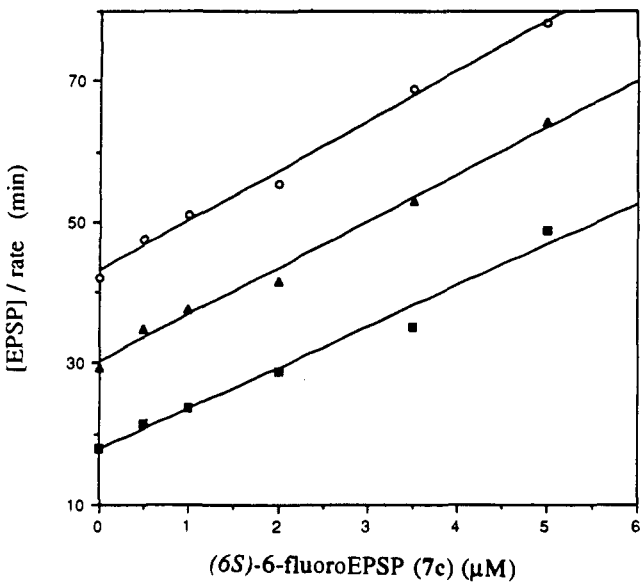

Figure 1. Cornish-Bowden plot showing competitive inhibition of chorismate synthase by (6*S*)-6-fluoro-EPSP (**7c**). UV assays (275 nm) were carried out at 25 °C, pH 7.0 (triethanolamine hydrochloride, 50 mM), and included 1.2 milliunts of chorismate synthase, 20 μM NADPH, 10 μM FMN, 50 mM KCl, and 2.5 mM $MgCl_2$ in addition to substrate EPSP (**7a**) and inhibitor (6*S*)-6-fluoro-EPSP (**7c**) in a final volume of 1 mL. Inhibitor concentrations were 0, 0.5, 1, 2, 3.5, and 5 μM, and substrate concentrations were (■) 20 μM EPSP, (▲) 35 μM EPSP, and (○) 50 μM EPSP.

compound under conditions which would have easily detected a turnover rate 0.2% that of EPSP itself.[17]

Competition experiments were performed in which chorismate synthase was assayed at various fixed concentrations of EPSP in the presence of a range of concentrations of **7b** or **7c**. Figure 1 shows a Cornish-Bowden plot[18] of the data obtained for (6*S*)-6-fluoro-EPSP (**7c**). The parallel plots clearly signify a competitive mode of inhibition. The inhibition constant K_i was determined from a Dixon plot.[19] It is found that both fluoro-EPSPs show clean competitive inhibition with **7c** having an affinity an order of magnitude greater than **7b**: K_i ((6*S*)-6-fluoro-EPSP) = 0.2 ± 0.1 μM, K_i ((6*R*)-6-fluoro-EPSP) = 3.0 ± 0.3 μM. These values compare with K_i (iso-EPSP **3**) = 8.7 μM,[7] and K_m (EPSP) = 2.2 μM.[8] The lack of irreversible inhibition by either compound was confirmed by incubation of *N. crassa* chorismate synthase with 50 μM of each inhibitor at 25 °C. Over a period 1 h, no loss of enzyme activity was observed relative to a control which lacked inhibitor.

The lack of irreversible inhibition is inconsistent with a mechanism involving a covalent enzyme–intermediate adduct such as **4**. While the observation that both 6-fluoro-EPSPs are potent competitive inhibitors does not itself support or preclude any of the other mechanisms in Scheme I, it does provide a useful tool for future mechanistic studies of the enzyme.

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Supplementary Material Available: Preparation of **7b,c** from **5b,c**, spectroscopic characterization of **5b,c** and **7b,c**, and Dixon plot showing inhibition of chorismate synthase by **7c** (4 pages). Ordering information is given on any current masthead page.

(16) Chorismate formation is normally monitored by the appearance of the diene chromophore which has its λ_{max} at 275 nm. In these experiments, absorbance was monitored in the range 240–300 nm in order to accommondate a possible shift in the absorbance maximum due to a fluorine substituent.

(17) This experiment does not rigorously preclude either compound being a substrate but puts an upper limit on their turnover rate.

(18) Cornish-Bowden, A. *Biochem. J.* **1974**, *137*, 143–144.

(19) Dixon, M. *Biochem. J.* **1953**, *55*, 170–171.

Modulation of Physical and Chemical Properties of η-H_2 Complexes of Osmium Ammines by Facile Substitution

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Since the discovery of the first dihydrogen complex by Kubas et al.,[1] many dihydrogen complexes have been synthesized. In 1971,[2] the preparation in our laboratory of $[Os(en)_2H_2]^{2+}$ as the chloride salt was reported. It was described as a dihydride and was assigned a cis configuration on the strength of ^1H NMR results which revealed two sets of amine protons in equal number. Our investigation of the analogous species $[Os(NH_3)_4H_2]^{2+}$, not heretofore reported, throws new light on that structural assignment and, as well, provides ready access to a series of complexes arrived at by the simple addition of a variety of ligands to these $16e^-$ moieties.

When $[Os(NH_3)_4H_2](B(C_6H_5)_4)_2$[3] (**1**) is dissolved in $(CD_3)_2CO$, the ^1H NMR spectrum reveals only two kinds of protons ascribable to the cation, in the abundance ratio 6:1 at δ = 3.82 ppm and −11.37 ppm, respectively. For the purposes of species differentiation, the value of T_1 for the coordinated hydrogen was also measured (T_1 = 572 ms, 20 °C), as it was for the other species to be dealt with. When a trace of acid, for example, HO_3SCF_3, is present, slow H/D exchange between the solvent and coordinated hydrogen ensues, and, in a partially exchanged sample, J_{HD} was measured as 4.0 Hz. When any of a large number of solutes is added in excess, among them acetonitrile (AN), pyridine (Py), imidazole (Im), I^-, Cl^-, D_2O, and Br^-, δ (ppm) J_{HD} (Hz), and T_1 (ms, 400 MHz) change and new characteristic values are registered. (See Table I.) In every case except with D_2O and $(CD_3)_2CO$ as addend, the corresponding solid salt was also prepared,[5] and dissolved, with no discernible differences in the ^1H NMR signals. Because the solute level is low (0.010 M), we can conclude, at least in the case of the labile systems I^-, Br^-, or Cl^- as addend, that in acetone the affinity of the osmium center for the ligand is very high. As expected, it is much reduced in D_2O as solvent.

Of potential anionic ligands, the only one among those we have introduced which does not change the values of δ, J_{HD}, and T_1 is PF_6^- (even $CF_3SO_3^-$ produces a set of characteristic values). This indicates that neither $B(C_6H_5)_4^-$ nor PF_6^- enters the coordination sphere of the osmium complex, a supposition which, in the case of the former at least, is reasonable and, in view of the bulk and almost spherical shape of PF_6^-, is reasonable for it also. However, it leaves open the question of whether $(CD_3)_2CO$ also is a ligand when the $B(C_6H_5)_4^-$ and PF_6^- salts are dissolved. That this is in fact the case is indicated by observations made for $[Os(en)_2H_2]^{2+}$, and it is therefore likely also the case for $[Os(NH_3)_4H_2]^{2+}$.

In preparing $[Os(en)_2H_2]^{2+}$, we followed the literature procedure,[2] but with the difference that, instead of Cl^-, $B(C_6H_5)_4^-$ was

(1) Kubas, G. J.; Ryan, R. R.; Swanson, B. I.; Vergamini, P. J.; Wasserman, J. J. *J. Am. Chem. Soc.* **1984**, *106*, 451–452.

(2) Malin, J.; Taube, H. *Inorg. Chem.* **1971**, *10*, 2403.

(3) $[Os(NH_3)_4H_2](B(C_6H_5)_4)_2$ was made by the folllowing procedure: $Os(NH_3)_4(O_3SCF_3)_3$[4] (100 mg) in 15 mL of H_2O was reduced by Zn/Hg (3 g) for 3 h, and then 15 mL of 0.2 M $NaB(C_6H_5)_4$ solution was added. The resulting precipitate was dried under vacuum. Microanal. Calcd for $[Os(NH_3)_4H_2](B(C_6H_5)_4)_2 \cdot 2H_2O$: C, 61.67; H, 6.25; N, 5.99. Found: C, 61.50; H, 6.20; N, 5.80. Yield: >70%.

(4) Li, Z.-W.; Harman, W. D.; Lay, P. A.; Taube, H. *Inorg. Chem.*, submitted.

(5) The preparation of the pyridine adduct is typical of the others. The compound $[Os(NH_3)_4(H_2)\cdot Py][B(C_6H_5)_4]_2$ (**2**) was prepared by dissolving **1** (100 mg) in pyridine (5 mL); after 1 h, ether was added to cause precipitation. The precipitate was collected, washed with ether, and dried. Yield: 90%. Microanal. Calcd for $[Os(NH_3)_4(H_2)\cdot Py][B(C_6H_5)_4]_2 \cdot 2H_2O$: C, 62.81; H, 5.82; N, 6.91. Found: C, 62.76; H, 6.03; N, 6.64. ^1H NMR in $(CD_3)_2CO$ (ppm): 8.83 (d, 2 H, Py), 8.14 (t, 1 H, Py), 7.75 (t, 2 H, Py), 7.40–6.70 (m, 40 H, C_6H_5), 3.74 (s, br, 12 H, 4 NH_3), −7.44 (s, 2 H, OsH_2).

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Table I. ^1H NMR Data[a] of Selected Dihydrogen Complexes $[Os^{II}(NH_3)_4(H_2)L]^{2+/1+}$

L	AN	Py	Im	I^-	Cl^-	D_2O	$(CD_3)_2CO$	Br^-
$\delta(H_2)$, ppm	−7.80[c]	−7.44	−7.43	−11.60	−10.78[c]	−11.35	−11.37[b,c]	−11.29
J_{HD}, Hz	20.3	19.6	17.1	12.5	10.2	8.1	4.0	<2.5
T_1, ms (20 °C)	221	148	131	142	1026	346	572	963
T_1, ms (low temperature)	62[d]	38 (min)	63 (min)	129[d]	125[d]	77 (min)	74[d]	107[d]

[a] In $(CD_3)_2CO$ as solvent, at 400 MHz; complexes at ca. 0.010 M level. [b] Identical values of parameters for PF_6^- and $B(C_6H_5)_4^-$ salts. [c] No new peaks at −95 °C or above. [d] No minimum observed at −95 °C or above.

Figure 1. Trans-to-cis isomerization.

used as precipitant. Elemental analysis shows the composition of the solid to correspond to $[Os(en)_2H_2Cl]B(C_6H_5)_4$.[6] The solid was dissolved in acetone and treated with $TlPF_6$ to extract Cl^-. After filtration, ether was added to the solution; the solid was collected, washed with ether, dried, and redissolved in $(CD_3)_2CO$, and the ^1H NMR spectrum was recorded. The proton signals for dihydrogen and for en prove to be different from those for the chloro complex.[7] In addition, a peak is observed at $\delta = 2.39$ ppm (3.7 H atoms counted at the first measurement), which disappears ($t_{1/2} \sim 10$ min) without attendant alterations in the other peaks. We ascribe this behavior to the replacement of coordinated $(CH_3)_2CO$ by $(CD_3)_2CO$.

We conclude that in solution each of the $16e^-$ species $[Os(NH_3)_4H_2]^{2+}$ and $[Os(en)_2H_2]^{2+}$ adopts an additional ligand and that the nitrogen ligands are coplanar in both cases. In contrast to NH_3, the amine moieties in $[Os(en)_2H_2]^{2+}$ are not free to rotate, and thus they respond to the different environments provided by the groups on the opposite faces of the molecular plane. X-ray crystallographic study of three compounds[8] confirmed the trans, octahedral stereochemistry (H_2 being counted as one ligand). All π-acid ligands which we have introduced lead to values of J_{HD} in the high range, suggesting that such ligands cause the hydrogen atoms to draw together. The addition of a very strong π-acid such as $C_6H_5CH_2NC$ leads to the immediate release of H_2. The product is a *trans*-$[Os(en)_2]^{2+}$ complex, which shows ^1H NMR signals only for en. Presumably the axial positions are occupied by $(CD_3)_2CO$.

In every one of at least 10 different systems, where the complexes in acetone are kept at room temperature for many hours, the ^1H NMR signals change.[9] For ammonia, the 12 protons appear in the ratio 2:1:1, the pattern expected for cis configurations (see Figure 1). For the bis(ethylenediamine) complexes in the cis configuration, a plane of symmetry is lacking even when the addend is a symmetrical ligand such as Cl^-, and for the eight amine as well as the eight methylene protons, four to eight peaks appear, the number depending on the symmetry of the addend. In all cases but one, conversion to the cis forms is more than 98% complete at equilibrium. The sole exception is $[Os(NH_3)_4(H_2)(CH_3CN)]^{2+}$, where the conversion is only 90% complete.

The facile addition of both saturated and unsaturated ligands to the $16e^-$ moieties dealt with above provides a unique opportunity for a systematic study of the variation of the chemical and physical properties of η^2 complexes with composition and, trans and cis forms being available, also with geometry. Moreover, the use of the moieties as recognition probes, particularly for biological molecules, also suggests itself. Studies along both lines are in progress.

Acknowledgment. Support of this work by National Institutes of Health Grant GM13638-25 is gratefully acknowledged.

(6) Microanal. Calcd for $[Os(en)_2(H_2)Cl]B(C_6H_5)_4$: C, 50.43; H, 5.69; N, 8.39; Cl, 5.32. Found: C, 50.18; H, 5.46; N, 8.22; Cl, 5.81. (en = $H_2NCH_2CH_2NH_2$). ^1H NMR (ppm, in $(CD_3)_2CO$): 5.31 (s, br, 4 H, NH_2), 4.03 (s, br, 4 H, NH_2), 2.67 (m, 4 H, $-CH_2-$), 2.11 (m, 4 H, $-CH_2-$), −12.57 (s, 2 H, OsH_2). $^1J_{HD} = 7.2$ Hz. T_1 (20 °C, 400 MHz) = 254 ms. T_1(min) = 61 ms (400 MHz).

(7) ^1H NMR in $(CD_3)_2CO$: 5.75 (s, br, 4 H, NH_2), 4.34 (s, br, 4 H, NH_2), 2.76 (m, 4 H, $-CH_2-$), 2.34 (m, 4 H, $-CH_2-$), 2.39 (s, 3.7 H, $(CH_3)_2CO$), −13.19 (s, 2 H, OsH_2). T_1 (20 °C, 400 MHz) = 145 ms. T_1(min) = 57 ms (400 MHz).

(8) X-ray crystal structure determinations on $[Os(en)_2(\eta^2\text{-}H_2)Cl]Cl$, $[Os(en)_2(\eta^2\text{-}H_2)Br]Br$, and $[Os(en)_2(\eta^2\text{-}H_2)py](PF_6)_2{\cdot}CH_3OH$ by T. Hasegawa of this laboratory and H. Hope of the University of California, Davis, confirm the trans geometry in these cases. Though data were taken at low temperature, they are not refined enough to place the atoms of dihydrogen. Arrangements have been made with T. F. Koetzle of the Brookhaven National Laboratory for structure determination by neutron diffraction.

(9) For example, on leaving **2** in $(CD_3)_2CO$ for 4 days, the original ^1H NMR peaks disappear and are replaced by a set of new peaks, which we assign as *cis*-$[Os(NH_3)_4(H_2)Py][B(C_6H_5)_4]_2$: 8.74 (d, 2 H, Py), 7.70 (t, 1 H, Py), 7.24 (t, 2 H, Py), 7.40-6.70 (m, 40 H, C_6H_5), 4.51 (s, br, 3 H, NH_3), 4.23 (s, br, 6 H, 2 NH_3), 3.58 (s, br, 3 H, NH_3), −7.32 (s, 2 H, OsH_2). $^1J_{HD}$ = 20.2 Hz; T_1 (20 °C, 400 MHz) = 104 ms, T_1(min) = 28 ms (400 MHz).

The Structures of Quartromicins A_1, A_2, and A_3: Novel Macrocyclic Antiviral Antibiotics Possessing Four Tetronic Acid Moieties

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In the course of a search for new antibiotics, novel antiviral antibiotics have been isolated from *Amycolatopsis orientalis* No. Q427-8. Non-ionic resin extraction of the fermentation broth (9.8 L) followed by chromatographic purification resulted in isolation of three active components, quartromicins A_1 (**1**; 57 mg), A_2 (**2**; 87 mg), and A_3 (**3**; 70 mg),[1] which contain a unique macrocarbocyclic ring possessing four tetronic acid moieties.

Quartromicin A_1 (**1**) [$C_{78}H_{88}O_{30}$, negative FABMS m/z 1525 $(M - 2 + Na)^-$, 1541 $(M - 2 + K)^-$] and quartromicin A_3 (**3**) [$C_{78}H_{92}O_{30}$, m/z 1545 $(M - 2 + K)^-$] were isolated as colorless amorphous salts.[2] The ^{13}C NMR spectra of **1** and **3** exhibit 39 well-defined carbon signals, and therefore, both antibiotics must possess symmetrical dimeric structures.

The ^1H and ^{13}C NMR spectra[3] of **1**, **2**, and **3** suggest the presence of a sugar, which was identified as D-galactose after isolation from the acid hydrolysis (MeOH/HCl) products. Although the aglycons, degalactosylquartromicins A_1-A_3,[4] were isolated, the yield was rather poor (ca. 50%) and the aglycons were poorly soluble in NMR solvents. Because **3** could be purified most easily, structure determination was performed largely on the salt of **3**. (Structure elucidation will be interpreted on the monomeric fragments hereafter.)

Routine 2D NMR[3] analyses led to partial structures **4** and **5**, which accounted for 33 of 39 carbons in the monomeric fragment of **3**, but the following spectral properties, which were assigned

(1) Tsunakawa, M.; Tenmyo, O.; Tomita, K.; Naruse, N.; Kotake, C.; Miyaki, T.; Konishi, M.; Oki, T. *J. Antibiot.*, submitted for publication.

(2) The metal composition of the salt (mol %) of **3** was analyzed as follows: Na, 70%: K, 19%: Ca, 10%: Mg, 1%.

(3) The 1D and 2D NMR spectra are available as supplementary material.

(4) Degalactosyl-**1**: $C_{66}H_{68}O_{20}$, negative FABMS m/z 1201 $(M - 2 + Na)^-$, 1217 $(M - 2 + K)^-$.

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