Insulin-Mimetic Peroxovanadium Complexes: Preparation and Structure of Potassium Oxodiperoxo(pyridine-2-carboxylato)vanadate(V), $K_2[VO(O_2)_2(C_5H_4NCOO)]\cdot 2H_2O$, and Potassium Oxodiperoxo(3-hydroxypyridine-2-carboxylato)vanadate(V), $K_2[VO(O_2)_2(OHC_5H_3NCOO)]\cdot 3H_2O$, and Their Reactions with Cysteine

Alan Shaver,*[1a] Jesse B. Ng,[1a] David A. Hall,[1a] Bernadette Soo Lum,[1a,b] and Barry I. Posner[1c]

Department of Chemistry, McGill University, 801 Sherbrooke Street West, Montreal, Quebec, Canada H3A 2K6, and Protein and Polypeptide Laboratory, Department of Medicine, Royal Victoria Hospital and McGill University, Montreal, Quebec, Canada H3A 2K6

Received September 30, 1992

The complexes potassium oxodiperoxo(pyridine-2-carboxylato)vanadate(V), $K_2[VO(O_2)_2(C_5H_4NCOO)]\cdot 2H_2O$ (**1**), and potassium oxodiperoxo(3-hydroxypyridine-2-carboxylato)vanadate(V), $K_2[VO(O_2)_2(OHC_5H_3NCOO)]\cdot 3H_2O$ (**2**), were prepared, and their structures were determined: **1**, *Cc*, $a = 16.952(4)$ Å, $b = 10.496(1)$ Å, $c = 7.492(1)$ Å, $\beta = 108.43(1)°$, $V = 1264.7$ Å^3, $Z = 4$; **2**, $P2_1/a$, $a = 7.078(2)$ Å, $b = 27.573(6)$ Å, $c = 7.222(3)$ Å, $\beta = 105.99(3)°$, $V = 1354.8$ Å^3, $Z = 4$. In both complexes, the pyridinecarboxylato group is coordinated to the vanadium atom via the nitrogen atom and one oxygen atom of the carboxylato group. The geometry about vanadium is pentagonal bipyramidal, with the two peroxo groups and the nitrogen atom of the pyridine ring defining the pentagonal plane while the vanadyl oxygen atom and the oxygen of the carboxylato group of the ligand occupy apical positions. The two complexes rapidly oxidize cysteine to cystine in aqueous solution. The results are discussed as a possible model for the insulin-mimetic activity of peroxovanadium complexes.

Introduction

Diabetes affects one person in 300 among the population of industrialized countries.[2] Although insulin injection combined with careful control of the diet is the best treatment at present, the well-known inadequacies of this regime[3] are a strong incentive to find alternatives. The insulin-mimetic ability of vanadium compounds, notably the vanadate anion (at pH 5–9:$H_2VO_4^-$),[4] has generated much interest since the original report by Shechter and Karlish in 1980.[5] For example, a report describing the use of bis(maltolato)oxovanadium(IV) in regulating the blood glucose level in diabetic rats via oral administration appeared recently.[6] In 1986, it was observed that mixtures of hydrogen peroxide and vanadate or vanadium(V) oxide were remarkable insulin mimics in a variety of different tests and were far more potent in controlling the blood glucose level in rats than either vanadate or hydrogen peroxide alone.[7] Unfortunately, the combination of the vanadate anion and H_2O_2 under physiological conditions generates several different peroxovanadium species.[8] Moreover, the aqueous preparations appeared to be unstable, losing their insulin-mimetic activity with time. Nevertheless, this observation raised the possibility that further research (i) might lead to clinically useful agents for the treatment of diabetes, (ii) provide some insight into the mode of insulin action, and (iii) extend the bioinorganic chemistry of vanadium.

We have recently found that a number of well-characterized and stable peroxovanadium complexes do indeed display strong insulin-mimetic activity both *in vivo* and *in vitro*.[9] Such compounds form a large new class of powerful insulin mimics. They are represented by the general formula $M_n[VO(O_2)_xL\text{-}L']\cdot yH_2O$, where M = NH_4^+ or K^+, $n = 0$–3, $x = 1$ or 2, and L-L' is normally a bidentate ligand such as 1,10-phenanthroline[10] although a single monodentate example (ammonia)[11] and a tridentate example (pyridine-2,6-dicarboxylato)[12] are also known. These complexes were originally intended to illustrate the chemistry and bonding modes of the peroxo group,[10–13] although some of them have been reported to have biological activity.[14]

Reported here is an improved synthesis of potassium oxodiperoxo(pyridine-2-carboxylato)vanadate(V), $K_2[VO(O_2)_2(pic)]\cdot 2H_2O$ (**1**),[15] and the preparation of the related new complex potassium oxodiperoxo(3-hydroxypyridine-2-carboxylato)vanadate(V), $K_2[VO(O_2)_2(OHpic)]\cdot 3H_2O$ (**2**), and their crystal structures. Preliminary tests in rats have shown that both complexes are extremely efficient insulin mimics.[9]

Results

Complexes **1** and **2** have been prepared by adding a solution (or slurry for **2**) of the pyridine ligand in ethanol to a mixture

* Author to whom correspondence should be addressed.

(1) (a) Department of Chemistry. (b) Present address: Dow Corning, Kanagawa, Japan. (c) Department of Medicine.
(2) (a) Atkinson, M. A. *Sci. Am.* **1990**, *263*, 62. (b) Stinson, S. C. *Chem. Eng. News* **1991**, *69* (39), 35.
(3) Reach, G.; Wilson, G. W. *Anal. Chem.* **1992**, *64*, 381A.
(4) (a) Shechter, Y. *Diabetes* **1990**, *39*, 1. (b) Heyliger, C. E.; Tahiliani, A. G.; McNeill, J. H. *Science* **1985**, *227*, 1474.
(5) Shechter, Y.; Karlish, S. J. D. *Nature* **1980**, *284*, 556.
(6) McNeill, J. H.; Yuen, V. G.; Hoveyda, H. R.; Orvig, C. *J. Med. Chem.* **1992**, *35*, 1489.
(7) (a) Posner, B. I.; Shaver, A.; Fantus, I. G. In *New Antidiabetic Drugs*; Bailey, C. J., Flatt, P. R., Eds.; Smith-Gordon: London, 1990; Chapter 8, pp 107–117. (b) Fantus, I. G.; Kadota, S.; Deragon, G.; Foster, B.; Posner, B. I. *Biochemistry* **1989**, *28*, 8864. (c) Kadota, S.; Fantus, I. G.; Deragon, G.; Guyda, H. J.; Hersh, B.; Posner, B. I. *Biochim. Biophys. Res. Commun.* **1987**, *147*, 259. (d) Kadota, S.; Fantus, I. G.; Deragon, G.; Guyda, H. J.; Posner, B. I. *J. Biol. Chem.* **1987**, *262*, 8252.
(8) (a) Howarth, O. W. *Prog. NMR Spectrosc.* **1990**, *22*, 453. (b) Harrison, A. T.; Howarth, O. W. *J. Chem. Soc., Dalton Trans.* **1985**, 1173. (c) Heath, E.; Howarth, O. W. *J. Chem. Soc., Dalton Trans.* **1981**, 1105. (d) Howarth, O. W.; Hunt, J. R. *J. Chem. Soc., Dalton Trans.* **1979**, 1388. (e) Campbell, N. J.; Dengel, A. C.; Griffith, W. P. *Polyhedron* **1989**, *8*, 1379. (f) Tracey, A. S.; Jaswal, J. S. *J. Am. Chem. Soc.* **1992**, *114*, 3835. (g) Jaswal, J. S.; Tracey, A. S. *Inorg. Chem.* **1991**, *30*, 3718.
(9) Posner, B. I., et al. Manuscript submitted for publication.
(10) Vuletic, N.; Djordjevic, C. *J. Chem. Soc., Dalton Trans.* **1973**, 1137.
(11) Drew, R. E.; Einstein, F. W. B. *Inorg. Chem.* **1972**, *11*, 1079.
(12) Drew, R. E.; Einstein, F. W. B. *Inorg. Chem.* **1973**, *12*, 829.
(13) Djordjevic, C.; Puryear, B. C.; Vuletic, N.; Abelt, C. J.; Sheffield, S. J. *Inorg. Chem.* **1988**, *27*, 2926.
(14) Djordjevic, C.; Wampler, G. L. *J. Inorg. Biochem.* **1985**, *25*, 51 and references therein.
(15) "Pic" is an abbreviation for "picolinic acid anion", which is the trivial name for the pyridine-2-carboxylic acid anion; the abbreviation OHpic follows as the abbreviation for the 3-hydroxypyridine-2-carboxylic acid anion.

0020-1669/93/1332-3109$04.00/0 © 1993 American Chemical Society

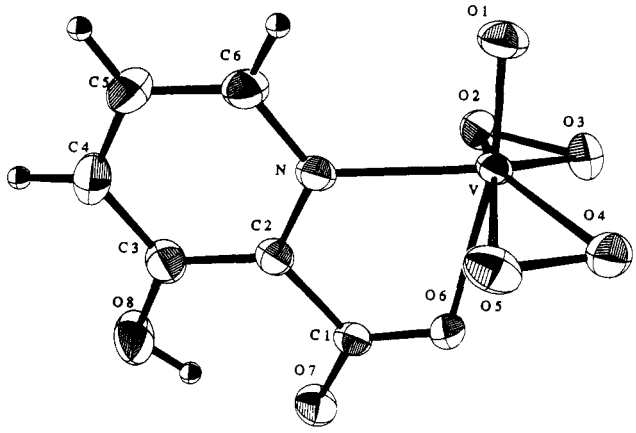

Figure 1. ORTEP diagram of $K_2[VO(O_2)_2(OHpic)]\cdot 3H_2O$ (**2**) excluding the H_2O molecules. Selected bond lengths (Å) and angle (deg) involving O(8) and H(O8): O(8)–C(3), 1.347(3); O(8)–H(O8), O.86(3); H(O8)–O(7), 1.69(4); O(8)–H(O8)–O(7), 158(3).

Table I. Crystallographic Data for $K_2[VO(O_2)_2(pic)]\cdot 2H_2O$ (**1**) and $K_2[VO(O_2)_2(OHpic)]\cdot 3H_2O$ (**2**)

	1	**2**
chem formula	$C_6H_8K_2NO_9V$	$C_6H_{10}K_2NO_{11}V$
fw	367.27	401.28
space group	*Cc* (No. 9)	$P2_1/a$ (No. 14)
a (Å)	16.952(4)	7.078(2)
b (Å)	10.496(1)	27.573(6)
c (Å)	7.492(1)	7.222(3)
β (deg)	108.43(1)	105.99(3)
V (Å^3)	1264.7	1354.8
Z	4	4
ρ_{calcd} (g cm^{-3})	1.929	1.967
λ (Å)	0.710 69	0.71069
μ(Mo Kα) (cm^{-1})	14.55	13.76
T (°C)	20 ± 1	20 ± 1
R^a	0.037	0.023
R_w^b	0.031	0.028

^{a} $R = \sum||F_o| - |F_c||/\sum|F_o|$. ^{b} $R_w = [\sum w(|F_o| - |F_c|)^2/\sum w|F_o|^2]^{1/2}$.

of vanadium(V) oxide and potassium hydroxide in hydrogen peroxide, as originally reported.[16] It was found that **1** thus prepared may contain additional peroxovanadate species which contain no ancillary ligand if the pH is too high or if the solution is stirred too long before the addition of ligand. Such impurities, identified by ^{51}V NMR spectroscopy, include $[VO(O_2)_2(H_2O)_2]^-$ (δ_V –697.3 ppm),[8f] $[V(O_2)_3(OH)]^{2-}$ (δ_V –726.7 ppm),[8g] and $[\{VO(O_2)_2\}_2(OH)]^{3-}$ (δ_V –752.2 ppm).[8g] On the other hand, addition of a solution of V_2O_5 and KOH in H_2O_2 to a cold solution of pyridine-2-carboxylic acid in aqueous KOH gave purer crops of **1**, but this method could not be extended to the preparation of **2** as the product obtained in this manner was consistently impure.

Both **1** and **2** are yellow crystalline complexes, stable indefinitely in the solid state and very soluble in water. They do, however, decompose slowly in aqueous solution (over a week) to give a mixture of vanadates. Crystals of **1** and **2** were obtained when solutions of the complexes in a mixture of water, H_2O_2, and ethanol were cooled at 5 °C. The structures of the two complexes have been determined, and that of **2** is depicted in Figure 1. The structure of **1** is visually identical except for the absence of the OH group and is shown in Figure 2 in the supplementary material. The crystal data for **1** and **2** are given in Table I, positional parameters in Table II, and selected bond lengths and angles in Table III.

The coordination sphere about the vanadium atom in **1** and **2** consists of six oxygen atoms and one nitrogen atom arranged in a distorted pentagonal bipyramid geometry with the oxo ligand

(16) Quilitzsch, U.; Wieghardt, K. *Inorg. Chem.* **1979**, *18*, 869.

Table II. Positional Parameters and Isotropic Thermal Parameters (Å^2) of the Non-Hydrogen Atoms for $K_2[VO(O_2)_2(pic)]\cdot 2H_2O$ (**1**) and $K_2[VO(O_2)_2(OHpic)]\cdot 3H_2O$ (**2**)

	x	*y*	*z*	B_{eq}
		Compound **1**		
V	0.5418	0.77468(8)	0.0477	2.55(2)
K(1)	0.39205(10)	0.9276(1)	0.2073(2)	3.29(3)
K(2)	0.5632(1)	0.5820(1)	0.5173(2)	3.90(3)
O(1)	0.4878(2)	0.8970(3)	–0.0437(5)	3.08(9)
O(2)	0.5355(2)	0.7863(4)	0.2958(5)	3.32(10)
O(3)	0.4767(2)	0.6950(4)	0.1772(6)	3.6(1)
O(4)	0.5090(3)	0.6512(4)	–0.1464(6)	4.1(1)
O(5)	0.5797(3)	0.7261(4)	–0.1580(6)	4.1(1)
O(6)	0.6437(2)	0.6332(4)	0.1998(5)	3.34(9)
O(7)	0.777(3)	0.6162(4)	0.3586(6)	5.3(1)
N(1)	0.6541(3)	0.8800(4)	0.1495(6)	2.7(1)
C(1)	0.7151(3)	0.6763(6)	0.2708(7)	3.0(1)
C(2)	0.7243(3)	0.8174(5)	0.2400(7)	2.8(1)
C(3)	0.8012(4)	0.8807(6)	0.3092(9)	3.7(2)
C(4)	0.8044(4)	1.0098(7)	0.281(1)	4.7(2)
C(5)	0.7326(5)	1.0730(6)	0.1914(9)	4.6(2)
C(6)	0.6565(4)	1.0055(6)	0.1248(8)	3.7(2)
OW(1)	0.4032(3)	0.8086(5)	0.5417(6)	6.0(1)
OW(2)	0.2331(3)	0.8650(4)	0.3315(9)	8.5(2)
		Compound **2**		
V	0.16670(5)	0.395717(12)	0.21324(5)	1.539(14)
K(1)	0.95437(8)	0.441635(19)	0.62766(7)	2.554(22)
K(2)	0.51446(8)	0.401212(19)	0.92526(7)	2.660(22)
O(1)	0.12720(24)	0.39750(5)	–0.01661(21)	2.41(7)
O(2)	0.44779(22)	0.38808(5)	0.28444(21)	2.24(6)
O(3)	0.38518(23)	0.43828(5)	0.29591(21)	2.47(7)
O(4)	–0.00037(23)	0.44414(6)	0.26069(21)	2.51(7)
O(5)	–0.09488(23)	0.39658(6)	0.23767(22)	2.66(7)
O(6)	0.22938(22)	0.37690(5)	0.53675(19)	1.96(6)
O(7)	0.31469(24)	0.31436(6)	0.73969(20)	2.70(8)
O(8)	0.2952(3)	0.22949(6)	0.61110(25)	3.51(9)
N	0.16138(25)	0.31837(6)	0.22920(23)	1.80(7)
C(1)	0.2547(3)	0.33277(8)	0.5729(3)	1.78(8)
C(2)	0.2161(3)	0.29839(7)	0.4062(3)	1.81(8)
C(3)	0.2369(3)	0.24842(8)	0.4324(3)	2.31(9)
C(4)	0.2000(4)	0.21849(9)	0.2716(4)	2.80(11)
C(5)	0.1426(4)	0.23958(9)	0.0927(3)	2.77(10)
C(6)	0.1249(3)	0.28961(8)	0.0747(3)	2.28(9)
OW(1)	0.7658(3)	0.47531(7)	–0.0891(3)	3.45(9)
OW(2)	0.3149(3)	0.48489(8)	0.6342(3)	3.69(9)
OW(3)	0.6938(3)	0.37108(7)	0.6502(3)	2.88(8)

O(1) and the carboxylato oxygen atom O(6) in the axial positions. The two peroxo ligands and the nitrogen atom lie in the pentagonal plane. In both anions the V=O, the V—O(peroxo), and the mean O—O bond lengths are comparable to those found in anions of similar geometry such as $[VO(O_2)_2\text{oxalato}]^{3-}$ [17] and $[VO(O_2)_2\text{bipyridine}]^-$.[17a,18] The long V—O(6) bond distances in **1** and **2** are also typical of bonds trans to the oxo ligand. In **2** there is a lengthening of the C=O bond (1.268(2) Å) compared with those in **1** (1.231(11) Å) and free pyridine-2-carboxylic acid (1.214 Å).[19] As shown in Figure 1, the 3-hydroxy group is not involved in the coordination sphere of the vanadium atom of **2** but is linked through a short hydrogen bond to the free carboxylato oxygen atom.

Reaction of **1** and **2** with cysteine for 4 h, under N_2, gave a fine white precipitate which was collected and identified as the disulfide cystine by its NMR spectrum. The reactions were conducted with molar ratios of cysteine to complex of 1:1, 2:1, and 4:1, and the conversion to cystine was compared to that observed for a control sample and to that for KVO_3 and H_2O_2 (Table IV). Under the conditions used, the conversion to cystine

(17) (a) Campbell, N. J.; Capparelli, M. V.; Griffith, W. P.; Skapski, A. C. *Inorg. Chim. Acta* **1983**, *77*, L215. (b) Begin, D.; Einstein, F. W. B.; Field, J. *Inorg. Chem.* **1975**, *14*, 1785.

(18) (a) Stomberg, R.; Szentivanyi, H. *Acta Chem. Scand., Ser. A* **1984**, *A38*, 121. (b) Szentivanyi, H.; Stomberg, R. *Acta Chem. Scand., Ser. A* **1983**, *A37*, 553.

(19) Takusagawa, F.; Shimada, A. *Chem. Lett.* **1973**, 1089.

Table III. Selected Bond Lengths (Å) and Angles (deg) for $K_2[VO(O_2)_2(pic)]\cdot 2H_2O$ (**1**) and $K_2[VO(O_2)_2(OHpic)]\cdot 3H_2O$ (**2**)

	1	2		1	2
V–O(1)	1.599(4)	1.606(2)	O(6)–C(1)	1.244(6)	1.247(3)
V–O(2)	1.899(4)	1.924(2)	O(7)–C(1)	1.231(6)	1.268(2)
V–O(3)	1.881(4)	1.902(2)	N–C(2)	1.341(7)	1.347(3)
V–O(4)	1.895(4)	1.877(2)	N–C(6)	1.333(7)	1.335(3)
V–O(5)	1.917(4)	1.908(2)	C(1)–C(2)	1.515(8)	1.497(3)
V–O(6)	2.290(4)	2.314(2)	C(2)–C(3)	1.408(8)	1.393(3)
V–N	2.123(5)	2.137(2)	C(3)–C(4)	1.375(9)	1.389(3)
O(2)–O(3)	1.464(5)	1.463(2)	C(4)–C(5)	1.364(9)	1.373(4)
O(4)–O(5)	1.458(6)	1.461(2)	C(5)–C(6)	1.417(8)	1.388(3)
O(1)–V–O(2)	99.6(2)	98.76(8)	O(6)–V–N	73.1(2)	73.92(6)
O(1)–V–O(3)	103.2(2)	101.56(8)	V–O(2)–O(3)	66.6(2)	66.70(8)
O(1)–V–O(4)	103.3(2)	103.17(7)	V–O(3)–O(2)	67.9(2)	68.35(9)
O(1)–V–O(5)	99.3(2)	101.43(8)	V–O(4)–O(5)	68.3(2)	68.42(9)
O(1)–V–O(6)	166.7(2)	168.73(7)	V–O(5)–O(4)	66.7(2)	66.17(9)
O(1)–V–N	93.6(2)	94.92(7)	V–O(6)–C(1)	117.1(4)	113.99(12)
O(2)–V–O(3)	45.6(2)	44.95(7)	V–N–C(2)	118.6(4)	117.03(13)
O(2)–V–O(4)	134.5(2)	133.33(7)	V–N–C(6)	121.9(4)	123.36(14)
O(2)–V–O(5)	160.2(2)	159.21(7)	C(2)–N–C(6)	119.5(6)	119.29(19)
O(2)–V–O(6)	79.7(2)	79.00(6)	O(6)–C(1)–O(7)	126.9(6)	125.49(19)
O(2)–V–N	86.4(2)	84.76(6)	O(6)–C(1)–C(2)	115.1(5)	117.76(17)
O(3)–V–O(4)	90.8(2)	90.14(7)	O(7)–C(1)–C(2)	118.0(5)	116.73(18)
O(3)–V–O(5)	134.2(2)	133.57(7)	N–C(2)–C(1)	116.0(5)	116.44(18)
O(3)–V–O(6)	85.9(2)	84.74(6)	N–C(2)–C(3)	121.7(6)	121.67(19)
O(3)–V–N	130.7(2)	128.60(7)	C(1)–C(2)–C(3)	122.2(6)	121.90(18)
O(4)–V–O(5)	45.0(2)	45.40(7)	C(2)–C(3)–C(4)	119.1(6)	119.07(20)
O(4)–V–O(6)	86.0(2)	86.00(6)	C(3)–C(4)–C(5)	118.8(6)	118.28(22)
O(4)–V–N	130.1(2)	132.76(7)	C(4)–C(5)–C(6)	120.1(6)	120.34(21)
O(5)–V–O(6)	80.5(2)	80.23(7)	N–C(6)–C(5)	120.7(6)	121.35(21)
O(5)–V–N	86.4(2)	88.59(7)			

Table IV. Oxidative Coupling[a] of Cysteine to Cystine (% Conversion) by **1**, **2**, KVO_3, and H_2O_2

ratio[b]	1	2	KVO_3	H_2O_2
1:1	12	44	7.7[c]	96
2:1	47	49	28[c]	92
4:1	38	36	5.9[c]	

[a] Reaction time = 4 h at room temperature, pH = 7.4 (phosphate buffer), cysteine concentration = 3.3×10^{-2} M. [b] Molar ratio cysteine: reagent. [c] Reaction time = 20 h.

is negligible for pure cysteine and for cysteine in the presence of KVO_3, but the presence of H_2O_2 results in almost complete conversion.

Studies of the reactions of **1** and **2** with cysteine (1:1) were monitored by their ^{51}V NMR spectra. These showed the appearance of peaks due to a number of species in addition to those due to significant amounts of starting material. Some of the new peaks appeared in the region associated with polyvanadates (–548 ppm, mono; –566 ppm, di; –574 ppm, tetra; –582 ppm, penta).[20] For **1** a peak also appeared at –630 ppm (–626 ppm for **2**), consistent[8e,g] with a monoperoxo species. At molar ratios of cysteine to complex of 2:1 and 4:1, very little of starting **1** or **2** remained and only those peaks associated with polyvanadates were observed. The ^{51}V NMR spectra of samples of cysteine and KVO_3 in the ratios 1:1, 2:1, and 4:1 showed the presence of polyvanadate species (di, tetra, penta), but loss of the signals occurred after 2.5 h at the 2:1 and 4:1 ratios.

Discussion

In addition to **1**, two other peroxovanadium complexes containing the pyridine-2-carboxylato ligand have been structurally characterized: $[VO(O_2)(pic)(H_2O)_2]$[21] and $[VO(O_2)(pic)(bipyridine)]\cdot H_2O$.[22] These monoperoxo complexes are quite different in structure from **1**. Complex **2** is the first example of a peroxovanadium complex containing the 3-hydroxypyridine-2-carboxylato ligand. The vanadate anion (VO_3^-) is reported[23] to interact only weakly with this ligand, possibly via interaction between the hydroxy group and the metal. The interaction between this ligand and the metal in **2** is through the nitrogen atom and one oxygen atom of the carboxylato group. There is no evidence in the structure or in the ^{51}V NMR spectrum which suggests the interaction of the hydroxy group with the vanadium atom.

Although peroxovanadates are insulin mimetic, little is known about the mechanism. Insulin interacts[24] with the extracellular α subunit of the insulin receptor which is attached to the plasma membrane by the β subunit which passes through the membrane. The result is activation of the protein kinase of the cytoplasmic β subunit. It is not clear how the interaction outside the cell is transmitted through the transmembrane domain; however, the α subunit of the receptor contains cysteine-rich regions at both termini and these may be important. Protein–tyrosine–phosphatases (PtPase) have been proposed[25] as acting synergistically with kinases as an essential part of the physiological response to insulin. It has been suggested that a very reactive cysteine residue at the active site of such PtPases forms a phosphate thioester intermediate[26] during catalysis. Although amino acids and peptides are reported not to coordinate with vanadate anion very strongly,[8f,27] the latter oxidatively couples cysteine to give cystine and a cysteine complex[28] and esterifies[29] the phenol group of *N*-acetyltyrosine ethyl ester. Since peroxovanadium complexes

(20) (a) Crans, D. C.; Rithner, C. D.; Theisen, L. A. *J. Am. Chem. Soc.* **1990**, *112*, 2901. (b) Butler, A.; Eckert, H. *J. Am. Chem. Soc.* **1989**, *111*, 2802.
(21) Mimoun, H.; Saussine, L.; Daire, E.; Postel, M.; Fischer, J.; Weiss, R. *J. Am. Chem. Soc.* **1983**, *105*, 3101.
(22) Szentivanyi, H.; Stomberg, R. *Acta Chem. Scand., Ser A* **1983**, *A37*, 709.
(23) Galeffi, B.; Tracey, A. S. *Inorg. Chem.* **1989**, *28*, 1726.
(24) Rosen, O. M. *Science* **1987**, *237*, 1452.
(25) Fischer, E. H.; Charbonneau, H.; Tonks, N. K. *Science* **1991**, *253*, 401.
(26) (a) Pot, D. A.; Dixon, J. E. *J. Biol. Chem.* **1992**, *267*, 140. (b) Guan, K.-L.; Dixon, J. E. *J. Biol. Chem.* **1991**, *266*, 17026.
(27) (a) Crans, D. C.; Bunch, R. L.; Theisen, L. A. *J. Am. Chem. Soc.* **1989**, *111*, 7597. (b) Rehder, D. *Inorg. Chem.* **1988**, *27*, 4312. (c) Jaswal, J. S.; Tracey, A. S. *Can. J. Chem.* **1991**, *69*, 1600.
(28) (a) Sakurai, H.; Yamada, Y.; Shimomura, S.; Yamashita, S. *Inorg. Chim. Acta* **1980**, *46*, L119. (b) Paumard, J.-P.; Cadiot, M. *Bull. Soc. Chim. Fr.* **1981**, I-441. (c) Sakurai, H.; Shimomura, S.; Ishizu, K. *Inorg. Chim. Acta* **1981**, *55*, L67. (d) Sakurai H.; Taira, Z.-E.; Sakai, N. *Inorg. Chim. Acta* **1988**, *151*, 85.
(29) Tracey, A. S.; Gresser, M. J. *Proc. Natl. Acad. Sci. U.S.A.* **1986**, *83*, 609.

are known oxidants,[21,30] it was of interest to compare the reactions of **1** and **2** with cysteine with that of vanadate ion.

The observation that **1** and **2** are much more efficient than vanadate at the oxidative coupling of cysteine is consistent with the much greater insulin-mimetic activity[7,9] of **1** and **2**. This also suggests that the mode of action of peroxovanadate complexes may be different from that of the vanadate anion, which is thought[29,31] to act as a phosphate analogue. Complexes **1** and **2** are also much better insulin mimics than H_2O_2; however, they are less efficient than H_2O_2 at the oxidative coupling of cysteine. This suggests that strong oxidative ability alone is not conducive to great insulin-mimetic activity. It may be that peroxovanadium complexes are particularly suited to being transported to a site where their oxidative capability is most effective. In this case their effectiveness may be susceptible to improvement by careful ligand design. It is worth noting that the use of pyridine-2-carboyxlate as a ligand in chromium complexes promotes membrane fluidity and the rate of insulin uptake.[32]

Experimental Section

Infrared spectra were recorded on an Analect AQS-18 FT-IR spectrometer with samples as KBr pellets or Nujol mulls. ^{51}V NMR spectra of solutions of the complexes in D_2O (98% D purity, MSD Isotopes) were obtained at ambient temperature on a Varian XL-300 NMR spectrometer operating at 78.891 MHz. Vanadium-51 chemical shifts were measured in parts per million (±1 ppm) from $VOCl_3$ as external standard at 0.00 ppm with upfield shifts considered negative. ^1H NMR spectra of samples in D_2O or in $CDCl_3$ (99% D, MSD Isotopes) were obtained with a Varian XL-200 NMR spectrometer using HOD at 4.63 ppm or residual $CHCl_3$ at 7.24 ppm as reference, respectively. Elemental analyses were performed by Spang Microanalytical Laboratories, Eagle Harbor, MI.

The compounds V_2O_5 (99.99%), pyridine-2-carboxylic acid (picolinic acid), and 3-hydroxypyridine-2-carboxylic acid (3-hydroxypicolinic acid) were supplied by Aldrich Chemical Co. and used as received. Solutions of H_2O_2 (30% by volume) were purchased from ACP Chemicals Inc. The preparations of $K_2[VO(O_2)_2(pic)]\cdot 2H_2O$ are a modification of that reported in the literature.[16] Distilled water was used in all preparations.

$K_2[VO(O_2)_2(pic)]\cdot 2H_2O$ (1). Method A. A clear pale green solution was obtained after heating a mixture of V_2O_5 (1.82 g, 10.0 mmol) and KOH (2.47 g, 44.0 mmol) in H_2O (20 mL) in a 125-mL Erlenmeyer flask. To this solution was added 30% H_2O_2 (17 mL, 25.8 mmol), upon which the solution became deep yellow with some effervescence. The mixture was stirred for 10 min and cooled at 5 °C in a water bath. A solution of picolinic acid (2.71 g, 22.0 mmol) in ethanol (35 mL) was then added, resulting in a color change to orange-red with the formation of some yellow precipitate. About 10 mL of H_2O_2 was added to dissolve the precipitate, and the solution was allowed to stand for 30 min. Then, ethanol (10 mL) was added to the solution, which was then cooled at 5 °C for 3 days, whereupon yellow crystals of **1** formed. They were collected by suction filtration, washed with cold ethanol (2 × 5 mL), and dried overnight *in vacuo*. A second crop was also obtained after the mother liquor was cooled further (total yield: 5.21 g, 72%).

Method B. A mixture of V_2O_5 (1.81 g, 9.95 mmol) in KOH (1.30 g, 23.2 mmol) in 15 mL of H_2O was heated to give a pale green solution, which was filtered. The solution was cooled (cold water bath), and hydrogen peroxide (10 mL, 88.2 mmol) was added, resulting in an orange-brown solution, which was stirred for 90 min. Any precipitated solid was removed by filtration and redissolved with a minimum amount of H_2O_2 (about 2 mL), and the solution was added to the filtrate. Then, this solution was added dropwise via a dropping funnel to an aqueous solution (12 mL) of pyridine-2-carboxylic acid (2.79 g, 22.7 mmol) in KOH (1.41 g, 25.1 mmol), and the resulting solution was stirred for 2 h to give a slightly cloudy yellow mixture. The latter was filtered, 10–12 mL of absolute ethanol was added, and the solution was cooled at 5 °C. Yellow crystals of **1** were generally obtained after 24 h (yield: 6.9 g, 94%). IR (Nujol): ν(CO) 1625 (s), 1598 (vs); ν(VO) 953 (vs); ν(OO)[33] 859 (vs), 874 (m) cm^{-1}. ^{51}V NMR (D_2O): δ −743.6. ^1H NMR (D_2O): δ 7.16 (s), 7.44 (m), 7.57 (m), 8.60 (s). Anal. Calcd for $C_6H_8K_2NO_9V$: C, 19.62; H, 2.74; N, 3.81. Found: C, 18.85; H, 2.16; N, 3.81.

$K_2[VO(O_2)_2(OHpic)]\cdot 3H_2O$ (2). A mixture of V_2O_5 (1.30 g, 7.1 mmol) and KOH (1.84 g, 33.0 mmol) in H_2O (15 mL) in a 125-mL Erlenmeyer flask was heated and stirred for 20 min to give a light green solution. To the cooled solution was added 30% H_2O_2 (1 mL, 8.8 mmol), causing an immediate color change to yellow. After being stirred for 20 min, the solution was filtered through a medium-porosity frit. Additional H_2O_2 (12 mL, 20.8 mmol) was added to the filtrate, and the resulting yellow solution was stirred for 30 min. Any yellow solid which precipitated during that time was filtered out and redissolved with H_2O_2 (6 mL, 8.8 mmol). The solution was then cooled to 0 °C, and a slurry of 3-hydroxypicolinic acid (2.01 g, 14.0 mmol) in absolute ethanol (30 mL) was slowly added, resulting in a bright yellow suspension after stirring for 30 min at room temperature. The yellow powder was filtered out, washed with cold ethanol, and dried *in vacuo*. The washings were added to the mother liquor, and the mixture was stored at 5 °C for 48 h to give a second crop, which was similarly washed and dried (total yield: 4.3 g, 81%). Elemental analysis was consistent with loss of two water molecules from the sample upon prolonged drying under vacuum. IR (KBr): ν(O–H) 2792 (m); ν(CO) 1630 (s); ν(VO) 927 (s); ν(OO)[33] 862 (s), 868 (m) cm^{-1}. ^{51}V NMR (D_2O): δ −740.8. ^1H NMR (D_2O): δ 8.78 (s, 1H), 7.63 (s, 2H). Anal. Calcd for $C_6H_6K_2NO_9V$: C, 19.73; H, 1.66; N, 3.83. Found: C, 19.48; H, 1.82; N, 3.79.

Oxidation of Cysteine to Cystine with 1, 2, KVO_3, and H_2O_2. Stock solutions of L-cysteine (1×10^{-1} M) and the oxidants **1** (5×10^{-2} M), **2** (5×10^{-2} M), KVO_3 (5×10^{-2} M), and H_2O_2 (1×10^{-1} M) were prepared in a phosphate buffer solution (pH 7.4) which had been thoroughly degassed by a vigorous stream of N_2. Solutions of cysteine and the oxidants in the molar ratios 1:1, 2:1, and 4:1 (1:2, 1:1, and 1:2 for H_2O_2) were prepared under N_2 in a three-necked flask by adding 10 mL of the cysteine stock solution to the appropriate amount of oxidant, followed by dilution with buffer to 30-mL total volume. A control sample (10 mL of cysteine solution plus 20 mL of the buffer) was prepared for each experiment. All the reactions were stirred for 4 h except those with KVO_3, which were allowed to react for 20 h. Then the samples were filtered through preweighed sintered-glass filters, which were dried under vacuum overnight and reweighed to give the yield of cystine, which was identified in each case by comparison of its ^1H NMR spectrum to that of an authentic sample.

Structure Determinations. $K_2[VO(O_2)_2(pic)]\cdot 2H_2O$ (1). A yellow parallelpiped crystal of **1** (0.30 × 0.20 × 0.50 mm), obtained via method A above, was mounted on a glass fiber. Cell constants were obtained using 25 carefully centered reflections in the range $35.63° \le 2\theta \le 44.58°$. The assignment of the space group as *Cc* was based on the systematic absences, packing considerations, a statistical analysis of intensity distribution, and the successful solution and refinement of the structure. The data, 2363 reflections, were collected using the ω–2θ scan technique where $2\theta \le 50.0°$ on a Rigaku AFC6S diffractometer with graphite-monochromated Mo Kα radiation ($-20 \le h \le 20$; $0 \le k \le 12$; $-8 \le l \le 8$); of these, 2233 were unique ($R_{int} = 0.030$) and 2016 having $I \ge 3.00\sigma(I)$ were used in the final cycle of full-matrix least-squares refinement. The intensities of three representative reflections remained constant throughout data collection. An empirical absorption correction using the program DIFABS was applied. The TEXSAN crystallographic software package[34] was used for all calculations. The structure was solved by direct methods to give the position of all the non-hydrogen atoms, which were refined anisotropically. The hydrogen atoms were placed in calculated positions with C–H = 0.96 Å and $B_{eq}(H) = 1.1B_{eq}(C)$ but not refined. Refinement using the mirror image gave higher agreement factors.

$K_2[VO(O_2)_2(OHpic)]\cdot 3H_2O$ (2). Yellow square prisms crystals of **2** (0.33 × 0.30 × 0.30 mm) were obtained by cooling a hydrogen peroxide–ethanol solution of the complex at 5 °C and were sealed in a capillary tube. Standard intensities monitored over 24 h showed no change for the first 20 h and then decreased by 5%. Following data collection, the crystal decayed rapidly and no absorption correction was performed. Cell dimensions were obtained from 20 reflections with $10.00° \le 2\theta \le 24.00°$. A total of 2594 reflections having $2\theta \le 49.9°$ were collected on a Rigaku

(30) (a) Bonchio, M.; Conte, V.; DiFuria, F.; Modena, G. *J. Org. Chem.* **1989**, *54*, 4368. (b) Bonchio, M.; Conte, V.; DiFuria, F.; Modena, G.; Padovani, C.; Sivak, M. *Res. Chem. Intermed.* **1989**, *12*, 111. (c) Ghiron, A. F.; Thompson, R. C. *Inorg. Chem.* **1990**, *29*, 4457.
(31) Gresser, M. J.; Tracey, A. S. In *Vanadium in Biological Systems*; Chasteen, N. D., Ed.; Kluwer: Dordrecht, The Netherlands, 1990; pp 63–79.
(32) Evans, G. W.; Bowman, T. D. *J. Inorg. Biochem.* **1992**, *46*, 243.
(33) Schwendt, P.; Volka K.; Suchanek, M. *Spectrochim. Acta* **1988**, *44A*, 839.
(34) TEXSAN-TEXRAY Structure Analysis Package, Molecular Structure Corp., 1985.

AFC6S diffractometer using the ω scan mode with grahite-monochromated Mo Kα radiation ($-8 \leq h \leq 8$; $0 \leq k \leq 32$; $0 \leq l \leq 8$); of these, 2392 were unique and 2102 having $I \geq 2.5\sigma(I)$ were employed in the solution and refinement of the structure using the NRCVAX system of crystallographic software.[35] Merging R for 202 pairs of symmetry-related relections was 2%. The structure was solved by direct methods, which gave the positions of all non-hydrogen atoms. Hydrogen atoms were located in a difference map and were refined isotropically; all other atoms were refined anisotropically.

(35) Gabe, E. J.; LePage, Y.; Charland, J. P.; Lee, F. L.; White, P. S. *J. Appl. Crystallogr.* **1989**, *22*, 384.

Acknowledgment. Financial support for this work was provided by the Medical Research Council (MRC) of Canada, the Department of Education of Quebec, and Nordic Merrell Dow Research Inc. of Montreal. Dr. James Britten and Dr. Rosemary Hynes are thanked for solving the solid-state structures of **1** and **2**, respectively.

Supplementary Material Available: An ORTEP diagram of **2** (Figure 2) and listings of anisotropic temperature factors (Tables V and VII) and hydrogen atom coordinates and temperature factors (Tables VI and VIII) for **1** and **2** (3 pages). Ordering information is given on any current masthead page.