University of Münster, for the preliminary NMR measurements at 270 MHz.

Supplementary Material Available: ^1H NMR spectra (270 MHz) of cyclo[Pro-Bzl-Gly_2], cyclo[Pro_2Sar], and cyclo[Bzl-$Gly]_3$ (Figures 3-5) and interpretation of conformational behavior in cyclotripeptides (Table I) (6 pages). Ordering information is given on any current masthead page.

References and Notes

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H. Kessler,* P. Kondor, G. Krack, P. Krämer
Institute of Organic Chemistry, Laboratory Niederrad Theodor-Stern-Kai 7, D-6000 Frankfurt a.M. 70, Germany
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cyclo-Triphosphorus (δ-P_3) as a Ligand in Cobalt and Nickel Complexes with 1,1,1-Tris(diphenylphosphinomethyl)ethane. Formation and Structures

Sir:

Compounds formed by the reaction of white phosphorus with metal complexes are quite rate. Only recently some rather unstable compounds of the types $[RhCl(PR_3)_2(P_4)]$,[1] $[Fe(CO)_4]_3P_4$,[2] and $[Fe(CO)_3P_2]_n$,[2] which have been suggested to contain P_4 or P_2 molecular units, have been reported. However definitive conclusions about their structures have not been reached. The compound $[CoPCp]_4$ has been found to possess a cubane-like geometry with phosphorus and cobalt atoms at the vertices of a distorted cube.[3]

We have been using over several years the tri(tertiary phosphine), 1,1,1-tris(diphenylphosphinomethyl)ethane, $CH_3C(CH_2PPh_2)_3$, L, as an efficient ligand to form metal complexes with coligands of various types.[4] By reacting white phosphorus with cobalt(II) and nickel(II) aquoions in presence of L, the complexes $[CoL(P_3)]$ and $[LNi(P_3)NiL]Y_2$ (Y = BF_4, BPh_4) containing the *cyclo*-triphosphorus δ-P_3 group as a ligand were obtained. To our knowledge the existence of this molecular unit either free or bound has not been ascertained before.

Equimolecular quantities of $Co(BF_4)_2 \cdot 6H_2O$ in butanol and L in THF were allowed to react, at 50 °C and under inert gas atmosphere, with an excess of white phosphorus. After ~10 min yellow-orange crystals of the $[CoL(P_3)]$ complex (**1**) precipitated. They were filtered off and recrystallized from methylene chloride–butanol. Anal. Calcd for $C_{41}H_{39}CoP_6$: C, 63.41; H, 5.06; Co, 7.58; P, 23.93. Found: C, 63.56; H, 5.28; Co, 7.30; P, 24.92.

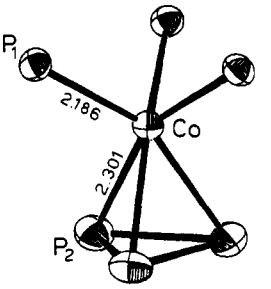

Figure 1. Inner skeleton of $[CoL(P_3)]$. The P–Co–P angles formed by the L and P_3 ligands are 93.6 (1) and 55.5 (1)°, respectively.

The $[LNi(P_3)NiL](BF_4)_2$ complex (**2**) was obtained by reaction at room temperature and under nitrogen atmosphere of $Ni(BF_4)_2 \cdot 6H_2O$ (1 mmol), L (1 mmol), and white phosphorus (excess), in THF–butanol solution. By concentration of the resulting solution, red-brown crystals precipitated. Anal. Calcd for $C_{82}H_{78}B_2F_8Ni_2P_9$: C, 60.29; H, 4.81; Ni, 7.18; P, 17.06. Found: C, 60.00; H, 5.14; Ni, 6.95; P, 17.17. The solution of this complex in acetone was added to a solution of $NaBPh_4$ in butanol and large crystals of the $[LNi(P_3)NiL](BPh_4)_2 \cdot 2(CH_3)_2CO$ complex (**3**) precipitated. Anal. Calcd for $C_{136}H_{130}B_2Ni_2O_2P_9$: C, 73.76; H, 5.91; Ni, 5.30; P, 12.58. Found: C, 73.61; H, 6.57; Ni, 5.15; P, 12.92.

All complexes are air stable, also in solution of THF, methylene chloride, nitroethane.

The cobalt derivative is diamagnetic and a nonelectrolyte in methylene chloride solution. The complex **3** is 1:2 electrolyte in nitroethane solution. The effective magnetic moments of the compounds **3** and **2**, respectively, are equal to 1.92 μ_B (at 293 K) and to 1.9 ± 0.1 μ_B (from 85 to 293 K) for the dimeric units. This is in agreement with the existence of one unpaired electron in both dimers.

The structures of **1** and **3** were established by single-crystal x-ray diffraction studies. Complex **1** crystallizes in space group $R3$ with the following cell constants: a = 10.57 (1) Å, α = 109.5 (1)°, Z = 1. Compound **3** belongs to space group $P\bar{1}$ with a = 17.53 (1), b = 15.86 (1), c = 13.88 (1) Å; α = 111.7 (1), β = 91.2 (1), γ = 115.4 (1)°; Z = 1.

Intensity data were collected on a Philips computer controlled PW 1100 diffractomerter (Mo Kα monochromatized radiation λ = 0.7107 Å) by the ω–2θ scan technique within $2\theta \leq 55°$ and $2\theta \leq 45°$ for complex **1** and **3**, respectively. Both structures were solved by the heavy-atom method and refined at the present stage to R = 0.048 over 1340 observed reflections ($I \geq 3\sigma(I)$) and R = 0.11 over 4285 observed reflections ($I \geq 3\sigma(I)$) for complex **1** and **3**, respectively. The rather high R value for complex **3** is due to the quality of the data affected by decomposition of the crystal and by the presence of disordered acetone molecules in the lattice which have not been yet included in the model. Refinement of both structures is still in progress.

The inner coordination geometries of the two complexes are shown in Figures 1 and 2. The metal atom in the cobalt complex is coordinated by the three phosphorus atoms of L and by the three phosphorus atoms of the P_3 unit. The two ligands are in a staggered position. The configuration of the nickel derivative is that of a triple-decker sandwich compound, with the P_3 unit bridging the two NiL moieties. The P–P distances in the *cyclo*-triphosphorus units of the two complexes, ranging from 2.13 to 2.16 Å, are indicative of covalent P–P bonds.

The electronic configurations of the complexes may be approached as follows. The 3d and 4s metal orbitals, which span the a_1 and e representations in C_{3v} symmetry, have nonzero overlap with the orbitals containing the lone pairs of the three

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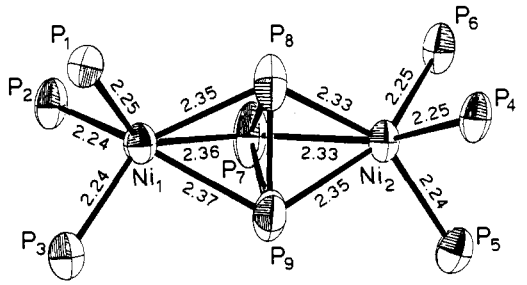

Figure 2. Inner skeleton of $[LNi(P_3)NiL]^{2+}$. Values of P_n-Ni-P_m angles: 89-96° (n, m = 1-6), 53-55° (n, m = 7-9).

phosphorus atoms of L and with the p_π orbitals of the P_3 molecule. If the latter are considered to provide 3 electrons and the former to provide 6 electrons, the total number of 18 electrons is attained for complex **1.** By the same approach, the number of 33 electrons is obtained for the nickel derivatives, which has never been reported for triple-decker sandwich compounds.[5,6] The presence of an odd number of electrons is confirmed by the value of the magnetic moment which corresponds to one unpaired electron for dimer.

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M. Di Vaira, C. A. Ghilardi
S. Midollini, L. Sacconi*
Istituto di Chimica Generale ed Inorganica Università e Laboratorio C.N.R. Via J. Nardi, 39, Firenze, Italy
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Catalytic Irreversible Inhibition of Mammalian Ornithine Decarboxylase (E.C. 4.1.1.17) by Substrate and Product Analogues

Sir:

The diamine putrescine and the polyamines spermidine and spermine which are derived from it have been implicated in the regulation of growth processes.[1] In attempts to delineate the still controversial roles of these bioamines, several reversible inhibitors of the pyridoxal phosphate (PLP)-dependent enzyme L-ornithine carboxylyase (ODC, E.C. 4.1.1.17) which catalyzes the conversion of L-ornithine to putrescine, have been prepared.[2] A new and elegant approach to specific, irreversible enzyme inactivation is to design inhibitors possessing latent reactive groupings which are unmasked at the enzyme's active site as a result of the normal catalytic turnover.[3] Such known inhibitors are analogues of the normal enzyme substrate, but, less obviously, in view of the microscopic reversibility principle, they may conceptually be analogues of the product. This communication discloses that, not only the ornithine analogues **1,** but also the putrescine analogues 5-hexyne-1,4-diamine (**2**) and *trans*-hex-2-en-5-yne-1,4-diamine (**3**) are irreversible inactivators of ODC, and that, in each case, the mechanism of inhibition demands activation of the inhibitor by the target enzyme.

Incubation of the enzyme preparation, obtained from the livers of thioacetamide-treated rats[4] at pH 7 with **1a, 1b, 1c, 2,** and **3** in each case resulted in a time-dependent loss of enzyme activity which followed pseudo-first-order kinetics for at least two half-lives (Table I). Over longer time periods, the semilogarithmic plots deviated from linearity.[5] However, incubation with **1a,** the most efficient inhibitor among the ornithine analogues, or **2** at 0.1 mM concentration resulted in 95% inactivation of ODC after 10 min. Prolonged (24 h) dialysis of enzyme previously inactivated by **1a** or **2** against a buffer solution containing phosphate (30 mM), pyridoxal phosphate (0.1 mM), and dithiothreitol (5 mM) (conditions where the native enzyme is stable) did not lead to regeneration of enzyme activity, thus demonstrating the irreversibility of the process. That the inhibition of ODC is active site directed is shown by the protective effects of the natural substrate L-ornithine, of a competitive inhibitor 2-methylornithine[6] and of putrescine, the product of decarboxylation, against induced inactivation. The presence of dithiothreitol (5 mM) in the preincubation medium and the absence of lag time before the onset of inhibition rule out the possibility of inhibition via an affinity labeling mode by a diffusible alkylating species.

Table I. Kinetic Constants for the Irreversible Inhibition of Rat Liver ODC[a]

Compd	K_I, μM[c]	τ_{50}, min[c]	k_{inact}, s^{-1}[c]
1a	39	3.1	3.7×10^{-3}
1b	*d*	*d*	
1c	8700	29	0.4×10^{-3}
2	2.3	9.7	1.2×10^{-3}
[^{2}H]-**2**	4.3	9.6	1.2×10^{-3}
3	1	5	2.3×10^{-3}

[a] The composition of the stock solution of ODC follows: proteins (16 mg/mL), sodium phosphate buffer (30 mM, pH 7.1), dithiothreitol (5 mM), pyridoxal phosphate (0.1 mM). The specific activity of this stock solution was 0.12 nmol of CO_2/min per mg of protein.[b] For a typical experiment 320 μL of this stock solution were mixed at time 0 with 80 μL of a solution of inhibitor in water and incubated at 37 °C. At different times 50-μL aliquots were transferred into a 1-mL assay medium containing sodium phosphate (30 mM, pH 7.1), dithiothreitol (5 mM), pyridoxal phosphate (0.1 mM), L-ornithine (0.081 μmol), and DL-[1-^{14}C]ornithine (0.043 μmol, 58 Ci/mol, Amersham) in a closed vessel in which a filter paper moistened with 50 μL of hyamine hydroxide (1 M) was fitted. The reaction was allowed to proceed for 60 min at 37 °C and then terminated by the addition of 0.5 mL of 40% trichloroacetic acid. After an additional 30 min the CO_2 absorbed on the filter paper was counted in a standard scintillation cocktail. [b] Partially purified preparations of similar specific activity have been used by others[2b] in their assessment of potential irreversible inhibitors of ODC. [c] K_I (apparent dissociation constant), τ_{50} (half-life or $t_{1/2}$ at infinite concentration of inhibitor), and k_{inact} (inactivation rate constant) were calculated according to the method of Kitz and Wilson.[8] [d] No saturation kinetics were apparent with **1b**; at a concentration of 0.1 mM, $t_{1/2}$ is 22 min.

Further evidence for the involvement of the enzyme's active site in the inhibitory process comes from the observed saturation effect (Table I) on the rate of inactivation (demonstrated by plotting $t_{1/2}$ as a function of $1/I$ according to Kitz and Wilson[8]). Moreover, with both **1a** and **2,** the inhibitory activity resides with only one optical isomer ((−)-**1a** and (−)-**2**), the other isomer being essentially inactive.[9]

When the rate of inhibition induced by 4-deuterio-5-hexyne-1,4-diamine ([^{2}H]**2**) was compared with that observed with **2,** no kinetic isotope effect on the inactivation rate constant was observed, but rather a primary kinetic isotope effect on the apparent Michaelis constant is measured ($K_{H/D}$ = 1.9). Proton abstraction hence must occur, but is not rate limiting. Presumably, the rate-determining step involves covalent linkage of transformed inhibitor to the enzyme.

A straightforward mechanism (Scheme I) can be considered for the inactivation of ODC by **1a, 1b,** and **1c.** The first steps

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