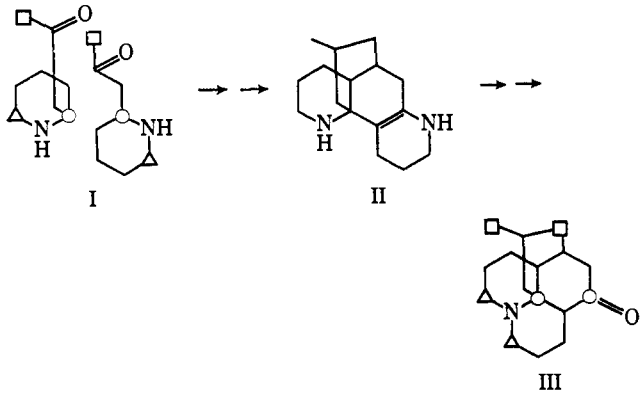
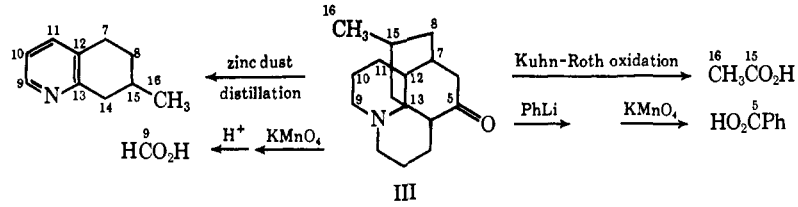
Scheme I



I II III

is shown in Scheme I. We now report the results of an experimental examination of the biosynthetic relationship of pelletierine and lycopodine.

Scheme II



III

Labeled pelletierine was synthesized[3] by the reaction of acetoacetate with Δ^1-piperideine, which in turn was prepared by the oxidation of lysine. The following radiomers of pelletierine, prepared by this method, were administered to *Lycopodium tristachyum*:[4] [4,5-$^3H_2$,2-^{14}C]pelletierine (^{3}H:^{14}C 9.5 ± 0.1), [6-^{14}C]-pelletierine, and [2,3′-$^{14}C_2$]pelletierine, which was shown to contain 18 ± 0.3% of its label in the C-methyl group (C-3′) and 79 ± 2% of its label at C-2 of the piperidine ring.

Table I. Incorporation of Pelletierine into Lycopodine

Product	Precursor: [2-^{14}C]-Pelletierine[a]	[2,3′-$^{14}C_2$]-Pelletierine	[6-^{14}C]-Pelletierine
	Relative specific activity, %		
Lycopodine	100 ± 2	100 ± 2	100 ± 2
7-Methyl-5,6,7,8-tetrahydroquinoline (C-7 to -16)	94 ± 4	96 ± 3	
Acetic acid[b] (C-15,16)		18 ± 1	
Formic acid[b] (C-9)			104 ± 3
Benzoic acid (C-5)	1 ± 0.1		

[a] The data in this column refer to the ^{14}C activity of the sample of lycopodine derived from [4,5-$^3H_2$,2-^{14}C]pelletierine. [b] Isolated as the α-naphthylamide.

Radioactive lycopodine was isolated from each of the three feeding experiments. The sample from the experiment with [4,5-$^3H_2$,2-^{14}C]pelletierine showed a ^{3}H:^{14}C ratio (^{3}H:^{14}C 9.6 ± 0.1) identical with that of the precursor.

(3) R. N. Gupta and I. D. Spenser, *Can. J. Chem.*, 47, 445 (1969).

(4) A voucher specimen of the plant used in our experiments is now deposited in the herbarium of Algonquin Provincial Park, Ontario. It is unfortunate that an erroneous designation of the species used in our work was reported[2] in our earlier communication.

The lycopodine samples were partially degraded to locate the sites of ^{14}C labeling. The degradation products which were isolated are shown in Scheme II.[5]

The relative specific activities of these products, obtained from the active samples of lycopodine, are presented in Table I.

The recovery, from the experiment with [^{3}H,^{14}C]-pelletierine, of lycopodine whose ^{3}H:^{14}C ratio matched that of the precursor was consistent with the hypothesis that lycopodine is a modified dimer of pelletierine. The absence of activity derived from [2-^{14}C]pelletierine at C-5 of lycopodine (benzoic acid) was not, however. The hypothesis demands 50% of activity at this site. It is evident that the C_8 unit of the lycopodine molecule which includes C-5 (*i.e.*, C-1 to -8) is not derived from pelletierine in the predicted manner. Since all activity derived from [2-^{14}C]pelletierine was confined to the portion of the molecule represented by 7-methyl-5,6,7,8-tetrahydroquinoline (C-7 to -16), it seemed likely that, whereas the C_8 unit, C-1 to -8, was not derived from pelletierine at all, a pelletierine unit did indeed serve as the precursor of the other C_8 unit of the lycopodine molecule, C-9 to -16. This conclusion is supported by the recovery from C-9 (formic acid) of all activity derived from [6-^{14}C]pelletierine. Intact incorporation of a pelletierine moiety into the C_8 unit, C-9 to -16, is clearly established by the results of the experiment with [2,3′-$^{14}C_2$]pelletierine. Not only was all activity from this precursor present in the quinoline derivative (C-7 to -16), but the fraction of this activity, recovered in the Kuhn–Roth acetate (C-15,16), was identical with the fraction of activity at the C-methyl group of the precursor.

It is thus demonstrated that an intact pelletierine unit serves as a specific precursor of C-9 to -16 of lycopodine, as predicted. Contrary to prediction, however, lycopodine is not a modified dimer of pelletierine, since C-1 to -8 are not derived from this precursor. The origin of the latter C_8 unit is under investigation.

(5) We are greatly indebted to Dr. W. A. Ayer for informing us, prior to publication, of the results of D. A. Law (Ph.D. Thesis, University of Alberta) bearing on the oxidative cleavage of the C-9,10 bond of lycopodine and the isolation and identification of the resulting N-formylamino acid from which formic acid (representing C-9 of lycopodine) was obtained by hydrolysis.

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An "Artificial Enzyme" Combining a Metal Catalytic Group and a Hydrophobic Binding Cavity

Sir:

Although rate increases of up to 10^9 have been attained in some metal ion promoted reactions,[1] such

Table I. Pseudo-First-Order Rate Constants[a] for the Deacetylation of *p*-Nitrophenyl Acetate (*p*NPA) and 8-Acetoxyquinoline-5-sulfonate (AQS) in the Presence of Added Catalysts and Inhibitors at 30.0°

Substrate	Catalyst (m*M*)	Cyclohexanol inhibitor, *M*	k_{obsd},[b] min^{-1}
*p*NPA	None		7.1×10^{-5} (25°)[c]
	I-Ni^{2+} (5.0)		2.2×10^{-4} (55°)[d]
	Cyclohexaamylose and Ni^{2+}		2.2×10^{-4} (55°)[d]
	II (5.09)[e]		$9.91 \pm 0.04 \times 10^{-2}$
	II (10.0)		$19.3 \pm 0.1 \times 10^{-2}$
	PCA-Ni^{2+} (5.01)		$2.60 \pm 0.06 \times 10^{-2}$ [f]
	PCA-Ni^{2+} (10.0)		$5.06 \pm 0.24 \times 10^{-2}$
	II (5.09)	0.050	$5.99 \pm 0.32 \times 10^{-2}$
	II (5.09)	0.134	$4.78 \pm 0.20 \times 10^{-2}$
	PCA-Ni^{2+} (5.01)	0.050 or 0.134	$2.61 \pm 0.20 \times 10^{-2}$
AQS	II (5.09)		$11.9 \pm 0.9 \times 10^{-2}$
	PCA-Ni^{2+} (5.00)		$21.4 \pm 1.3 \times 10^{-2}$

[a] Rate of production of *p*-nitrophenol and 8-hydroxyquinoline-5-sulfonate determined spectrophotometrically at 370 nm in 0.10 *M* acetate buffer, pH 5.17 ± 0.03, I = 1.0 (NaCl), [ester] ~ 1×10^{-4} *M*, with 1% (v/v) added acetonitrile. [b] Average of two or more runs. Error values reflect reproducibility between separate runs. Individual runs were nicely first order with correlation coefficients 0.9998 or better. [c] W. P. Jencks and J. Carriulo, *J. Amer. Chem. Soc.*, **82**, 1778 (1960). [d] Reaction 22% complete in 1160 min. I-Ni^{2+} showed no signs of decomposition after 35 hr at 55°. [e] II was stable indefinitely at 30°. [f] k_{obsd} = $3.15 \pm 0.05 \times 10^{-2}$ min^{-1} in the presence of 5.0×10^{-2} *M* cyclohexaamylose and pyridine-2,5-dicarboxylic acid.

effects are limited to substrates which can bind strongly to the metal ions. By contrast, metalloenzymes seem to bind their substrates principally by use of the same kinds of forces, including hydrophobic interactions,[2] common to all enzymes. Thus in order to extend the powerful catalytic effects of metals to nonligand substrates and to furnish better models for metalloenzymes, we have set out to attach a metal-binding group to a molecule with a hydrophobic cavity. For this we have selected cyclohexaamylose, a toroidal polysaccharide which has been shown[3] to bind simple hydrocarbon groups into its hydrophobic center. We wish to report the preparation of such a molecule (I) and the demonstration that an appropriate metal derivative (II) will catalyze the hydrolysis of *p*-nitrophenyl acetate by attack of the functional group onto a substrate molecule bound in the cavity.

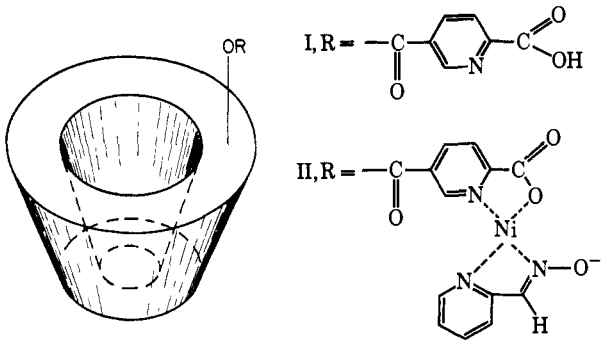

Figure 1. Schematic structural drawings. The functional groups are attached to a secondary alcohol group on the more open face of the toroidal cyclohexaamylose. The *p*-nitrophenyl acetate substrate binds in the cavity.

The metal-binding group was attached to cyclohexaamylose by taking advantage of Bender's finding[4] that esters of *m*-nitrophenol acylate a secondary hydroxyl group of the cycloamylose very rapidly. Thus treatment of an aqueous solution of cyclohexaamylose with 1 equiv of the 5-*m*-nitrophenyl ester of pyridine-2,5-dicarboxylic acid for 3 min at 28° and pH 9.1, followed by neutralization and careful gel filtration chromatography on Sephadex G-15, afforded the cyclohexaamylose ester of pyridine-2,5-dicarboxylic acid (I) in 55–65% yield, uncontaminated with cyclohexaamylose. Solutions of I (λ_{max} 272 nm (ϵ 7.0×10^3)) were stable indefinitely at pH 4–6, but underwent clean first-order hydrolysis at pH 9.1 ($t_{1/2}$ = 50 min) to liberate 1.0 ± 0.2 equiv of pyridine-2,5-dicarboxylic acid (λ_{max} 270 nm (ϵ 6.15×10^3)). The addition of 1 equiv of $NiCl_2$ to solutions of I afforded the nickel chelate (I-Ni^{+2}) which showed, besides the λ_{max} at 272 nm, shoulders characteristic of the nickel picolinate complex at 267 and 279 nm.

This was converted to II with 1 equiv of pyridinecarboxaldoxime (PCA), and the kinetics of hydrolysis of *p*-nitrophenyl acetate (*p*NPA) by II were studied. The nitrophenyl group has previously been shown to bind in the cyclohexaamylose cavity.[3,4] As we have found[5] with simple PCA-Ni^{2+}, the hydrolysis is a two-step process—acetylation of the PCA oxygen, followed by metal-catalyzed hydrolysis of the PCA acetate. Only the first step should in principle be especially facilitated in II, so the data for this step are listed in Table I.

Table I shows that 0.01 *M* II is nearly four times more reactive toward *p*NPA than is an equivalent concentration of the nickel-PCA complex (PCA-Ni^{2+}), corresponding to a rate acceleration of greater than 10^3 over the uncatalyzed rate. Control experiments have established that this increase in rate of II relative to PCA-Ni^{2+} does not arise from the presence of cycloamylose or pyridine-2,5-dicarboxylate. We therefore suggest that this increased reactivity of pNPA toward II is a result of binding and reaction within the II-*p*NPA complex. Consistent with this interpretation is the observation that the hydrolysis of *p*NPA in the presence of II can be competitively inhibited (K_i = 0.05–0.07 *M*)

(1) *E.g.*, R. Breslow, R. Fairweather, and J. F. Keana, *J. Amer. Chem. Soc.*, **89**, 2135 (1967).
(2) *Cf.* W. P. Jencks, "Catalysis in Chemistry and Enzymology," McGraw-Hill Book Co., Inc., New York, N. Y., 1969.
(3) F. Cramer, W. Saenger, and H.-Ch. Spatz, *J. Amer. Chem. Soc.*, **89**, 14 (1967), and references therein.
(4) R. L. VanEtten, J. F. Sebastian, G. A. Clowes, and M. L. Bender, *ibid.*, **89**, 3242 (1967); R. L. VanEtten, G. A. Clowes, J. F. Sebastian, and M. L. Bender, *ibid.*, **89**, 3253 (1967).
(5) R. Breslow and D. Chipman, *ibid.*, **87**, 4195 (1965); J. Malmin, Ph.D. Thesis, Columbia University, 1969.

by the addition of cyclohexanol, while the rate of the PCA–Ni^{2+} catalyzed reaction is unaffected by added cyclohexanol. Also consistent with this interpretation is the observation that 8-acetoxy-5-quinolinesulfonate (AQS), a molecule which does not fit into the cyclohexaamylose cavity, is only 57% as reactive toward II as toward PCA–Ni^{2+}. That in fact AQS is not bound into the cavity of II is further evident from the inability of cyclohexanol to inhibit the reaction of II with AQS. The dissociation constant for the II–*p*NPA complex must be greater than 0.05 *M* since saturation kinetics were not observed. Accordingly V_{max} for the acyl transfer under our conditions is at least 1 min^{-1}. Turnover occurs, and at pH 5.17 and 30.0° the overall pseudo-first-order rate constant, corresponding to the hydrolysis of the acetylated intermediate, is $3.95 \pm 0.60 \times 10^{-3}$ min^{-1}.

It should be pointed out that in contrast to the intracomplex reaction[4] of *m*-nitrophenyl acetate with cycloamylose at high pH, the present reaction occurs rapidly near neutrality (both the acylation and deacylation are base catalyzed,[5] and the rate of hydrolysis at pH 7.0 would be even greater than that observed at pH 5.1). However, the extent to which the very effective catalysis by our functional group has been increased by hydrophobic binding is only modest, of the same general magnitude as Cramer has observed[6] for some imidazole-substituted cycloamyloses. Presumably the difficulty is that even though models show that the PCA oxygen can reach the acetyl group of bound *p*NPA, several degrees of rotational freedom must be frozen for this to occur. As greater rigidity is built into the catalyst this problem should diminish.[7,8]

(6) F. Cramer and G. Mackensen, *Angew. Chem. Intern. Ed. Engl.*, **5**, 601 (1966).

(7) Support of the work by the National Institutes of Health, through a research grant and a postdoctoral fellowship to L. E. O., is gratefully acknowledged.

(8) NOTE ADDED IN PROOF. We have recently found a related phenomenon in the observation that the copper(II) complex of I is a six fold better catalyst for the hydrolysis of the *p*-nitrophenyl ester of glycine than is an equivalent concentration of copper(II) or the copper(II) pyridine-2,5-dicarboxylic acid complex. This acceleration is also competitively inhibited by added cyclohexanol.

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A Molecular Palladium(II) Complex Containing Both Sulfur- and Nitrogen-Bonded Thiocyanate Groups

Sir:

Metal complexes containing both S- and N-bonded nonbridging thiocyanate ions have been difficult to prepare. Although preparation of S- and N-bonded complexes has been claimed on the basis of infrared and other evidence,[1] no X-ray studies have been carried out on these compounds.[2] Since the bonding of thiocyanate to palladium appears to be strongly influenced by the other ligands,[3] mixed chelating ligands have been utilized to prepare the elusive mixed thiocyanate complexes.[4] We report herein the first X-ray structural study of a mixed thiocyanato–isothiocyanato complex, Pd(PN)(NCS)(SCN), where PN is $(C_6H_5)_2PCH_2CH_2CH_2N(CH_3)_2$.[5]

The complex Pd(PN)(NCS)(SCN) is a nonelectrolyte in nitromethane (Λ_M = 4.2 cm^2 ohm^{-1} mol^{-1}). The infrared spectrum of the solid (Nujol mull) has a very sharp peak at 2120 cm^{-1} and a relatively broader peak at 2080 cm^{-1}, while in dichloromethane the sharp peak is at 2125 cm^{-1} and the broad peak at 2087 cm^{-1}. These results suggest that the mixed mode of thiocyanate coordination is present in both the solid and in solution.

The orange, polyhedral crystals are monoclinic, space group $P2_1/c$ with unit cell dimensions of a = 11.684 (3), b = 12.961 (4), c = 14.641 (3) Å, and β = 110.04 (1)°. The density calculated for four molecules per unit cell is 1.574 g/cm^3, and the observed density is 1.567 g/cm^3. Three-dimensional intensity data were measured using Cu Kα radiation and an automatic diffractometer. All the unique reflections with $2\theta \leq 135°$ were measured followed by a measurement of all the reflections within these limits in a hemisphere defined by our quarter-circle single-crystal orienter. After averaging the equivalent reflections, 2176 reflections were considered to be observed and used in the analysis.

The structure was solved by locating the palladium atom in the Patterson function and the light atoms in successive Fourier syntheses. The structure was refined by least-squares methods using individual isotropic and then anisotropic thermal parameters for all atoms. The refinement converged to an R (the usual residual) of 4.1%. No attempt was made to locate the hydrogen atoms.

The palladium atom is square planar with the four donor atoms being the phosphorus and nitrogen atoms from the PN ligand and one nitrogen and one sulfur atom from each thiocyanate group. The molecule projected onto the plane of the four coordinated atoms is illustrated in Figure 1. The two palladium thiocyanate linkages are different, and thus the compound is correctly formulated as Pd(PN)(SCN)(NCS), isothiocyanatothiocyanato(1-diphenylphosphino-3-dimethylaminopropane)palladium(II).

The steric constraints imposed by the PN ligand require the thiocyanate groups to be *cis* coordinated. The observed structure has the Pd–SCN bond *trans* to the amine nitrogen atom and the Pd–NCS linkage *trans* to the phosphorus atom. The Pd–S–CN angle is 107.3 (3)° and the Pd–N–CS angle is 177.7 (6)°. Other metal isothiocyanates have M–N–CS angles as low as 156°, whereas metal thiocyanates have M–S–CN angles up to 120°.[6] These results suggest that Pd—N≡C—S and Pd—S—C≡N are the major resonance forms in

(1) (a) I. Bertini and A. Sabatini, *Inorg. Chem.*, **5**, 1025 (1966); (b) G. C. Kulasingam and W. R. McWhinnie, *Chem. Ind.* (London), 2200 (1966); (c) K. N. Raymond and F. Basolo, *Inorg. Chem.*, **5**, 1632 (1966); (d) P. E. Nicpon and D. W. Meek, *ibid.*, **6**, 145 (1967); (e) A. J. Carty and A. Efraty, *Can. J. Chem.*, **47**, 2573 (1969).

(2) A referee pointed out that the formulation of Cu(tren)(NCS)(SCN)[1c] was contrary to the infrared and conductance data which suggested an ionic five-coordinated species. Subsequently, P. C. Jain and E. C. Lingafelter, *J. Amer. Chem. Soc.*, **89**, 6131 (1967), demonstrated that the compound was indeed $[Cu(tren)(NCS)]^+SCN^-$.

(3) D. J. Hewkins and A. J. Poe, *J. Chem. Soc.*, *A*, 1884 (1967), and J. L. Burmeister, *Coord. Chem. Rev.*, **1**, 205 (1966), and references contained therein.

(4) D. W. McPeek, P. E. Nicpon, and V. I. Meek, *J. Amer. Chem. Soc.*, in press; C. A. McAuliffe and D. W. Meek, *ibid.*, in press.

(5) *Anal.* Calcd for $C_{19}H_{22}N_3PPdS_2$: C, 46.20; H, 4.49; S, 12.98. Found: C, 46.19; H, 4.68; S, 13.10.

(6) A summary of the dimensions can be found in ref 2.