_h}^{\pi} + \rho_{C_i}^{\pi}])/(-22.5\rho_{C_i}^{\pi}) \quad (3)$$

$$\approx -6.2 + 2.5(\rho_{C_h}^{\pi} + \rho_{C_i}^{\pi})/\rho_{C_i}^{\pi} \quad (4)$$

$$\sigma_{con}^{CH_3}/\sigma_{con}^{CH_3} = (\gamma_H/\gamma_C)(-14.0\rho_{C_k}^{\pi}/27.5\rho_{C_k}^{\pi}) \quad (5)$$

$$\approx -2.1 \quad (6)$$

Because only the resonances of the α-, β-, *ortho*-, *meta*-, and methyl carbons were assigned with real certainty, the following discussion will be centered around these positions. It is readily seen that the predicted ($\sigma_{con}^{C_i}/\sigma_{con}^{H_i}$) and observed ($\sigma_{obsd}^{C_i}/\sigma_{obsd}^{H_i}$) values are in major disagreement for the β and *meta* positions whereas good agreement is obtained for $\sigma_{con}^{CH_3}/\sigma_{con}^{CH_3}$. However, in all cases, the signs of the ratio, predicted to be negative because $(\rho_{C_h}^{\pi} + \rho_{C_i}^{\pi})/\rho_{C_i}^{\pi}$ is negative, of the carbon and corresponding proton isotropic shifts are reproduced experimentally. No experimental determinations for the ratio are possible for the α and *ortho* positions because $\rho_{C\text{-}1}^{\pi}$ and $\rho_{C\text{-}2}^{\pi}$ are unknown; however, if the calculated values[2a] of these parameters are used it is readily seen that the predicted ratios are too low. The discrepancies between the predicted and observed ratio of contact shifts for carbon and hydrogen are sizable but might be regarded as tolerable in view of the approximate nature of the Karplus–Fraenkel treatment[5b] and the serious reservations that have been suggested for its application.[5b] However, in our view, the discrepancies fall into a pattern which indicates that there is an additional important mechanism contributing to the isotropic shifts and involve transmission of spin density by the σ framework of the ligand. The argument is based on the expectation that positive spin density arising from σ delocalization at the β and *meta* position would reduce the predicted upfield carbon shifts and increase the downfield proton isotropic shifts.[6] The overall result would be to reduce $\sigma_{con}^{C_i}/\sigma_{con}^{H_i}$ for these two positions, in agreement with experiment. The reverse situation is expected for the α and *ortho* positions, again in accord with experiment. The methyl group should exhibit a ratio in better agreement with theory, because effect of σ delocalization should be markedly reduced at this position as the result of attenuation through the intervening bonds. Because a tetrahedral Ni(II) complex has only unpaired electrons of π symmetry available for interaction with the ligand, a likely pathway of introducing spin density into the σ framework would be an indirect π–σ polarization.

Further evidence in support of positive spin density in the σ framework of the ligand comes from previous pmr studies. It has been noted[2a,c] that, if the *p*-tolyl group is replaced by ethyl or *n*-propyl, large negative contact shifts are observed for the CH_2 and CH_3 groups of the chain. The only reasonable mechanism of placing spin density at these positions is *via* the σ framework, and thus it is not unreasonable to expect nuclei at ring positions to also exhibit isotropic shifts resulting from such a mechanism. On this basis, the observed small isotropic shifts of C-1 and C-2 seem quite reasonable. The σ and π effects, though large, should be opposite in sign and could be close to equivalence at these positions. The present results indicate that spin densities calculated from proton isotropic shifts, assuming purely π delocalization, are likely to be unreliable.

This study is the first reported of the ^{13}C nmr spectrum of a paramagnetic transition metal complex capable of showing multiple resonances, and although not all of the carbon resonances were observed, it seems clear that ^{13}C contact shifts will be extremely useful in probing the nature of metal–ligand interactions.

Acknowledgments. We are indebted to Dr. R. E. Benson of the Central Research Department of E. I. du Pont de Nemours and Company for samples of the ligand and complex and the reviewers for their helpful comments.

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(5) (a) H. M. McConnell, *J. Chem. Phys.*, **24**, 632, 764 (1956); *Proc. Nat. Acad. Sci.*, **43**, 741 (1957); (b) M. Karplus and G. K. Fraenkel, *J. Chem. Phys.*, **35**, 1312 (1961); G. K. Fraenkel, *Pure Appl. Chem.*, **4**, 143 (1962).

(6) The predictions about the direction of the shifts are based on consideration of π–π configurational interactions[2a] which suggest that negative spin density at the carbon will produce positive spin density at the proton and a downfield shift.

The Structure of a Novel Lipid from the Antibiotic Diumycin

Sir:

The isolation and characterization of a new group of phosphorus-containing antibiotics, the diumycins, have been reported.[1,2] The antibiotics are highly active *in vitro* against gram-positive bacteria and exhibit a remarkable duration of action *in vivo*. A single dose of 6.7 mg/kg of diumycin, administered subcutaneously to mice 14 days prior to challenge with a lethal dose of *Streptococcus pyogenes* C_{203}, provides protection to 50% of the mice injected.[1]

Recently, structures have been assigned to the optically inactive lipids obtained by hydrolysis of the related antibiotics prasinomycin[3] and moenomycin.[4] We now wish to present evidence for the structures of the

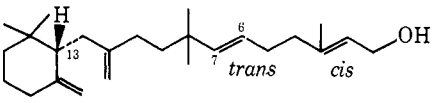
(1) E. Meyers, D. S. Slusarchyk, J. L. Bouchard, and F. L. Weisenborn, *J. Antibiot.*, **22**, 490 (1969).

(2) The diumycins are members of a family of antibiotics that includes: prasinomycin,[2a] moenomycin,[2b] 11,837RP,[2c] 8036RP,[2d] 19,402RP,[2e] and macarbomycin.[2f] (a) F. L. Weisenborn, J. L. Bouchard, D. Smith, F. Pansy, G. Maestrone, G. Miraglia, and E. Meyers, *Nature (London)*, **213**, 1092 (1967); (b) G. Huber, U. Schacht, H. L. Wiedenmuller, J. Schmidt-Thome, I. Duphorn, and R. Tschesche, *Antimicrob. Ag. Chemother. 1965*, 737 (1966); (c) Rhone-Poulenc, Belgian Patent 653,168 (1965); (d) Rhone-Poulenc, South African Patent 65/6204 (1966); (e) Rhone-Poulenc, Netherlands Patent 68,02093 (1968); (f) S. Takahashi, A. Okanishi, R. Utahara, K. Nitta, K. Maeda, and H. Umezawa, *J. Antibiot.*, **23**, 48 (1970).

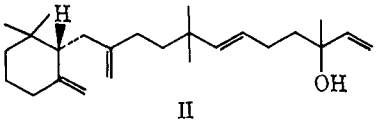
(3) W. A. Slusarchyk and F. L. Weisenborn, *Tetrahedron Lett.*, 659 (1969).

(4) R. Tschesche, F.-X. Brock, and I. Duphorn, *ibid.*, 2905 (1968); R. Tschesche, F.-X. Brock, and I. Duphorn, *Justus Liebigs Ann. Chem.*, 720, 58 (1968).

optically active lipids, diumycinol (I), isodiumycinol (II), and diumycene (III), derived from diumycin.



I



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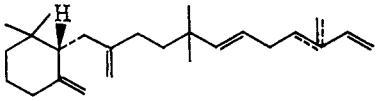
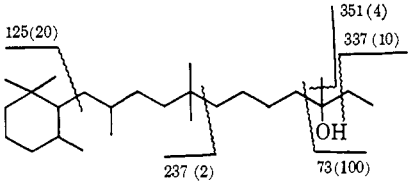


III

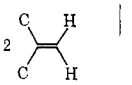
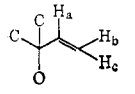


IV

Hydrolysis (1 *N* HCl, 100°, 30 min) of either the individual diumycins or the diumycin mixture yields a chloroform-soluble oil that can be resolved by silica-gel tlc in the system benzene–$CHCl_3$–MeOH (8:1:1) to give an alcohol I, R_f 0.68, $[\alpha]^{26}D$ +5.6° (EtOH), a second alcohol II, R_f 0.80, $[\alpha]^{25}D$ +8° (EtOH), and a hydrocarbon mixture III, R_f 0.95.

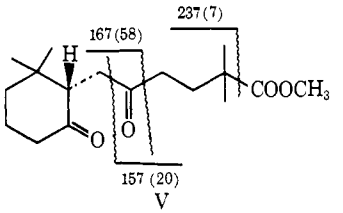
Alcohol II, by elemental analysis and mass spectrometry, *m/e* 358 (M^+), has the formula $C_{25}H_{42}O$ and shows: ir (neat) 3400 (OH), 1800–1600 (C=C), 1360 and 1380 (suggesting CH_3–C–CH_3), and 995, 975, 925, and 890 cm^{-1} (terminal methylene and terminal vinyl); uv (EtOH) no absorption above 205 mμ (no conjugated double bonds). The pmr spectrum of alcohol II ($DCCl_3$, 60 MHz) allowed the proton assignments shown in Table I.

Table I

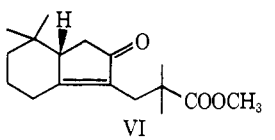
Signal	Assignment
τ 9.14 (s, 3 H)	C-C(CH_3)$_2$C
τ 9.07 (s, 3 H)	
τ 9.03 (s, 6 H)	C-C(CH_3)$_2$—C=C
τ 8.72 (s, 3 H)	CH_3—C—O
τ 7.7–8.65 (m, ~18 H)	Methylene protons
τ 5.20–5.55 (m, 3–4 H)	
τ 4.61 (m, 2 H)	Vinylic protons
τ 4.96 (q, 1 H, J = 10, J = 2) H_b τ 4.82 (q, 1 H, J = 17, J = 2) H_c τ 4.05 (q, 1 H, J = 17, J = 10) H_a	

Alcohol II consumes 4 equiv of hydrogen (PtO_2, H_2, EtOH, or EtOAc) to yield a saturated alcohol IV, $C_{25}H_{50}O$, with molecular ion at *m/e* 366 and base peak at *m/e* 73; alcohol II, therefore, must contain a ring and four olefinic bonds. On the basis of this initial information and the probable biogenetic relationship of isodiumycinol to isomoenocinol[3,4] it was possible to assign structure II to isodiumycinol. Confirmatory evidence that II is the correct structure was provided by two degradation schemes.

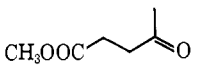
Degradation of alcohol II (1, $KMnO_4$–$NaIO_4$; 2, CrO_3–H^+; 3, CH_2N_2) yields, after glc, a monocyclic diketo ester analyzed as $C_{17}H_{28}O_4$ (*Anal.* Calcd: C, 68.89; H, 9.52; molecular ion, 296.1996. Found: C, 69.04; H, 9.68; molecular ion, 296.1988), and consistent with structure V. The ester has: $[\alpha]^{25}D$ +13° (EtOH); ir (neat) 1728 (ester C=O), 1706 cm^{-1} (C=O); pmr ($DCCl_3$) τ 9.27 (s, 3, CH_3–), 8.97 (s, 3, CH_3–), 8.82 (s, 6, $(CH_3)_2$C–COO), 6.33 (s, 3, ester CH_3O–); mass spectrum *m/e* 296 (4) (M^+) and *m/e* 69 (base).



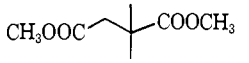
V



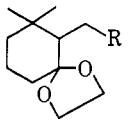
VI



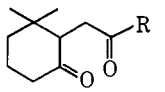
VII



IX



XI, R = –OH
XII, R = $-OSO_2C_6H_4CH_3$
XIII, R = –CN



VIII, R = $-OCH_3$
X, R = –OH

Degradation of alcohol II (1, $KMnO_4$–$NaIO_4$; 2, Ag_2O–NaOH; 3, CH_2N_2) yields a $C_{17}H_{26}O_3$ ester VI (molecular ion, calcd: 278.1967; found: 278.1924) and methyl levulinate (VII),[5] both isolated by glc. The ester VI, formed by intramolecular aldol condensation of a diketone of type V, has: $[\alpha]^{25}D$ +2.8° (EtOH); uv max (EtOH) 239 mμ (ε 12,000); ir (neat) 1730 (ester C=O), 1700 and 1640 cm^{-1} (C=C—C=O); pmr ($DCCl_3$) τ 9.33 (s, 3, CH_3–), 9.00 (s, 3,CH_3–), 8.84 (s, 6, $(CH_3)_2$C–COO), 7.53 (s, 2, unsplit allylic methylene), and 6.33 (s, 3, ester CH_3O–); mass spectrum *m/e* 278 (M^+) and *m/e* 178 (base).

Further evidence for the structure of VI was obtained by its conversion (1, O_3–CH_2Cl_2; 2, HIO_4; 3, CH_2N_2) to a keto ester VIII and 2,2-dimethylsuccinic acid dimethyl ester (IX).[6] The structure assigned to the keto ester was confirmed by: (1) mass spectral and glc comparisons with an authentic sample and (2) comparison of its 2,4-dinitrophenylhydrazone derivative, mp 168–170 (EtOH), with that of an authentic sample, by melting point, mixture melting point, ir (KBr), and mass spectrum. Authentic VIII was obtained by con-

(5) Methyl levulinate was identified by comparison of its glc retention times on two different columns and its pmr and ir spectra with those of an authentic sample.

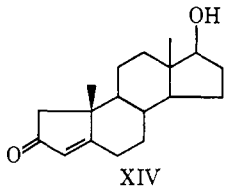
(6) 2,2-Dimethylsuccinic acid dimethyl ester was identified by pmr, ir, and glc comparisons with an authentic sample.

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.

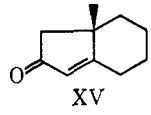
In another degradation scheme (1, O_3–CH_2Cl_2; 2, Zn–AcOH; 3, Ag_2O–NaOH; 4, CH_2N_2), alcohol I, alcohol II, and the hydrocarbon mixture III all yielded the ester VI and methyl levulinate (VII). Additionally, alcohol I yielded the diketo ester V in the scheme (1, O_3–CH_2Cl_2; 2, Zn–AcOH; 3, CrO_3–H^+; 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.

Acknowledgment. We thank Mr. J. Alicino and his staff for elemental analyses, Dr. A. I. Cohen and his staff for mass spectra and 60-MHz pmr spectra, and Miss B. Keeler and her associates for ir spectra.

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Received March 6, 1970

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-

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(8) L. Ruzicka and C. F. Seidel, *ibid.*, **33**, 1285 (1950).
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(10) C. Djerassi, "Optical Rotatory Dispersion," McGraw-Hill, New York, N. Y., 1960, p 67.
(11) C. Djerassi and J. E. Gurst, *J. Amer. Chem. Soc.*, **86**, 1755 (1964).

(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.