mmol) in THF (500 mL). After stirring at room temperature for 15 min the reaction mixture was diluted with H_2O (800 mL) followed by extraction with EtOAc. The organic layer was dried and evaporated to a small volume. On addition of Et_2O, crystals separated, which were dried in a vacuum oven at 120 °C for 10 h to yield 49.6 g (96%) **22a**: mp 161-164 °C; $[\alpha]^{20}_D$ (MeOH, c 1) -173.7°. Anal. ($C_{17}H_{17}N_3O_4$) C, H, N.

Crystal Data. 3b: $C_{18}H_{18}N_2O_4$; M = 326.4; monoclinic; $P2_1/c$; a = 1503.1 (4) pm; b = 803.1 (3) pm; c = 1397.5 (4) pm; α = 90°; β = 96.99° (4); γ = 90°; V = 1674.4 × 10^6 pm^3; Z = 4; ρ_x = 1.295 g cm^{-3}; μ(Cu K$_\alpha$) = 7.240 cm^{-1}; F(000) = 688; no. of reflections with $I \geq 3\sigma(I)$ = 1931; no. of refinement parameters = 272; final R values, R = 0.050; R_w = 0.048.

18e: $C_{16}H_{16}N_4O_3$; M = 312.4; orthorhombic; $Pca2_1$; a = 1075.8 (2) pm; b = 966.8 (2) pm; c = 1489.7 (4) pm; $\alpha = \beta = \gamma$ = 90°; V = 1549.4 × 10^6 pm^3; Z = 4; ρ_x = 1.339 g cm^{-3}; μ(Cu K$_\alpha$) = 7.487 cm^{-1}; F(000) = 656; no. of reflections with $I \geq 3\sigma(I)$ = 2568; no. of refinement parameters = 208; final R values, R = 0.047; R_w = 0.049.

21a: $C_{27}H_{29}N_3O_7$; M = 507.6; orthorhombic; $P2_12_12_1$; a = 1097.5 (2) pm; b = 1853.9 (3) pm; c = 1301.1 (2) pm; $\alpha = \beta = \gamma$ = 90°; V = 2647.1 × 10^6 pm^3; Z = 4; ρ_x = 1.273 g cm^{-3}; μ(Cu K$_\alpha$) = 7.314 cm^{-1}; F(000) = 1072; no. of reflections with $I \geq 3\sigma(I)$ = 4454; no. of refinement parameters = 335; final R values, R = 0.057; R_w = 0.053.

Antihypertensive Studies in Conscious Spontaneously Hypertensive Rats. Compounds were tested for antihypertensive activity in conscious spontaneously hypertensive male rats (280-330 g; blood pressure >180 mmHg; origin: Okamoto strain). Mean arterial pressure was recorded directly via an aortic catheter in unrestrained animals. A HSE setup (Statham pressure transducer, Watanabe recorder, HSE oscilloscope) was used for the recording of arterial blood pressure. Blood pressure was recorded continuously over a period from 1 h before to 3.5 h after administration of the substance; to assess the effects of the substance, the mean of the maximum individual changes in the 3.5-h period after administration was used. For each compound 1 mg/kg was administered orally as a screening dose; 2-4 additional doses of selected compounds which proved active at 1 mg/kg in reducing blood pressure were tested, and an ED_{30} (= dose in μg/kg which reduces blood pressure by 30 mmHg) was calculated from a linear regression of effect vs log dose. The substances were suspended in 5% gum arabic and administered orally by gavage.

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Supplementary Material Available: X-ray crystallographic data, including positional parameters, bond distances, bond angles, and anisotropic displacement parameter expressions, for **3b**, **18e**, and **21a** (29 pages). Ordering information is given on any current masthead page.

Anti-HIV-1 Activity, Toxicity, and Stability Studies of Representative Structural Families of Polyoxometalates

Craig L. Hill,*[‡] Mark S. Weeks,[‡] and Raymond F. Schinazi*[†]

Department of Chemistry, Emory University, Atlanta, Georgia 30322, and Veterans Affairs Medical Center, and Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine, Atlanta, Georgia 30033.
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The anti-HIV-1 activity and toxicity of representative structural families of polyoxotungstates in human lymphocytes was determined. The 21 compounds examined include those derived from the following structural families: $[NaSb_9W_{21}O_{86}]^{18-}$ (HPA-23), $X^{n+}W_{12}O_{40}^{(6-n)-}$ (Keggin), $P_2W_{18}O_{62}^{6-}$ (Wells-Dawson), $W_6O_{19}^{2-}$ (Lindqvist), $[NaP_5W_{30}O_{110}]^{14-}$ (Preyssler), and $W_{10}O_{32}^{4-}$ (decatungstate). The molecular architecture of each of these structural families is constituted principally by a network of bonds between d^0 W^{VI} and oxide ions. Of these, 10 show median effective concentration (EC_{50}) values of approximately 1 μM and six have marked toxicity with a median inhibitory concentration (IC_{50}) of less than 50 μM. Only compounds containing more than six metal atoms showed appreciable antiviral activity. Beyond this, however, no marked correlation existed between the molecular size, charge, or charge density of the polyoxometalates and their anti-HIV-1 activity. Examination of an exemplary class of polyoxotungstates, the phosphotungstates of formula A- and B-$PW_9O_{34}^{9-}$ under physiological conditions (buffered neutral aqueous media), illustrates that both isomers equilibrate rapidly to generate the same distribution of products and that this distribution depends principally on the buffer. These heretofore unappreciated complexities in the chemistry of these compounds under neutral aqueous conditions indicates interpretation or evaluation of these compounds in cell culture and other biological screens must be done with care.

Introduction

Various nucleoside analogues show high activity against human immunodeficiency virus type 1 (HIV-1), the causative agent in acquired immunodeficiency syndrome (AIDS), and substantial promise for the treatment of this disorder.[1] Despite this early promise, the one drug to garner full approval by the FDA for treatment of AIDS, 3′-azido-3′-deoxythymidine (AZT), exhibits several manifestations of toxicity.[1,2] Furthermore, recent research has also indicated that a high percentage of patients maintained on AZT for a period of 6 months or longer develop resistance to the drug.[3] Although a number of other

* Address correspondence to Professor Craig L. Hill, Department of Chemistry, Emory University, Atlanta, GA 30322.

† Veterans Affairs Medical Center, and Laboratory of Biochemical Pharmacology, Department of Pediatrics, Emory University School of Medicine.

‡ Department of Chemistry, Emory University.

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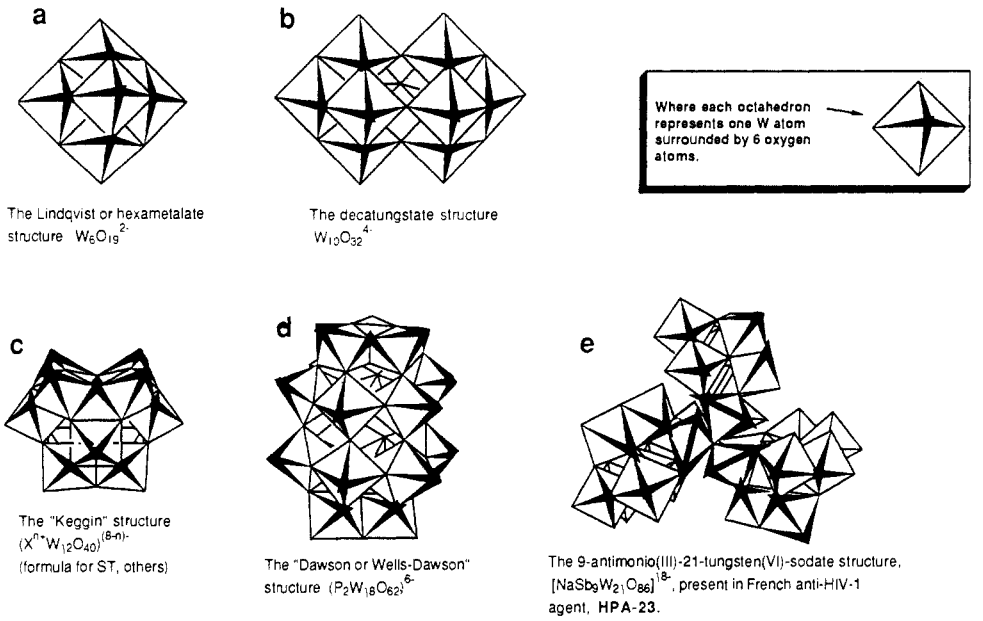

Figure 1. Five representative structural types of polyoxotungstates in polyhedral notation. In this notation, each diamond of octahedral structural subunit, as indicated on the figure itself, represents one W atom surrounded by six oxygen atoms. In each WO_6 octahedron, the W atom is displaced toward the one terminal oxygen atom. The Lindqvist structure (O_h point group symmetry and molecular formula $W_6O_{19}^{2-}$, structure a) and the decatungstate structure (D_{4h} point group symmetry and molecular formula $W_{10}O_{32}^{4-}$, structure b) are isopolytungstates. The "Keggin" structure (T_d molecular point group symmetry and molecular formula $(X^{n+}W_{12}O_{40})^{(8-n)-}$, structure c), the "Wells–Dawson" structure (the predominant α isomer is D_{3h} point group symmetry and molecular formula $P_2W_{18}O_{62}^{6-}$, structure d), and the nonaantimonio(III)–heneicosatungsten(VI)–sodate structure (C_{3h} point group symmetry and molecular formula $[NaSb_9W_{21}O_{86}]^{18-}$, structure e) are heteropolytungstates. The heptadecaammonium, mono sodium (or hydrogen) salt of e is the anti-HIV-1 agent HPA-23.

nucleoside analogues currently under development may well prove to be less toxic and less susceptible to engendering resistance, the search for an effective and minimally toxic non-nucleoside or inorganic antiviral agent that may be less susceptible to the latter problem should be possible.

One class of inorganic complexes with anti-HIV-1 activity are the early transition-metal polyoxometalates.[4] The most prominent member of this very large class of inorganic mineral compounds is HPA-23 (1, molecular formula = $(NH_4^+)_{17}(H^+)[NaSb_9W_{21}O_{86}^{18-}]$).[5] Polyoxometalates are polyanionic, condensed oligomeric aggregates of transition-metal ions, usually in their d^0 electronic configurations, and oxide ions, held together only by metal–oxygen bonds. Based on energetic factors (e.g., relative bond strengths) and structural factors (e.g., ionic radii ratios), only five types of ions form these complexes, V^V, Nb^V, Ta^V, Mo^{VI}, and W^{VI}, with the latter two forming by far the largest number.[4] The principal units that make up most polyoxometalates are MO_6 octahedra, that is, metal ions each surrounded by six oxide ions. The "polyhedral notation", used by investigators that prepare and study polyoxometalates and related materials including many metal oxides, reflects the preponderance of these octahedral units. Figure 1 illustrates four representative polyoxometalate structural families in polyhedral notation.

Deviations of the MO_6 units from pure octahedral symmetry can be substantial with the metal always being displaced toward the terminal or doubly bonded oxygen atoms. The MO_6 units in polyoxometalates can be linked together by a single oxygen atom, termed a μ-oxo linkage. Octahedra joined in this manner are said to be "corner sharing". Usually present in the same polyoxometalate molecule as corner-sharing octahedra are edge-sharing octahedra. In the latter are two MO_6 units linked together by two oxygen atoms, termed a di-μ-oxo linkage. These two types of MO_6 unit connectivities are perhaps most readily seen in the decatungstate structure (b in Figure 1). The two equivalent halves of decatungstate are joined by four corner-sharing connections while each of the five WO_6 units in each half are joined by edge-sharing connections. Two large subcategories of polyoxometalates exist: the "isopoly" and the "heteropoly" compounds. The former are constituted only by metal and oxygen atoms while the latter contain one or more "heteroatoms" at well-defined geometrical sites in the molecule in addition to the metal and oxygen atoms. Up to 75% of the elements in the periodic table can function as heteroatoms in polyoxometalates.[4] The drug first developed and evaluated in France, 1, is a heteropolytungstate, a polyoxometalate based on tungsten. A final point concerns the redox chemistry of polyoxometalates. Although some classes of polyoxometalates are reducible to highly colored species with d^1 or nd^1 electronic configurations, the redox potentials for all the most active antiviral polyoxometalates are at the lowest end of the physiologically accessible potential range. Furthermore there is no experimental evidence either in these studies or other polyoxometalate antiviral studies in the literature that redox chemistry plays a

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substantive role in the activities and toxicities of such species. However, the possible role of such redox processes with these compounds cannot yet be ruled out.

Polyoxotungstates of various classes have been known for 20 years to exhibit potent antiviral activity. The Keggin heteropolytungstates (structure c, Figure 1), and in particular the acid salt of silicotungstate (α-$H_4SiW_{12}O_{40}$), have been shown to have antiviral activity against several viruses in cell culture. These include rubeola,[6] Moloney,[7] vesicular stomatitis,[8] and rubella.[8] Likewise 1 has been shown to have potent antiviral activity against a range of viruses,[9] and most recently against HIV-1.[10] Although 1 proceeded to clinical trial in both France and the US, marked dose-related hematological abnormalities associated with administration of the drug caused the clinical trials to be discontinued.[11]

Two factors have kept the further development of polyoxometalates as potential anti-HIV-1 agents to a minimum, the toxicity of the one compound extensively examined (1) and, more significantly, the unfamiliarity of nearly all investigators in biomedical and even chemical disciplines with these complex inorganic compounds.

This paper reports the anti-HIV-1 activity and toxicity of a range of polyoxotungstates (polyoxometalates of tungsten) in human peripheral blood mononuclear (PBM) cells. In addition, fundamental stability studies in buffered aqueous solutions of one representative class of polyoxotungstates, the trivacant phosphotungstates derived from the parent compound α-$H_3PW_{12}O_{40}$, indicate that the chemistry of these compounds in aqueous media is buffer dependent and more complicated than heretofore appreciated. These studies indicate that care needs to be exercised in interpreting structure–activity data in cell culture, pharmacokinetics, and other biological assays involving polyoxometalates.

Results and Discussion

Activity and Toxicity in Cell Culture. Twenty one known polyoxometalate compounds (18 polyoxotungstates representing all five structural classes illustrated in Figure 1 and two polyoxomolybdates) were evaluated in human PBM cells for their anti-HIV-1 activity and toxicity. The formulas and molecular weights of these compounds along with their median effective (EC_{50}) and inhibitory concentration (IC_{50}) values are given in Table I. The compounds in the lacunary and trivacant fragment structural classes (entries 8, 9, and 10 in Table I, respectively) are known to be derived from the "Keggin" structural class (entries 2–7, Table I). The compounds in the fifth structural class in Table I, the bis-trivacant Keggin fragment sandwich (BTKS) compounds, are derived from the trivacant species in the fourth structural class. In the BTKS compounds two trivacant Keggin fragments are the "buns" and four d^n ($n \neq 0$) adjacent transition metal ions (Cu^{II} or Co^{II} for 11 and 12 in Table I, respectively), are the "meat" in an idealized "inorganic hamburger". The structural and chemical relationships between the compounds in the remaining structural categories are not well established and, in any case, not simple.

Table I. Anti-HIV-1 Activity and Toxicity of Seven Structural Categories of Polyoxometalates in Human PBM Cells

	molecular formula	mol wt	EC_{50},[a] μM	IC_{50},[b] μM
	HPA-23			
1	$(NH_4)_{17}Na[NaSb_9W_{21}O_{86}]$	6940	0.39	35
	"Keggin"			
2	$H_3PW_{12}O_{40}$	2988	14.0	>100
3	$H_4SiW_{12}O_{40}$	3004	0.12	>200
4	$H_5BW_{12}O_{40}$	3042	0.46	126
5	$H_6ZnW_{12}O_{40}$	3097	0.90	12
6	$Na_6H_2W_{12}O_{40}$	2986	0.34	>100
7	$[(DMA)_2H]_3PMo_{12}O_{40}$[c]	2348	>100	>100
	"Keggin" Lacunary			
8	$Na_7PW_{11}O_{39}$	3081	10.8	>100
9	$K_8SiW_{11}O_{39}$	2987	0.15	>100
	Trivacant "Keggin" Fragment			
10	$Na_9HSiW_9O_{34}$	2849	2.4	>100
	Bis-Trivacant "Keggin" Fragment Sandwich (BTKS)			
11	$K_{10}Cu_4(H_2O)_2(PW_9O_{34})_2$	5464	4.4	>100
12	$K_{10}Co_4(H_2O)_2(PW_9O_{34})_2$	5482	0.8	44
	"Wells–Dawson"			
13	$H_6P_2W_{18}O_{62}$	4763	0.52	6.2
14	$(NH_4)_6P_2W_{18}O_{62}$	4865	0.91	1.8
	"Lindqvist"			
15	$(n\text{-}Bu_4N)_2W_6O_{19}$	1892	107	>100
16	$(n\text{-}Bu_4N)_2Mo_6O_{19}$	1365	>100	>100
	"Preyssler"			
17	$(NH_4)_{14}[NaP_5W_{30}O_{110}]$	8264	0.32	7.7
	Decatungstate			
18	$(NH_4)_4W_{10}O_{32}$	2423	1.8	115
19	$(Me_4N)_4W_{10}O_{32}$	2648	3.1	>100
	Miscellaneous			
20	α-$(n\text{-}Bu_4N)_4Mo_8O_{26}$	2153	55.3	>100
21	$K_4W_4O_{10}(O_2)_6$	1352	>50	>100
	AZT (Zidovudine)—Non-Polyoxometalate Anti-HIV-1 Agent			
22	$C_{10}H_{13}N_5O_3$	267	0.004	>100

[a] EC_{50} = median effective (antiviral) concentration. [b] IC_{50} = median inhibitory (toxicity) concentration. [c] DMA = *N*,*N*-Dimethylacetamide.

The following points regarding anti-HIV-1 activity were noted. First, none of the complexes containing fewer than eight metal ions (entries 15, 16, 20, and 21, Table I) have marked activity; all have EC_{50} values >50 μM. In contrast, the remaining 17 polyoxometalate compounds with the exception of the Keggin polyoxomolybdate (entry 7) had demonstrable activity. Of this group, all but two have EC_{50} values below 10 μM and 10 have EC_{50} values at or below 1 μM. Second, the activity of five of the compounds was as high or higher than that of HPA-23 and none was as active as AZT (control entry 22, Table I). Third, although there appears to be some correlation with the size, shape, or molecular charge of the polyoxometalates and their anti-HIV-1 activity, the correlation was not a strong one. Importantly, the stability studies presented below argue that the interpretation of such structure–activity rela-

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tionships needs to be done with some care.

Examination of the data in Table I also established that the toxicity of most of the polyoxometalates in PBM cells was not very high. The therapeutic indices in these human cells was quite high for several of the compounds. Some of the compounds had higher therapeutic indices than that of 1. The highest was observed with entry 3 (Table I), also referred to as silicotungstic acid or ST, despite the well-known acidity of this compound.

Stability Studies. A fundamental limitation in interpreting any results on the behavior of polyoxometalate compounds in physiological media, from cell culture studies to mammalian pharmacokinetics to clinical investigations, derives from the nature of the compounds themselves. Unlike most of the nucleoside and purely organic antiviral agents, many polyoxometalate structures are not stable in water but degrade or rearrange to a complex mixture of inorganic products.[4] As a consequence, it is usually very difficult to know what the actual form of the drug is in vivo. In the one pharmacokinetics study of a polyoxometalate in mammals, in which 1 radiolabeled with ^{185}W was shown to accumulate in the liver, kidneys, spleen, and brain,[12] it is far from clear what actual W-containing species are deposited in these tissues. The ^{185}W could be in the form of intact 1, monomeric tungstate, WO_4^{2-}, the likely form that is ultimately excreted in the urine, or one of a number of fragments of undetermined structure and molecular weight intermediate between 1 and WO_4^{2-}. In addition, it is often not a simple matter to quantify all the distinct polyoxometalate species present in dilute aqueous solution. In the few cases where fairly thorough studies addressing the kinetic and thermodynamic stabilities of different polyoxometalate fragments or isomers in aqueous media have been performed, there remain several questions about the structures of some species and key features of the mechanisms and rate laws for these rearrangements and/or degradations are not understood. For many polyoxometalates, virtually nothing is known about their degradation chemistry in aqueous solution. Furthermore, virtually nothing is known about the possible role of enzymes or other physiologically occurring biological macromolecules with respect to enhancing the breakdown and/or rearrangements of polyoxometalate species. It is in context with these points that we felt one well-studied polyoxometalate system should be further investigated in the presence of different buffers at physiological pH to assess the possibility of further complexity in the chemistry.

One logical choice of compound for this work was the phosphotungstates derived from the parent Keggin phosphotungstate α-$PW_{12}O_{40}^{3-}$. These compounds are as thoroughly studied in aqueous solution as any polyoxometalate.[4a] As with most polyoxometalates based on W and Mo, generally degradation to smaller and more highly charged species is observed as one proceeds from acidic media to more basic media. As of the mid-1980s, the major species generally accepted to exist in the aqueous Keggin phosphotungstate system as a function of pH are those illustrated in Figure 2.[4,13] Subsequently a host of other phosphorus-containing polyoxotungstates have been made.[13] Some of these new species may well exist in

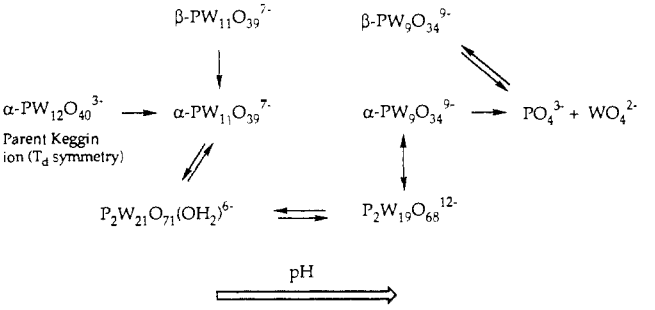

Figure 2. Some of the known species identified thus far from investigations of the chemistry of the phosphotungstates derived from the parent Keggin phosphotungstate α-$PW_{12}O_{40}^{3-}$ as a function of pH. The pH increases from left to right.[4a]

aqueous solution but others clearly do not and have no relevance to the development of anti-HIV-1 chemotherapeutic agents. Recent work has indicated that two types of isomers, designated A and B, of the trivacant phosphotungstates of formula $PW_9O_{34}^{9-}$ not only exist but are independently isolable. The A isomer results from removal of one W from three of the four W_3O_{13} units that constitute the parent Keggin structure and B results from removal of one complete W_3O_{13} unit (refer to c, Figure 1). Both A and B isomers themselves, like the parent structures c and d (Figure 1), can exist in α and β isomers. The β isomers are distinguished from the α isomers in that the former are formed from the more symmetrical α isomers by rotating the capping or terminal W_3O_{13} unit in each of the structures by 60°. Only two of the trivacant isomers (one set of α and β isomers) are shown in Figure 2. Given the stability–pH profile, it appeared that both the α,A and α,B isomers of the trivacant phosphotungstates of formula $PW_9O_{34}^{9-}$ (henceforth referred to as just A- and B-$PW_9O_{34}^{9-}$ for simplicity) should have sufficient stability at pH 7 that their chemistry could be assessed spectroscopically. ^{31}P NMR was chosen as the principal physical method for this study as no other spectral method permitted the ready quantitation of the starting complexes and the possible breakdown products. The time required for ^{183}W NMR data collection (many hours or days for acquisition of spectra with acceptable signal to noise levels) and the intricacy of the spectra for complex product distributions are sufficiently uninterpretable that this technique was of marginal value in this particular set of experiments. ^{17}O NMR suffers not only from the fact that the reactant complexes must be labeled with ^{17}O for sufficient sensitivity but also from the fact that the label washes out in facile exchanges with the H_2O of the medium.

Figure 3 illustrates the results of preparing and characterizing solid-state crystalline samples of A-$PW_9O_{34}^{9-}$ and B-$PW_9O_{34}^{9-}$ and then recording the ^{31}P spectra of both complexes dissolved in three aqueous solutions all buffered at pH 7 but using three unrelated buffers: sulfite, tris, and ethylenediamine (en). All three buffer species at pH 7 are known to be unreactive with polyoxotungstates generally: polyoxotungstates, including α-$PW_{12}O_{40}^{3-}$, are known to be stable in aqueous solutions of alcohols (cf. tris or tris-H^+),[14] stable and often recrystallized in the presence of protonated amines (cf. en-H^+ and tris-H^+),[15] and stable

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(13) For example, see: Constant, R.; Tézé, A. *Inorg. Chem.* **1985**, *24*, 4610. (b) Thouvenot, R.; Tézé, A.; Constant, R.; Hervé, G. *Ibid.* **1988**, *27*, 524. See also the new phospho*molybdate* structures of Haushalter and Lai: Haushalter, R. C.; Lai, R. W. *Ibid.* **1989**, *28*, 2905.

(14) For example, see: (a) Hill, C. L.; Bouchard, D. A. *J. Am. Chem. Soc.* **1985**, *107*, 5148. (b) Akid, R.; Darwent, J. R. *J. Chem. Soc.*, Dalton *Trans.* **1985**, 395. (c) Fox, M. A.; Cardona, R.; Gaillard, E. *J. Am. Chem. Soc.* **1987**, *109*, 6347. (d) Papaconstantinou, E. *Chem. Soc. Rev.* **1989**, *18*, 1. (e) Yamase, T.; Watanabe, R. *J. Chem. Soc., Dalton Trans.* **1986**, 1669 and references cited in each.

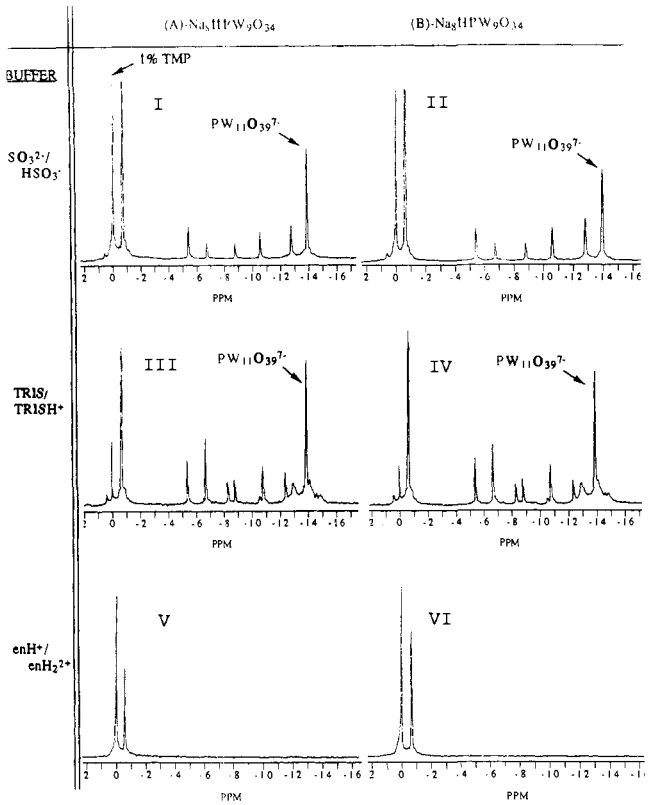


Figure 3. Effect of medium at a constant pH of 7.0 on the actual distribution of phosphorus-containing species present upon dissolution and equilibration of two isomerically distinct trivacant polytungstophosphate reactant complexes, A-$PW_9O_{34}^{9-}$ and B-$PW_9O_{34}^{9-}$, as monitored by ^{31}P NMR. The three spectra in the left column are those obtained by dissolving the former complex; those in the right column are those obtained by dissolving the latter complex. The top two spectra are recorded in sulfite buffer (SO_3^{2-}/HSO_3^-), the middle two spectra are recorded in tris-hydroxymethyl amino methane buffer (tris/tris-H^+), and the bottom two spectra are recorded in ethylenediamine buffer (en/en-H^+). All spectra were recorded at 295 K and all shifts are relative to trimethyl phosphate (TMP), 1% solution in D_2O in coaxial tube. (H_3PO_4 is −3.06 ppm relative to TMP in D_2O.) All solutions were approximately 0.06 M in polyoxometalate, except for the solution buffered with en. The latter were at ~0.01 M as a consequence of solubility limitations.

to reduction by sulfite.[16] The ^{31}P NMR chemical shifts are enumerated in Table II.

There are several key points related to this study. First, it is clear despite the fact that the two reactant complexes were distinct by infrared spectroscopy prior to dissolution that they formed identical distributions of phosphorus-containing products when placed in the buffered media. Second, it is also very clear that the distribution of these products was quite different among the three buffered solutions. Third, although no parent Keggin complex, α-$PW_{12}O_{40}^{3-}$, was formed, a substantial quantity of the monodefected or lacunary structure, α-$PW_{11}O_{39}^{7-}$, was formed with two of the buffers (sulfite and tris), but not with the third (ethylenediamine). Both starting complexes were free of the presence of this complex. Fourth, changes in buffer concentration produced no changes in the peaks but changes in ionic strength did produce small changes. Fifth, the ^{31}P NMR spectra of authentic pyrophosphate, linear triphosphate, metacyclotriphosphate, and phosphate (mono-, di-, and tribasic) established that none of these new phosphorus-containing compounds was formed upon dissolution of the reactant complexes in any of the buffers. Most of the new peaks are likely new phosphorus-containing polytungstophosphates. The exact identity of all these complexes is not yet known and will doubtless not be quickly established given the known experimental difficulties associated with this aqueous polyoxometalate chemistry.

Table II. ^{31}P NMR Chemical Shifts of A-PW_9 and B-PW_9 in Buffered Solution[a]

sulfite		tris[b]		en[b]	
A-PW_9[c]	B-PW_9[c]	A-PW_9[c]	B-PW_9[c]	A-PW_9[c]	B-PW_9[c]
0.62	0.61				
		0.42	0.44		
				−0.56	−0.67
−0.66	−0.60	−0.67	−0.64		
−5.40	−5.39	−5.41	−5.41		
−6.73	−6.68	−6.71	−6.69		
		−8.31	−8.30		
−8.75	−8.74				
		−8.82	−8.80		
−10.56	−10.53	−10.50	−10.53		
		−10.84	−10.79		
		−12.44	−12.40		
−12.77	−12.75				
		−13.04	−13.00		
−13.90	−13.89	−13.95	−13.95		

[a] All shifts in ppm relative to TMP (1% D_2O solution in coaxial tube). (H_3PO_4 is −3.06 ppm relative to TMP in D_2O.) All solutions were approximately 0.06 M in polyoxotungstate, except for the solutions buffered with en. The latter solutions were at a lower concentration, approximately 0.01 M, as a result of solubility limitations. [b] Tris = tris(hydroxymethyl)aminomethane; en = ethylenediamine. [c] A-PW_9 and B-PW_9 are abbreviations for A-$PW_9O_{34}^{9-}\cdot nH_2O$ and B-$PW_9O_{34}^{9-}\cdot nH_2O$, respectively.

Conclusions

The polyoxometalate compounds examined in this study represent only a small sample of the possible complexes in this class that should be readily synthesized, accessible in quantity, and exhibit high therapeutic indices in cell culture against HIV-1. The tremendous molecular diversity of polyoxometalates and the recent work that indicates the toxicity of these materials is greatly variable and to some extent controllable[17] suggests that some serious attention should be given to defining further the fundamental chemistry, biochemistry, virology, and pharmacology of these complexes.

From the studies here indicating a major effect of buffer at constant pH (~7) and ionic strength on the observed phosphotungstate species present, it is only prudent to use care in interpreting structure–activity relationships associated with polyoxometalates and, in general, all their attributes under biological conditions. The actual species

(15) Salts constituted by polyoxometalate anions and protonated amine cations are some of the most routinely prepared and stable forms of polyoxometalates; cf.: Yamase, T. *J. Chem. Soc., Dalton Trans.* **1985**, 2585. (b) Fuchs, v. J.; Hartl, H.; Schiller, W.; Gerlack, U. Z. *Acta Crystallogr.* **1976**, *B32*, 740 and references cited in each.

(16) The presence of any reduced polyoxometalate is readily detected with the unaided eye and quantified by simple UV–visible absorption spectroscopy. The chromophores of the reduced (generally d^1) forms are extremely intense and appear in the visible region of the spectrum in contrast to those of the parent oxidized polyoxometalates. At no time could any reduced polyoxotungstates, produced in situ by reaction of the oxidized forms with sulfite, be detected in these NMR experiments.

(17) Hill, C. L.; Hartnup, M.; Faraj, M.; Weeks, M.; Prosser-McCartha, C. M.; Brown, R. B.; Schinazi, R. F.; Sommadossi, J.-P. In *Advances in Chemotherapy of AIDS, Pharmacology and Therapeutics*; Diasio, R., Sommadossi, J.-P., Eds., in press.

present may not be those seen in the solid state and complicated distributions of polyoxometalate species may be established on time scales that are short with respect to the time for the drug to be administered and to take effect.

Experimental Section

Materials. The polyoxometalates were prepared by standard literature procedures.[4a,5a,14a,18] All polyoxometalates were purified by recrystallization, and their purity was assessed spectroscopically with ^{183}W and ^{31}P NMR, UV-visible, and infrared absorption spectroscopies.

Spectroscopic Methods. The ^{31}P NMR spectra were taken on an IBM WP-200SY instrument. Chemical shifts are referenced to a 1% TMP (trimethyl phosphate) solution in D_2O, by running the reference in a 5-mm coaxial tube, inside the 10-mm sample tube. The D_2O in the coaxial tube proved sufficient to provide a lock, so D_2O was not used in the buffer solutions. Infrared spectra were run on a Perkin-Elmer 1430 ratio recording spectrophotometer. Samples were run as KBr pellets (2-4 wt % in KBr). Electronic absorption spectra were run on a Hewlett-Packard Model 8451A diode-array spectrophotometer.

Cell Culture Assays. The compounds were evaluated in human mitogen-stimulated PBM cells infected with HIV-1 (strain LAV-1), as described previously.[19] Virus was harvested after 6 days. Stock solutions (2 mM) of the compounds were freshly prepared in water prior to testing. Compounds **7**, **15**, **16**, and **20** were dissolved in DMSO and then diluted with medium so that the final concentration of DMSO was less than 0.05%. At that concentration, DMSO has no effect on the viral yield.

Cytotoxicity Assays in PBM Cells. The drugs were evaluated for their potential toxic effects on uninfected mitogen-stimulated human PBM cells. Flasks were seeded so that the final cell concentration was 2×10^5 cells/mL. The cells were cultured with and without drug for 6 days at which time aliquots were counted for cell viability, as assessed by the trypan blue dye-exclusion method using a hemacytometer.[19] The EC_{50} and IC_{50} were calculated by using the median effect method.[20]

Acknowledgment. This work was supported by Public Health Service Grants AI 26055 (CLH and RFS) from the National Institutes of Health, and the Department of Veterans Affairs (RFS). The excellent technical assistance of D. Cannon, B. Oswald, and V. Saalmann is gratefully acknowledged.

(18) (a) Deltcheff, C.; Fournier, M.; Franck, R.; Thouvenot, R. *Inorg. Chem.* **1983**, *22*, 207. (b) North, E. O. *Inorg. Synth.* **1939**, *1*, 129. (c) Bouchard, D. A. Master's Thesis, Emory University, 1986. (d) Hartnup, M. Master's Thesis, Emory University, 1988. (e) Hill, C. L.; Bouchard, D. A.; Kadkhodayan, M.; Williamson, M. M.; Schmidt, J. A.; Hilinski, E. F. *J. Am. Chem. Soc.* **1988**, *110*, 5471.

(19) Schinazi, R. F.; Cannon, D. L.; Arnold, B. H.; and Martino-Saltzman, D. *Antimicrob. Agents Chemother.* **1988**, *32*, 1784.

(20) Chou, J.; Chou, T.-C. *Dose-Effect Analysis with Microcomputers: Quantitation of ED_{50}, LD_{50}. Synergism, Antagonism, Low-Dose Risk, Receptor Binding, and Enzyme Kinetics. A Computer Software for Apple II Series and IBM-PC and Instruction Manual*; Elsevier-Biosoft, Elsevier Science Publishers: Cambridge, U.K., 1985.

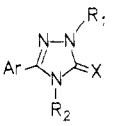
2,4-Dihydro-3*H*-1,2,4-triazol-3-ones as Anticonvulsant Agents

John M. Kane,* Bruce M. Baron, Mark W. Dudley, Stephen M. Sorensen, Michael A. Staeger, and Francis P. Miller

Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, Ohio 45215. *Received March 9, 1990*

A series of 5-aryl-2,4-dihydro-3*H*-1,2,4-triazol-3-ones was evaluated for anticonvulsant activity. In general the members of this series were prepared by the alkaline cyclization of 1-aroyl-4-alkylsemicarbazides. The resulting 2-unsubstituted 3*H*-1,2,4-triazol-3-ones were then alkylated, yielding 2,4-dialkyl-3*H*-1,2,4-triazol-3-ones. Approximately one-third of the compounds examined exhibited activity against both maximal electroshock- and pentylenetetrazole-induced seizures in mice. Receptor-binding studies suggest that this activity was not a consequence of activity at either benzodiazepine or NMDA-type glutamate receptors. From this series, compound **45** was selected for further evaluation where it was also found to be active against 3-mercaptopropionic acid, bicuculline, and quinolinic acid induced seizures in mice. In addition, **45** also protected gerbils from hippocampal neuronal degeneration produced by either hypoxia or intrastriatal quinolinic acid injection.

We have recently been investigating a series of 2,4-dihydro-3*H*-1,2,4-triazole-3-thiones **1** as potential antidepressant agents.[1] Selected members of this series exhibited potent behavioral effects in test systems traditionally used to detect these agents. The mechanism of action of these compounds, however, remains uncertain. While attempting to establish some correlation between the structure and the activity of these triazoles, we prepared several 2,4-dihydro-3*H*-1,2,4-triazol-3-ones **2**. Surprisingly, these compounds were completely devoid of antidepressant-like activity and instead exhibited anticonvulsant activity against a variety of convulsant stimuli. In order to more fully investigate these findings, we have prepared additional derivatives of **2** and we now report the anticonvulsant activities associated with members of this series.



1, X=S
2, X=O

Chemistry

The triazol-3-ones (Table I) which were evaluated in this study were prepared via the three routes depicted in Scheme I. The synthesis of 2,4-dihydro-5-phenyl-3*H*-1,2,4-triazol-3-one (**3**) was accomplished by the method of Lipkin.[2] Thus, condensation of benzoic acid hydrazide (**4**) and urea (**5**) gave **3** in a single, low-yielding step. The synthesis of 2,4-dihydro-2-methyl-5-phenyl-3*H*-1,2,4-triazol-3-one (**6**) was accomplished via a new route which involved S-methylation of triazole-3-thione **7**.[3] The resultant thioether **8**[4] was then oxidized with 2.5 equiv of *m*-chloroperoxybenzoic acid (MCPBA), affording sulfone **9**. Alkaline hydrolysis of **9** cleanly afforded **6**, which was

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