The essential features of $Rh_2(C_{22}H_{22}N_4)(CO)_4$ (III) can be summarized as follows (see Figure 2). (1) The ligand has an extremely warped conformation (via twisting about the C–N bonds of the five-membered chelate rings such that the nitrogen atom lone pairs are directed almost normal to the N_4 plane). It differs dramatically from the free ligand or any of its metal complexes structurally characterized to date.[11] (2) The twisted ligand conformation allows two Rh(I) atoms to coordinate to the macrocyclic ligand, each Rh(I) bound to one of the 2,4-pentanediiminato chelate rings.[12] (3) The cofacial arrangement of the dimer leads to an eclipsed arrangement of the coordination dimer. (4) The Rh–Rh separation of 3.086 Å is indicative of weak bonding interaction. (5) The average Rh–N and Rh–C distances are 2.071 (4) and 1.864 (6) Å, respectively.

The structure of $[Rh_2(C_{22}H_{23}N_4)(CO)_4]ClO_4$ (V) is similar to that of $Rh(C_{22}H_{22}N_4(CO)_4$ (III) but has a number of important differences. Protonation at the methine carbon has produced chelates with longer Rh–N bonds, 2.11 (1) Å, on the protonated side and a slightly shorter Rh–N bonds on the other side, 2.05 (1) Å. A slight shortening of the intramolecular Rh–Rh distance to 3.057 (3) Å is observed. Most importantly, the $[Rh_2(C_{22}H_{23}N_4)(CO)_4]^+$ cations are located near a crystallographic inversion center producing four-atom Rh(I) chains comprised of two $[Rh_2LH(CO)_4]^+$ units[15] with closest intermolecular Rh–Rh contacts of 3.268 (4) Å. This solid-state interaction accounts for the pronounced color differences between the solution and solid-state species (Figure 3). A closely related structure has recently been found for the platinum compound "*cis*-diammineplatinum α-pyridone blue" which contains four-atom chains of Pt(II) ions. The Pt(II) ions bridged by the α-pyridone are separated by 2.779 Å; the bridging units are related by a crystallographic inversion center yielding Pt(II)–Pt(II) distances of 2.885 Å between dimers.[16]

The complete insolubility of the blue-black dichloride salt, $[Rh_2(C_{22}H_{22}N_4(CO)_4]Cl_2$ (II) and the similarity of its infrared and electronic spectra to those of the monoprotonated species obtained from solid-state spectra strongly indicate an extended linear arrangement of Rh(I) atoms in this structure as well. Intramolecular steric repulsions between carbonyl groups and the methyl groups of the macrocyclic ligand are apparent in Figure 3 and probably account for the existence of polymorphs of this compound.[17]

The structure of a porphyrinbis[dicarbonylrhodium(I)] complex has recently been reported[18,19] but differs in the important respect that the Rh(I) atoms are on opposite sides of the porphyrin plane. The Rh–Rh separation of 3.094 Å and other bond parameters about the Rh atoms do not differ appreciably from the parameters reported herein. The possibility of protonating the porphyrin ligand to alter its electronic structure or the possibility of extended Rh–Rh interactions are less likely.

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References and Notes

(1) J. B. Keister and J. R. Shapley, *J. Am. Chem. Soc.*, **98**, 1056 (1976), and references cited therein.
(2) O. Cambino, R. P. Ferrar, M. Chinone, and G. A. Vaglio, *Inorg. Chim. Acta*, **12**, 155 (1975), and references cited therein.
(3) S. A. R. Knox, J. W. Koepke, M. A. Andres, and H. D. Kaesz, *J. Am. Chem. Soc.*, **97**, 3942 (1975).
(4) V. W. Day, S. S. Abdel-Meguid, S. Dabestan, M. G. Thomas, W. R. Pretzer, and E. L. Muetterties, *J. Am. Chem. Soc.*, **98**, 8289 (1976), and references cited therein.
(5) A. H. Reis, Jr., V. S. Hagley, S. W. Peterson, *J. Am. Chem. Soc.*, **99**, 4186 (1977).
(6) H. Isci and W. R. Mason, *Inorg. Chem.*, **13**, 1175 (1974).
(7) K. Krogmann, *Angew. Chem., Int. Ed. Engl.*, **8**, 35 (1969).
(8) K. R. Mann, J. G. Gordon II, and H. B. Gray, *J. Am. Chem. Soc.*, **97**, 3553 (1975), and references cited therein.
(9) D. Benlian and M. Bigorgne, *Bull. Soc. Chim. Fr.*, 1583 (1963); G. Winkhaus and H. Singer, *Chem. Ber.*, **99**, 11 (1966).
(10) W. H. Woodruff, R. W. Pastor, and J. C. Dabrowiak, *J. Am. Chem. Soc.*, **98**, 7999 (1976).
(11) (a) V. L. Goedken, J. J. Pluth, S.-M. Peng, and B. Bursten, *J. Am. Chem. Soc.*, **98**, 8014 (1976); (b) M. C. Weiss, B. Bursten, S.-M. Peng, and V. L. Goedken, *J. Am. Chem. Soc.*, **98**, 8021 (1976).
(12) A similar mode of coordination has been proposed for tungsten and molybdenum complexes of the composition $M(C_{22}H_{23}N_4)(CO)_4$. Only one metal is coordinatd per ligand and metal–metal interactions are absent. L. G. Bell and J. C. Dabrowiak, *J. Chem. Soc., Chem. Commun.* 512 (1975).
(13) V. L. Goedken, S.-M. Peng, J. Molin-Norris, and Y.-A. Park, *J. Am. Chem. Soc.*, **98**, 8391 (1976).
(14) Crystal data for $Rh_2(C_{22}H_{22}N_4)(CO)_4$: M = 646; triclinic, $P\bar{1}$; a = 10.385 (2), b = 11.003 (2), c = 12.022 (1) Å; α = 111.34 (1), β = 94.87 (1), γ = 95.28 (1)°; U = 1263.7 Å^3; D_m = 1.70, D_c = 1.67 g cm^{-3}; and Z = 2. Crystal data for $[Rh_2(C_{22}H_{23}N_4)(CO)_4]ClO_4 \cdot C_7H_8$: M = 838.5; monoclinic, $C2/m$; a = 17.439 (8), b = 18.480 (9), c = 12.176 (8) Å; β = 113.39 (5)°; U = 3601.4 Å^3; D_m = 1.59, D_c = 1.55 g cm^{-3}; and Z = 4. Intensity data for both structures were collected on an automated diffractometer and the structures solved by Patterson and Fourier techniques and refined by full-matrix least-squares analysis varying positional and anisotropic thermal parameters for nonhydrogen atoms and including hydrogen atoms as fixed contributions. At convergence conventional and weighted R values for $Rh_2(C_{22}H_{22}N_4)(CO)_4$ were 4.62 and 6.30%, respectively, for 5302 independent reflections with $|Fs| > 3\sigma(F)$. The refinement of $[Rh_2(C_{22}H_{23}N_4)(CO)_4]ClO_4 \cdot C_7H_8$ was considerably less satisfactory because of crystal decomposition during data collection, unusually rapid decrease of intensity data as a function of θ, and severe disorder of both the perchlorate anion and the solvate toluene molecule. At convergence conventional and weighted R values were 6.6 and 7.5%, respectively, for 1259 independent reflections with $|Fs| > 3\sigma(F)$.
(15) The nearest Rh(I)–Rh(I) distance between Rh(I) atoms outside the four chains is 7.801 Å. For the molecular species, $Rh_2(C_{22}H_{22}N_4)(CO)_4$, the closest approach of noninteracting Rh(I) atoms is 4.982 Å.
(16) J. K. Barton, H. N. Rabinavitz, D. J. Szalda, and S. J. Lippard, *J. Am. Chem. Soc.*, **99**, 2827 (1977).
(17) Three different polymorphs of $Rh_2(C_{22}H_{24}N_4)(CO)_4Cl_2$ have been isolated. Two are blue-black in color, and are presumed to contain a linear arrangement of Rh atoms; they differ in the nature of the fine structure of the carbonyl region (2100–2000 cm^{-1}) of the infrared spectra. The third polymorph is orange and is assumed to be monomeric on the basis of its solubility and the similarity of its electronic spectrum with that of the orange-red perchlorate salt. The orange and blue-black forms are interconvertible in Nujol mulls. Subjecting the monomeric form to high pressures produces a color change from orange to blue-black with corresponding changes occurring in the IR spectrum. The reverse process, although requiring hours to days, occurs when the pressure is released.
(18) A. Takemak and Y. Sasada, *Acta Crystallogr., Sect. B*, **1**, 1 (1975).
(19) For reactivities of related porphyrin complexes, see H. Ogoshi, T. Omura, and Z. Yoshida, *J. Am. Chem. Soc.*, **95**, 1966 (1973); Z. Yoshida, H. Ogoshi, T. Omura, E. Watanabe, and T. Kurosake, *Tetrahedron Lett.*, **No. 11**, 1077 (1972); A. Takenaka, Y. Sasada, T. Omura, H. Ogoshi, and Y. Yoshida, *J. Chem. Soc., Chem. Commun.*, 792 (1973).

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Unusual Reactivity of Trifluoroacetyl Peptide Chloromethyl Ketones with Pancreatic Elastase

Sir:

In the course of a recent investigation,[1] it was found that trifluoroacetyl peptides (TFA-peptides) were much more potent reversible inhibitors of elastase than the corresponding acetylated ones (Ac-peptides). This prompted us to study trifluoroacetyl peptide chloromethyl ketones (TFA-peptide-CMK), first in the hope of getting potent irreversible inhibitors of elastase[2-4] which might be useful therapeutic agents, and second to permit the use of ^{19}F NMR for investigating the interactions of these peptides with the enzyme.

The compounds listed in Table I were prepared as follows. Z-Ala-CH_2Cl was obtained by reacting Z-Ala-CHN_2 with anhydrous HCl.[2] Deblocking of this compound was performed

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Table I. Irreversible Inhibition of Porcine Pancreatic Elastase by TFA-peptide-CMK[a]

Inhibitors	k_2 (s^{-1} 10^3)	K_I (10^{-3} × M)	k_2/K_I (M^{-1} s^{-1})	Range of inhibitor concn, mM
TFA-Ala-Ala-CH_2Cl	0.3	4	0.07	2–5
TFA-Ala-Ala-Ala-CH_2Cl	0.4	0.1	4	0.1–0.5
TFA-Ala-Ala-Ala-Ala-CH_2Cl	6	0.1	60	0.02–0.07
Ac-Ala-Ala-Ala-CH_2Cl[b]	6.3	1.5	4.2	0.1–0.5
Ac-Ala-Ala-Ala-Ala-CH_2Cl[b]	20	0.4	50	0.04–2.0

[a] Elastase, 1 μM; inactivation medium, 190 mM Tris-HCl + 5% v/v dimethylformamide; pH 8.0; T, 25 °C. [b] Data from Powers and Tuhy;[2] pH 6.5; 5% v/v CH_3OH; T, 30 °C.

Table II. ^{19}F NMR Properties of the Reversible (EI) and Irreversible (EI′) Complexes Formed between TFA-peptide-CMK and Pancreatic Elastase in 5.10^{-2} M Tris Buffer[a]

Inhibitor	Estimated chemical shift of EI,[b] Hz	Chemical shift of EI′,[b] Hz	Line width of EI′, Hz
TFA-$(Ala)_3$-CH_2Cl	≥100	−8	7
TFA-$(Ala)_4$-CH_2Cl	≥100	38	5

[a] pD 8.0, 34 °C. [b] The chemical shifts, referenced to those of the free inhibitor, are positive if the resonance of the complex is downfield.

at room temperature using HBr in CH_3COOH. Ala-CH_2Cl was then transformed into Boc-$(Ala)_n$-CH_2Cl by treating it stepwise with Boc-Ala (mixed anhydride procedure). The later compounds were deblocked at room temperature with HCl in CH_3COOH and trifluoroacetylated either with CF_3COOCH_3 for $n = 3$ or with the mixed anhydride method for the other peptides. All compounds were fully characterized (combustion analysis, IR, NMR, chromatography). Yields were usually low.

The inhibitors were dissolved in dimethylformamide prior to use. Inactivation experiments were started by adding an aliquot of inhibitor solution to a buffered enzyme solution; 10-μL samples of this medium were then diluted at different times with 1 mL of a substrate solution (succinyl-$(Ala)_3$-*p*-nitroanilide)[5] to measure the residual activity of elastase.

^{19}F NMR experiments were performed at 34 °C using a Fourier transform equipped Varian XL 100 spectrometer.

The kinetic data were analyzed assuming the classical scheme[6]

$$E + I \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EI \xrightarrow{k_2} EI'$$

k_2 and k_1 (i.e., k_{-1}/k_1) could be determined separately from double reciprocal plots.[6] The results are presented in Table I together with those of Powers and Tuhy[2] concerning Ac-peptide-CMK.

It appears that the acetyl compounds have much higher k_2 values than the corresponding TFA inhibitors, although they have been tested at a lower pH. On the other hand, the former inhibitors have much lower affinities than the later ones. Such a behavior (i.e., better binding leads to lower reactivity) may be accounted for by the fact that the TFA-peptide-CMK form nonproductive complexes with the extended active center of elastase.

To check this hypothesis, we have investigated the reaction between TFA-peptide-CMK and elastase using ^{19}F NMR spectroscopy. The interaction between elastase and TFA-$(Ala)_3$-CH_2Cl is characterized initially by a broadened line which is shifted downfield with respect to the line corresponding to the free peptide (see Figure 1). As the reaction proceeds, this line shifts upfield and its intensity decreases. Concomitantly, a new line appears, whose position and width do not depend upon the extent of the reaction. These observations are interpreted in the following way. The initial line corresponds to a peptide which is in exchange between its free and its complexed form at an intermediate rate on the NMR time scale. The upfield shift of this line and the decrease of its intensity are due to the decrease of the free enzyme concentration resulting from the formation of the irreversible complex whose resonance yields the second line. With TFA-$(Ala)_4$-CH_2Cl, analogue results were obtained except that the irreversible complex appeared faster, in agreement with the above enzymatic data.

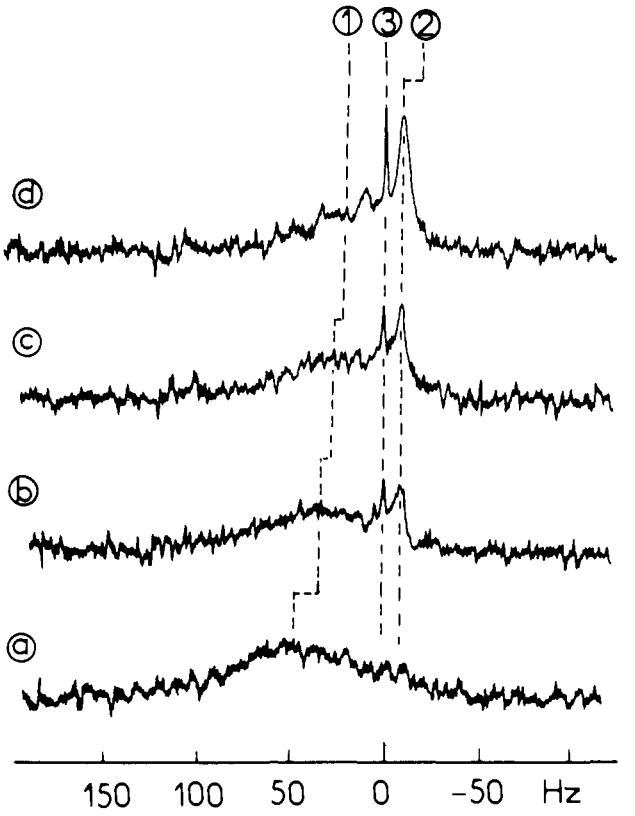

Figure 1. Time evolution of the ^{19}F NMR spectrum of 1.2×10^{-3} M TFA-$(Ala)_3$-CH_2Cl in the presence of 8.8×10^{-4} M elastase in Tris buffer 2×10^{-1} M, pD 8, 3% DMF, at 34 °C: a, b, c, and d correspond, respectively, to 20, 40, 60, and 100 min after mixing. The lines 1 and 2 are, respectively, attributed to the reversible and irreversible complexes. On the other hand, the line 3 is attributed to free trifluoroacetic acid from partial hydrolysis of the TFA peptide. The chemical shifts are referenced relative to the shift of the free peptide.

The large differences in chemical shifts (see Table II) indicate that the TFA group experiences very different environments within the reversible and the irreversible complexes. These results are compatible with the occurrence of nonproductive reversible complexes as already suggested by the enzymatic results. Nevertheless, it cannot a priori be excluded that the environment of the TFA group changes although the reversible complex is productive, but this hypothesis implies that the formation of the covalent bond with HIS 57 leads to an important reorganization of the enzyme-peptide complex, a view which is not in accord with the available x-ray diffraction data on elastase-inhibitor complexes.[7]

Our NMR data demonstrate thus that the replacement of an acetyl substituent by a trifluoroacetyl one leads to peptide-CMK which are able to form two kinds of reversible

complexes with elastase, i.e., productive ones able to transform into irreversibly inhibited enzyme and nonproductive ones unable to undergo further reaction. This explains unambiguously why the TFA-peptide-CMK possess higher affinities and lower rate constants than the corresponding acetyl-peptide-CMK (Table I).

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References and Notes

(1) J.-L. Dimicoli, J. Bieth, and J. M. Lhoste, *Biochemistry*, **15**, 2230–2236 (1976).
(2) J. C. Powers and P. M. Tuhy, *J. Am. Chem. Soc.*, **94**, 6544–6545 (1972).
(3) J. C. Powers and P. M. Tuhy, *Biochemistry*, **12**, 4767–4774 (1973).
(4) R. C. Thompson and E. R. Blout, *Biochemistry*, **12**, 44–47 (1973).
(5) J. Bieth, B. Spiess, and C. G. Wermuth, *Biochem. Med.*, **11**, 350–357 (1974).
(6) R. Kitz and I. B. Wilson, *J. Biol. Chem.*, **237**, 3245–3249 (1962).
(7) D. M. Shotton, N. J. White, and H. C. Watson, *Cold Spring Harbor Symp. Quant. Biol.*, **36**, 91–105 (1971).

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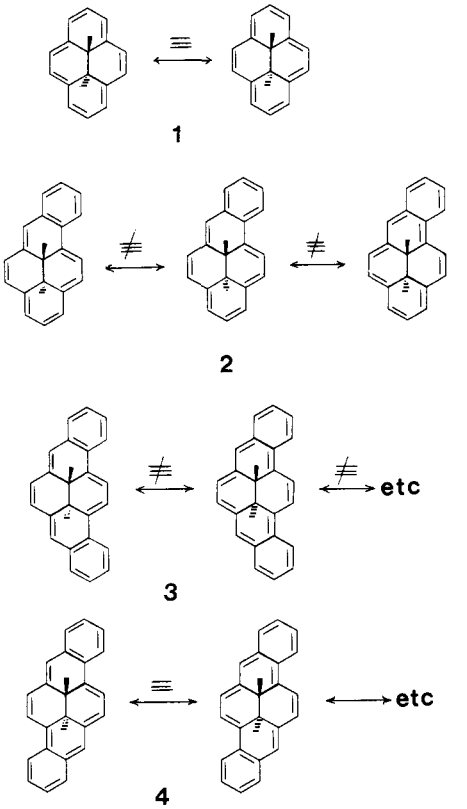
A Clear Demonstration of Importance of Symmetrical Kekulé Structures to Diatropicity (Aromaticity). Synthesis of a Stable, Highly Diatropic, Dibenzannulene[1]

Sir:

Although the concept of aromaticity is often introduced by consideration of the two identical Kekulé structures of benzene, when the aromaticity of larger annulenes is discussed (usually by comparison of diatropicities), such structures are normally neglected. We present here a clear experimental demonstration that, all other things being equal, the equivalence of Kekulé structures can have a profound effect on the diatropicity of an annulene. Nakagawa[2] has obtained results in the [18]annulene series which in our opinion also support the hypothesis, even though he suggests that his more recent[3] results throw doubt on his initial conclusions. This may be, however, because he is not comparing geometrically equivalent systems, or because of the inclusion of cumulated bonds. We believe that Boekelheide's[4] *trans*-15,16-dimethyldihydropyrene[5] (**1**) with its planar,[6] rigid [4n + 2] π-electron periphery, and easily discernable, highly shielded (δ −4.25) internal methyl protons is an excellent system to study to detect any such effect.

We thus now report the synthesis and properties of the mono- and bisbenzannelated derivatives of **1**, namely **2**, **3**, and **4**.



Reaction of 1,3-bis(bromomethyl)-2-methylnaphthalene,[7a,8] respectively, with 2,6-bis(mercaptomethyl)toluene[7b] and 1,3-bis(mercaptomethyl)-2-methylnaphthalene[8] (mp 88–89 °C prepared by the thiourea method[7b]) under high dilution conditions yielded the thiacyclophanes **5** (mp 188–190 °C; ^{1}H NMR ($CDCl_3$, 60 MHz) δ 8.3–7.0 (m, 8 H, ArH), 4.16, 4.13, 3.75, 3.70, 3.40, 3.35 (all s, 1 H, 1 H, 2 H, 2 H, 1 H, 1 H, $-CH_2-$), 1.42 (s, 3 H, -Np-CH_3), and 0.92 (s, 3 H, -PhCH_3))

Table I

Compd	Color of crystals	Mp, °C	NMR spectra: External H, δ	External C, ppm	Internal bridge C, ppm	Internal methyl H, δ	Internal methyl C, ppm
1[a]	Green		8.7–8.1	137–123	30.0	−4.25[b]	14.0
2	Orange-purple	115–116	8.7–7.1	139–117	35.5, 36.0	−1.60[b]	17.0, 17.7
3	Green	218–220	8.2–6.9	139–117	39.5	0.02[b]	19.2
4	Blue	195–196.5	9.8–7.8	137–124	32.8	−3.58[c]	15.9[d]

[a] R. DuVernet and V. Boekelheide, *Proc. Natl. Acad. Sci. U.S.A.*, **71**, 2961 (1974). [b] $CDCl_3$. [c] THF-d_8. [d] Tentative; this peak is very weak, possibly owing to relaxation time difficulties. The ^{1}H NMR spectra were recorded on a Perkin-Elmer R32 90-MHz spectrometer and ^{13}C NMR spectra in $CDCl_3$ on a Nicolet TT-14 60-MHz Fourier transform spectrometer, the chemical shifts being given in parts per million downfield from Me_4Si.

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