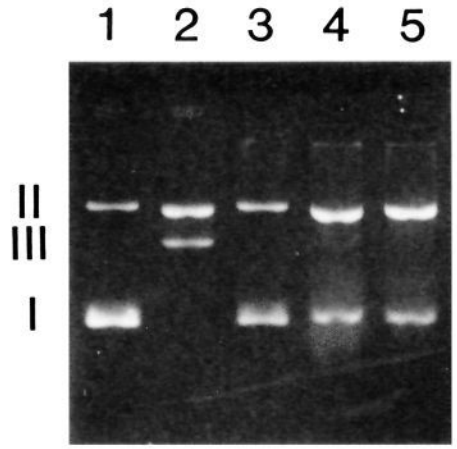

Figure 1. Strand scission of ΦX174 replicative form DNA. DNA samples (180 ng) in 40 μL of H_2O containing 3% dimethoxyethane were treated with 10 μM Fe(II) + 0.1% H_2O_2 (lane 2), 10 μM Cu(II) (lane 3), 20 μg of a CH_2Cl_2 extract of *Hakea trifurcata* + 10 μM Cu(II) (lane 4), 10 μg of extract + 10 μM Cu(II) (lane 5). Lane 1 contained untreated DNA. The reaction mixtures were incubated for 30 min at 25 °C and then analyzed by agarose gel electrophoresis. Form I DNA is supercoiled covalently closed circular DNA; form II DNA is relaxed (nicked) circular DNA; form III DNA is linear duplex DNA.

dihydroxybenzene groups linked through an unbranched alkene. As for **2**, a *cis*-olefin was indicated by ^{1}H NMR (δ 5.33, br t, J = 4.7 Hz); its location was determined by mass spectral analysis of a sample of **3** that had been acetylated and subjected to ozonolysis.[9] On this basis, compound **3** was assigned the structure 1,3-dihydroxy-5-(14′-(3″,5″-dihydroxyphenyl)tetradec-*cis*-6′-enyl)benzene.[11]

DNA cleavage by these 5-alk(en)ylresorcinols was found to be remarkable in a few different ways. First, the compounds exhibited Cu(II)-dependent DNA strand scission in spite of the absence of useful metal ion ligands. Second, although high concentrations of these compounds bound plasmid DNA sufficiently to alter its mobility on agarose gels (Figure 1), none has any functionality capable of mediating association with DNA by a well characterized mechanism (e.g., intercalation, groove binding, or electrostatic interaction[12]).

As regards DNA cleavage, preliminary mechanistic investigations provide some insight. During purification of the 5-alkylresorcinols, it was noted that after some fractionation steps the newly isolated material actually exhibited a *diminished* ability to mediate DNA strand scission. Interestingly, this activity increased upon storage of the sample or by incubation in aerated aqueous solution, especially where the solution was alkaline. It seems possible that DNA cleavage by such compounds may involve initial oxygenation of the benzene nucleus at C-4.[13] 1,3,4-Trihydroxybenzene derivatives produced in this fashion could then chelate Cu(II), providing a complex capable of initiating DNA degradation in the presence of O_2.[14,15] Of possible pertinence to the issue of DNA binding is the observation that DNA cleavage efficiency by 5-alkylresorcinol derivatives was found to be directly proportional to the length of the alkyl substituent. It seems conceivable that upon dissolution in an aqueous solution containing a DNA duplex, the lipophilic moiety of the 5-alkylresorcinols seeks to associate with the least polar component of the duplex, i.e., with the interior.[17]

Acknowledgment. We thank Dr. Jeffrey Ellena for assistance with the 2D NMR measurements. This work was supported by P.H.S. Grant CA 40291, awarded by the National Cancer Institute, D.H.H.S.

(11) This compound has been isolated previously.[7c]

(12) (a) Baguley, B. C. *Mol. Cell. Biochem.* **1982**, *43*, 167. (b) Feigen, J.; Denny, W. A.; Leupin, W.; Kearns, D. R. *J. Med. Chem.* **1984**, *27*, 450. (c) Dougherty, G. *Int. J. Biochem.* **1984**, *16*, 1179. (d) Neidle, S.; Abraham, Z. *CRC Crit. Rev. Biochem.* **1984**, *17*, 73.

(13) Certain of these transformations are known to be Cu(II)-catalyzed. See, e.g.: (a) Brackman, W.; Havinga, E. *Recl. Trav. Chim. Pays-Bas.* **1955**, *74*, 937, 1021, 1070, 1100, 1107. (b) Musso, H. *Angew. Chem., Int. Ed. Engl.* **1963**, *2*, 723.

(14) Although presently unproven experimentally, reductive activation of O_2 by the binary complex could lead to DNA strand scission.[16] Consistent with this scheme, DNA cleavage by 5-alkylresorcinols has been shown to be O_2 dependent (Singh, S., unpublished results).

(15) In fact synthetic 5-alkyl-1,3,4-trihydroxybenzene derivatives were found to cleave DNA at ~100-fold lower concentration than the respective 5-alkylresorcinols.

(16) (a) Wong, A.; Huang, C.-H.; Crooke, S. T. *Biochemistry* **1984**, *23*, 2939. (b) Wong, A.; Huang, C.-H.; Crooke, S. T. *Biochemistry* **1984**, *23*, 2946. (c) Sigman, D. S. *Acc. Chem. Res.* **1986**, *19*, 180.

(17) Consistent with this suggestion, we have found that oligonucleotide derivatives containing lipophilic substituents bound with substantially increased affinity to their single-stranded complementary oligonucleotides (Jager, A.; Levy, M. J.; Hecht, S. M., in preparation).

Biosynthesis of Riboflavin. The Structure of the Four-Carbon Precursor

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The initial steps in the biosynthesis of riboflavin lead from GTP to the pyrimidine **3** which reacts with a four-carbon moiety under formation of 6,7-dimethyl-8-ribityllumazine (**4**).[1] In vivo studies have shown that the four-carbon moiety originates from the pentose pool by the loss of C-4.[2] In vitro studies became feasible on the basis of the seminal observation by Shavlovsky and his co-workers that cell extracts of the yeast *Candida guilliermondii* catalyze the formation of **4** from **3** in the presence of ribose phosphate.[3] We could show that an intermediary carbohydrate phosphate designated as compound X can be formed from pentose phosphate by the yeast cell extract.[4]

We have purified the enzyme catalyzing the formation of compound X about 1000-fold from the cell extract of *C. guilliermondii*. Whereas crude cell extracts can use several pentose and pentulose phosphates as substrates for the production of compound X, the purified enzyme is limited to ribulose 5-phosphate (**1**) as substrate.

In light of the limited stability of compound X, it was advantageous to study its formation by NMR spectroscopy in the enzyme assay mixture without purification. Five ^{13}C NMR signals designated as a–e were observed after treatment of **1** with the purified enzyme from *C. guilliermondii* (Figure 1, Table I). The proton multiplicities of these signals were determined by DEPT spectroscopy. The ^{13}C NMR signal b at 173 ppm showed evidence for the presence of one proton and was clearly identified as formate by internal standardization with authentic formate. The production of formate by the enzyme-catalyzed reaction was also confirmed by ^{1}H NMR spectroscopy.

Treatment of the reaction mixture with alkaline phosphatase affects predominantly the chemical shifts of signals c and d and is accompanied by the loss of the fine structure of these signals. The dephosphorylated compound was identified as 3,4-dihydroxy-2-butanone on the basis of ^{1}H and ^{13}C NMR spectroscopic comparison with an authentic sample.[5] In experiments

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Scheme I. Biosynthesis of Riboflavin

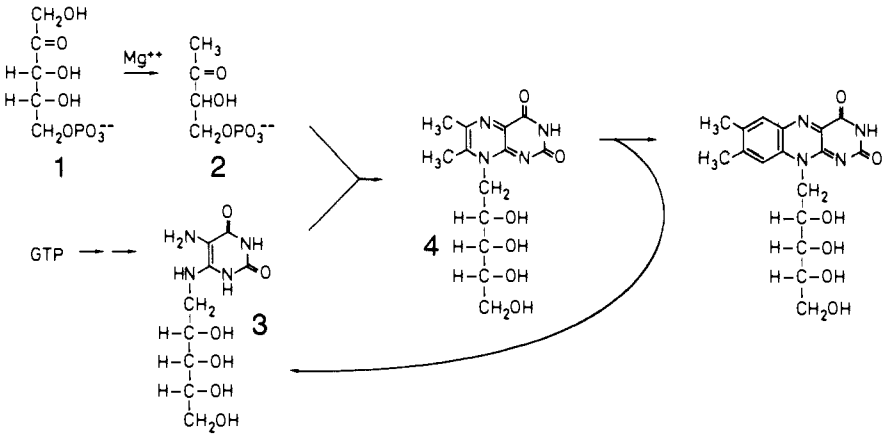

Table I. ^{13}C NMR Signals of Compound X[a]

signal	C atom	δ (ppm)	proton multiplicity	coupling constants
a	2	215.1	0	$^1J_{CC}$ = 40.9 Hz
c	3	79.4	1	$^1J_{CC}$ = 40.4 Hz, $^2J_{CC}$ = 13.1 Hz, $^3J_{PC}$ = 7.0 Hz
d	4	67.3	2	$^1J_{CC}$ = 39.6 Hz, $^2J_{PC}$ = 4.6 Hz
e	1	28.2	3	$^1J_{CC}$ = 40.8 Hz, $^2J_{CC}$ = 13.1 Hz

[a] For details see Figures 1 and 2.

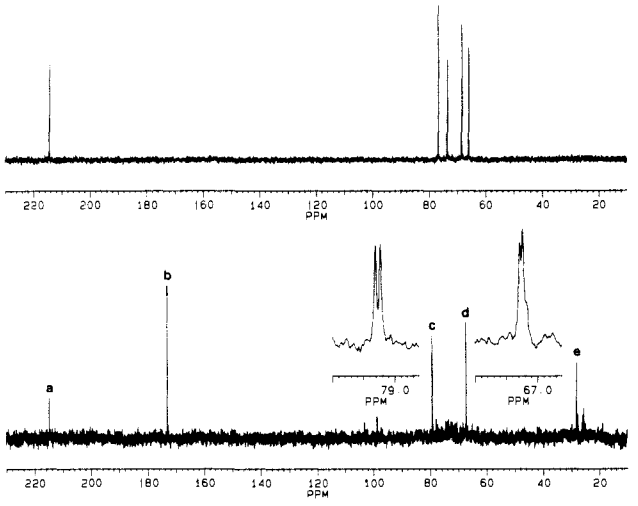

Figure 1. Proton-decoupled ^{13}C NMR spectrum (95.5 MHz) of ribulose 5-phosphate (**1**) (top) and of the product mixture (compound X + formate) generated enzymatically (bottom). A solution containing 50 mM **1**, 8 mM $MgCl_2$, 0.16 M phosphate buffer pH 7.5, and purified enzyme from *Candida guilliermondii* was incubated at room temperature for 5 days.

using internal standardization with the synthetic material, all respective signals were strictly coincident. These data identify compound X as a phosphoric acid ester of 3,4-dihydroxy-2-butanone. The ^{13}C–^{31}P coupling constants suggest the location of the phosphate residue at C-4 by comparison with ribulose phosphate.

Additional information was obtained by experiments with several ^{13}C-labeled samples of **1** prepared enzymatically from appropriately labeled glucoses.[6] Experiments using [1-^{13}C]-**1** and [5-^{13}C]-**1** showed unequivocally that C-1 and C-4 of **2** are derived from C-1 and C-5, respectively, of the precursor **1**. This is well in line with our earlier experiments.[2]

An NMR spectrum of the reaction mixture obtained after enzymatic treatment of [U-$^{13}C_5$]-**1** shows a singlet at 173 ppm giving additional evidence for the formation of formate. The other

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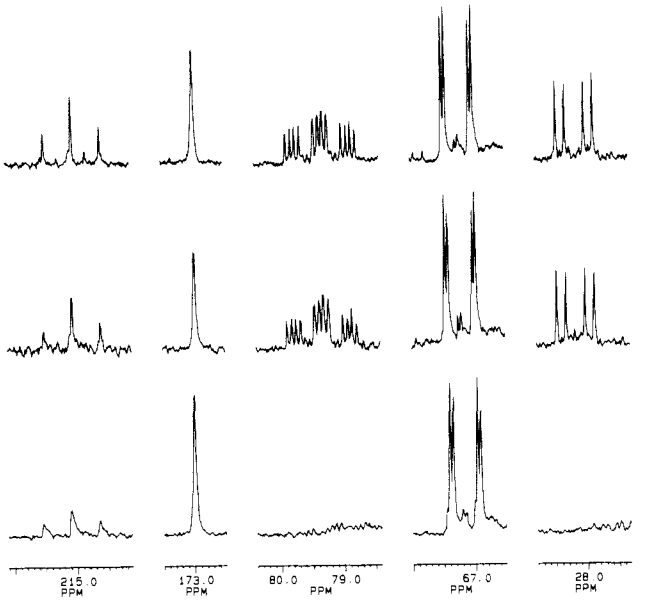
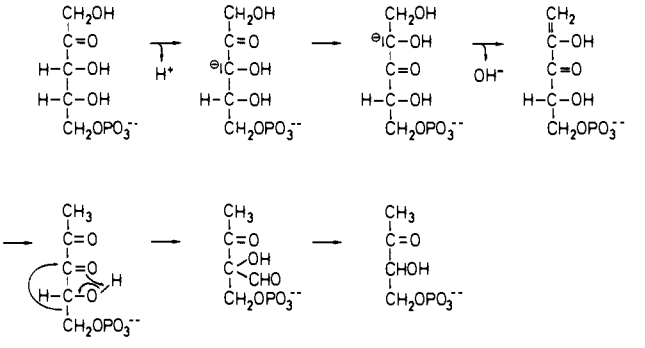


Figure 2. ^1H-decoupled ^{13}C NMR spectra (95.5 MHz) of compound X generated enzymatically from [U-$^{13}C_5$]-**1** (top), from a 1:1 mixture of [U-$^{13}C_5$]-**1** and **1** at natural abundance (center), and from [U-$^{13}C_5$]-**1** in D_2O (bottom).

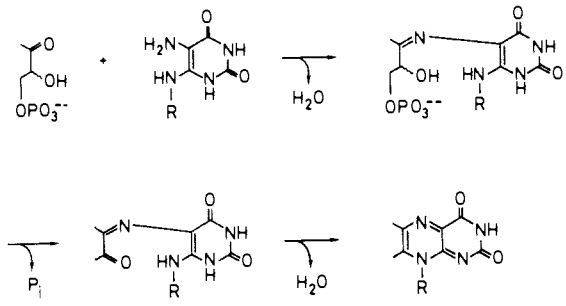
Scheme II. Hypothetical Mechanism for the Enzymatic Formation of 3,4-Dihydroxybutanone 4-Phosphate from Ribulose 5-Phosphate



signals appear as complex multiplets (Figure 2, top). The coupling patterns give additional confirmation for the structure assignment of compound X as 3,4-dihydroxy-2-butanone 4-phosphate. More important, the same multiplet pattern was found in an experiment with a mixture of [U-$^{13}C_5$]-**1** and **1** at natural abundance (Figure 2, center). This indicates that the removal of C-4 and the reconnection of C-3 with C-5 of the substrate **1** occurs by an intramolecular reaction.

Running the enzyme reaction in D_2O as solvent and with [U-$^{13}C_5$]-**1** as substrate resulted in severe broadening of the ^{13}C NMR signals of C-1 and C-3 of compound X, thus indicating the

Scheme III. Hypothetical Mechanism for the Enzymatic Formation of 6,7-Dimethyl-8-ribityllumazine (3)



introduction of deuterium from D_2O to positions 1 and 3 of **2** (Figure 2, bottom).

The available evidence is consistent with a hypothetical reaction mechanism starting with the generation of a methyl group by Lobry de Bryn–van Ekenstein reaction (Scheme II). Subsequent migration of C-5 to C-3 as anion followed by elimination of formate could complete the reaction.

The condensation of [1-^{13}C]-**2** with the pyrimidine **3** catalyzed by the β subunit of heavy riboflavin synthase from *Bacillus subtilis* yielded **4** predominantly labeled at the 6α methyl group in agreement with earlier studies.[2] A hypothetical mechanism for this reaction is summarized in Scheme III.

Acknowledgment. This work was supported by grants from the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. We thank Astrid Kōnig and Angelika Kohnle for expert technical and secretarial assistance.

Registry No. 1, 551-85-9; **2**, 114155-98-5; **3**, 17014-74-3; **4**, 2535-20-8; riboflavin, 83-88-5; riboflavin synthase, 9075-82-5.

Pillaring of Layered Double Hydroxides (LDH's) by Polyoxometalate Anions

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Layered silicate clays (LSC's) intercalated by pillaring polyoxocations are precursors to an important class of microporous catalysts[1] for a large number of reactions,[2] including shape selective petroleum cracking.[1,3] To date, smectite clays are the only host structures known to be pillarable by purely inorganic oxo ions. In the present work we report the oxo ion pillaring of a new family of lamellar ionic compounds, namely the layered double hydroxides (LDH's).

In LDH's, the structural polarity is the reverse of LSC's, i.e., the layers are two-dimensional hydroxo cations and the gallery species are anions. Typical compositions are $[M_{1-x}^{II}M_x^{III}(OH)_2][A^{n-}]_{x/n}\cdot yH_2O$, where M^{II} and M^{III} occupy octahedral positions in the hydroxide sheets, A is the gallery anion, and x = 0.17–0.33. Although many different oxo anions have been encapsulated in LDH's by topotactic ion exchange reactions,[4] the oxo ions have been small (CO_3^{2-}, SO_4^{2-}) and the interlayer gallery heights have been limited to values corresponding to one or two layers of space-filling oxygen.

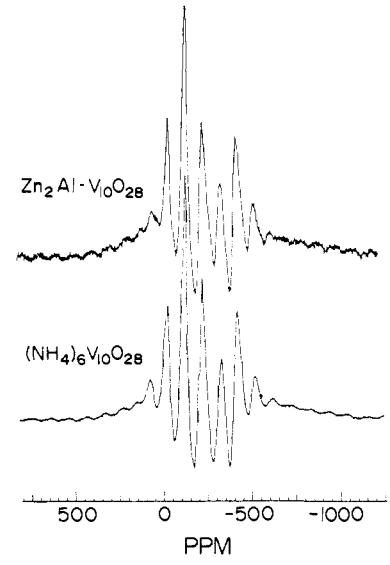

Figure 1. ^{51}V MAS NMR spectra for the Zn_2Al–$V_{10}O_{28}$ intercalate and for $(NH_4)_6V_{10}O_{28}\cdot 6H_2O$ obtained at 47.32 MHz and a spinning rate (4.8 KHz).

Polyoxometalates (POM's) should be ideal pillaring agents for LDH's. These anions generally possess structures consisting of multiple layers of space-filling oxygens as well as a wide range of charge densities.[5] Robust POM's should impart large gallery heights, and those with suitably high charge densities should give rise to large lateral anion spacings, thereby providing access to the intracrystalline gallery surfaces.

The decavanadate anion, $V_{10}O_{28}^{6-}$, was selected as our initial pillaring agent. LDH's with idealized formulas of $[Zn_2Al(OH)_6]Cl\cdot 2H_2O$, $[Zn_2Cr(OH)_6]Cl\cdot 2H_2O$, and $[Ni_3Al(OH)_8]Cl\cdot 2.3H_2O$ were prepared by previously reported coprecipitation methods.[6] Chemical analyses and X-ray basal spacings (d_{001} = 7.62 Å) were compatible with the indicated formulas. Pillaring was achieved by ion exchange of the chloride LDH with $[NH_4]_6[V_{10}O_{28}]\cdot 6H_2O$ at pH 4.5 and 25 °C.[7,8] For each product, chemical analysis indicated the absence of chloride and the presence of 0.17 mol $V_{10}O_{28}^{6-}$ per LDH equivalent, as expected for complete exchange. The basal spacings (d_{001} = 11.9 Å) corresponded to gallery heights of 7.1 Å (three oxygen planes) and to a $V_{10}O_{28}^{6-}$ orientation in which the C_2 axis is parallel to the host layers.

Further verification of intercalated $V_{10}O_{28}^{6-}$ was obtained from the ^{51}V MAS-NMR spectrum of the pillared Zn_2Al intercalate in comparison to the spectrum for the ammonium salt (Figure 1). Although the spectra were obtained at the spectrometer frequency (47.32 MHz) and spinning rate (4.8 KHz) which does not lead to isotropic averaging of chemical shifts, the spectra are qualitatively similar and indicative of the anion retaining its

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(6) Miyata, S. *Clays Clay Miner.* **1975**, *23*, 369.

(7) The pH during the exchange reaction was carefully monitored and controlled by the addition of dilute HCl in order to minimize the hydrolysis of $V_{10}O_{28}^{6-}$.[8] At pH values in the range of 5.5–10.0, formation of a LDH–$V_4O_{12}^{4-}$ phase was observed as a coproduct with d_{001} = 9.51 Å.

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