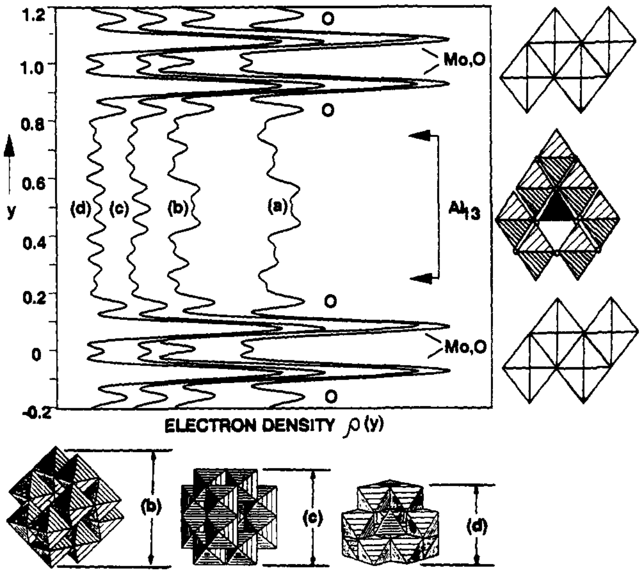

Figure 2. (a) Projection of the electron density of $[(Al_{13})_{0.047}(Na)_{0.003}(H_2O)_{1.0}]MoO_3$ along the interlayer axis, y. The scattering contributions from the lattice components are indicated on the figure. A schematic representation of the orientation of the Al_{13} cluster between the MoO_3 layers is shown on the right; (b) calculated projection with the C_2 axis of Al_{13} perpendicular to the layers, shown in (b) on the bottom; (c) calculated projection with the C_4 axis perpendicular to the layers, shown in (c) on the bottom; (d) calculated projection with the C_3 axis perpendicular to the layers, shown in (d) on the bottom.

contribution from each being roughly equal. The meso pores had a very narrow pore size distribution centered at a radius of 20 Å.

The "one-dimensional crystal structure" can be determined by 1-D electron density mapping. The Fourier transform of the structure factors obtained from the integrated X-ray intensities shown in Figure 1 resulted in the projection of the electron density along the interlayer axis shown in Figure 2a. Comparison with the calculated 1-D profiles for the Al_{13} in three different orientations (Figure 2b–d) shows that the Al_{13} cation is situated with the C_2 axis perpendicular to the layers. This may be responsible for the slightly larger interlayer expansion observed here, in comparison with that reported for clay minerals in which it is thought that the Al_{13} is oriented with the C_3 axis perpendicular to the layers.[1]

Treatment of the Li_xMoO_3 or Na_xMoO_3 dispersion with a Na-free, concentrated Ga–polyoxy cation solution resulted in a material which gave rise to a very similar diffraction pattern (d_{0k0} = 18.1 Å), Figure 1b, as that obtained for Al_{13}. ^{71}Ga NMR spectra of the hydrolyzed solutions showed that the NMR spectrum at 80 °C was similar to that of Al_{13};[10] namely, a single, narrow tetrahedral-site resonance was observed at 173.2 ppm (downfield from $Ga(H_2O)_6^{3+}$), with a very broad octahedral resonance barely visible at about 35 ppm. This, together with the almost identical values of interlayer expansion in the MoO_3 lattice, implies that the structure or composition of the Ga–polyoxy cation is very similar to Al_{13}.[11] After the initial submission of this paper, another report has appeared on the incorporation of a similar gallium species in clays, whose authors come to the same conclusion.[12]

Flocculation of the A_xMoO_3 with more dilute Ga–polyoxy cation solutions containing Na^+ (or dilute Al_{13} solutions) led to materials which also showed a very well-ordered diffraction pattern but which had an interlayer spacing of 25 Å. These were unstable, converting to a mixture of an 18 Å phase and A_xMoO_3 after a few hours. This suggests that they are due to a "alternate-stage" intercalation compound, in which an approximately 11-Å polyoxy cation is intercalated between every other MoO_3 layer. The intervening layers are occupied by either A^+ (A = Na, Li) or H^+. Such compounds have been observed under certain conditions for Al_{13} intercalated in montmorillonite clays.[13]

We have also been successful in forming pillared MoO_3 with other polyoxy cation clusters of Ti, Zr, and Cr.[14] The properties and thermal evolution of these materials will the subject of future investigations.

Acknowledgment. The authors thank the National Sciences and Engineering Council of Canada and the Ontario Center for Materials Research for financial support and NSERC for a postdoctoral fellowship for S.W.L.

Supplementary Material Available: Table I of XRD data for the 18-Å phase of $[(Al_{13})_{0.047}(Na)_{0.003}]MoO_3$ and the 25-Å phase of the Al_{13}-pillared MoO_3 and Table II of XRD data for the 18- and 25-Å phases of Ga polyoxycation pillared MoO_3 (2 pages). Ordering information is given on any current masthead page.

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[11] Chemical analysis of the pillared material gave a Ga/Mo ratio of 0.41, yielding a formulation of $[(Ga_{13})_{0.032}(Na)_{0.09}]MoO_3$. Note that some (or all) of the Na is present in a second phase (see Figure 1b).

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Alteration of the Substrate Specificity of 3-Oxo-Δ^5-steroid Isomerase by Mutation of the Catalytic Base (Asp-38 to Glu-38)

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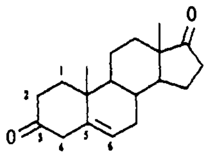
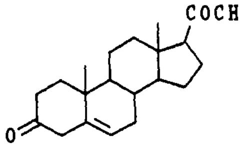
We report that mutation of Asp-38 to Glu-38 in 3-oxo-Δ^5-steroid isomerase alters the enzyme specificity, decreasing k_{cat} 300-fold toward 5-androstene-3,17-dione (**1a**) but only 32-fold toward 5-pregnene-3,20-dione (**1b**). This 10-fold difference in specificity is due to a modification of the catalytic base of the enzyme (Asp-38) rather than any change in the amino acids involved in binding.



1a



1b

3-Oxo-Δ^5-steroid isomerase (EC 5.3.3.1, also called Δ^5-3-ketosteroid isomerase, KSI) from *Pseudomonas testosteroni* catalyzes the conversion of a variety of 3-oxo-Δ^5-steroids to their conjugated Δ^4-isomers (Scheme I).[2] This reaction proceeds

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[2] For reviews on the mechanism of 3-oxo-Δ^5-steroid isomerase, see: (a) Pollack, R. M.; Bounds, P. L.; Bevins, C. L. In *The Chemistry of Enones*; Patai, S., Rappoport, Z., Eds.; Wiley: New York, 1989; p 559. (b) Batzold, F. H.; Benson, A. M.; Covey, D. F.; Robinson, C. H.; Talalay, P. *Adv. Enzyme Regul.* **1976**, *14*, 243.

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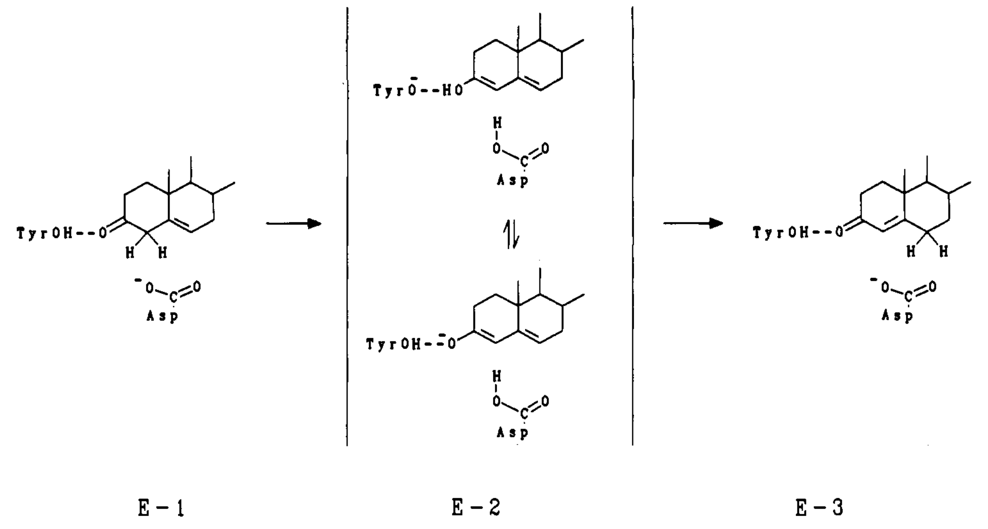
Scheme I



through the intermediacy of an enzyme-bound dienol(ate),[3] which is formed by abstraction of the 4β-hydrogen of the Δ^5-steroid (**1**) by Asp-38, with Tyr-14 acting to polarize the carbonyl,[4] by either hydrogen bonding or proton transfer. Subsequent protonation of the dienol (ate) (**2**) at C-6β by the conjugate acid of Asp-38 gives the α,β-unsaturated ketone (**3**), completing the reaction.

A mutant KSI with Glu-38 replacing Asp-38 (D38E) was prepared by using plasmid pKC2 obtained from W. F. Benisek.[5] A 1.2-kb *Pst*I restriction fragment containing the KSI gene was inserted into the *Pst*I site of M13mp8. Recombinant M13 bacteriophage were propagated on *Escherichia coli* strain JM103, and single-stranded M13 DNA was purified.[6] Oligonucleotide-directed mutagenesis was performed by the method of Eckstein,[7] as described elsewhere.[8] Mutants were identified by single-base sequence analysis and confirmed by sequencing of the entire gene. The Glu-38 KSI mutant gene was isolated from M13mp8 on a 1.2-kb *Eco*RI-*Hin*DIII fragment and inserted into the *Eco*RI and *Hin*DIII sites of pUC18. The protein was isolated from transformed *E. coli* JM83.

The wild-type (WT) and D38E enzymes were assayed with **1a** and **1b** at 25.0 °C and pH 7, where both enzymes show near maximum activity. Weighted least squares analysis of Lineweaver–Burk plots gave values for k_{cat} and K_m (Table I). Since replacement of Asp-38 by Glu involves movement of the carboxylate by about 1 Å, it might be expected that the activity of the D38E mutant would be significantly affected. In accord with this expectation, k_{cat} is decreased 300-fold for **1a**, with little change in K_m (2-fold). In contrast, k_{cat} is decreased only 32-fold toward **1b**. The difference in the responses of **1a** and **1b** toward this mutation is surprising since these two substrates differ only at C-17, ca. 8 Å away from the site of reaction. Thus, k_{cat} for the wild type is 12-fold greater for **1a** than it is for **1b**, whereas k_{cat} for the D38E mutant is similar for both **1a** and **1b**.

This alteration of specificity in k_{cat} for the D38E mutant can be rationalized if the mutation causes a change in the rate-limiting step for **1b**, but not for **1a** (Scheme II). For the WT, primary kinetic hydrogen isotope effects using $[4\beta\text{-}^2H]$-**1a** $[^{H/D}(k_{cat}/K_m) = 3.0, {}^{H/D}(k_{cat}) - 5.2\text{–}6.1]$[9] show that a chemical step, either deprotonation of **1** (k_3) or protonation of **2** (k_5), is at least partially rate limiting for both k_{cat} and k_{cat}/K_m.[10] Mutation of Asp-38 to Glu should decrease the rate for k_3 and/or k_5. Since one or both of these steps is rate-limiting, most of this effect will be observed, resulting in a substantial decrease in k_{cat} for **1a**.

Table I. Kinetic Parameters for Wild-Type and D38E Mutant 3-Oxo-Δ^5-steroid Isomerase with 5-Androstene-3,17-dione (**1a**) and 5-Pregnene-3,20-dione (**1b**)[a,b]

	k_{cat}, s^{-1}	K_m, μM	k_{cat}/K_m, M^{-1} s^{-1}
wild type			
1a	5.1×10^4	200	2.6×10^8
1b	4.2×10^3	6.8	6.2×10^8
D38E			
1a	1.7×10^2	109	1.6×10^6
1b	1.3×10^2	5.0	2.5×10^7

[a] Methanol 3.3%, 34 mM phosphate buffer, pH 7.0, 25.0 °C. [b] Standard deviations are approximately ±10–15% for k_{cat}/K_m and ±20–30% in k_{cat} and in K_m.

Scheme II

$$E+1 \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E\text{-}1 \underset{k_4}{\overset{k_3}{\rightleftharpoons}} E\text{-}2 \underset{k_6}{\overset{k_5}{\rightleftharpoons}} E\text{-}3 \xrightarrow{k_7} E+3$$

In contrast, the kinetic hydrogen isotope effect for the WT with $[4\beta\text{-}^2H]$-**1b** has been reported to be unity,[11] suggesting that the rate-limiting step for k_{cat} with **1b** is dissociation of **3** from E-3 (k_7). Thus, a lowered rate of deprotonation (k_3) or protonation (k_5) will not manifest itself in k_{cat} until the barrier for k_3 or k_5 becomes competitive with the barrier for k_7. Consequently, only a portion of the effect of the D38E mutation on the proton transfer steps shows up in the overall rate constant for **1b**, and the effect of mutation is less for **1b** than for **1a**.

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(10) We have recently investigated the energetics of this reaction; we found that the barriers to diffusion of E + **1** (k_1), deprotonation of **1** (k_3), and protonation of **2** (k_5) are all kinetically significant (Hawkinson, D. C.; Eames, T. C. M.; Pollack, R. M., unpublished results).

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Trishomocubane-Type Methoxide Cluster as a Novel Mediator in the Extension of Cube Size in Organometallic Oxide Clusters: Synthesis and Structures of $[(RhCp^*)_2Mo_3O_9(OMe)_4]\cdot MeOH$ and a Linear Quadruple Cubane-Type Cluster $[(RhCp^*)_4Mo_6O_{22}]\cdot 4CH_2Cl_2$ ($Cp^* = \eta^5\text{-}C_5Me_5$)

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In recent years, we have directed our efforts primarily toward the synthesis of integrated cubane-type clusters as potential models for inorganic solid surfaces to understand the chemistry[1] on them and have been reporting novel organometallic oxide clusters that contain soft as well as hard multimetal centers.[2-4] Examples include $[MCp^*MoO_4]_4$[2] and $[(MCp^*)_4V_6O_{19}]$ (M = Rh, Ir).[3]

The cluster $[RhCp^*MoO_4]_4$ (**1**) has triply fused cubane character with a central Mo_4O_4 framework. Having noted that the same framework can be recognized as a repeating unit in the infinite layer structure of MoO_3,[5] a heterogeneous catalyst for the oxidation of MeOH to CH_2O and Me_2O,[6] we were prompted to investigate the potential utility of **1** in the transformation of methanol. Consequently, the exploitation of the reaction system of **1** with MeOH was undertaken to reveal serendipitous conversion, outlined in Scheme I, of the triple cubane-type cluster **1** to a trishomocubane-type[7] cluster $[(RhCp^*)_2Mo_3O_9(OMe)_4]$ (**2**) and then to a quadruple cubane-type cluster $[(RhCp^*)_4Mo_6O_{22}]$ (**3**). Here, we report brief accounts of novel fragmentation and extension of cube size in the organometallic oxide cluster **1** as well as the structural characterization of **2**·MeOH and **3**·$4CH_2Cl_2$.

Cluster **1** is very soluble and stable in MeOH at ambient temperature. In the presence of excess *p*-hydroquinone (HQ),

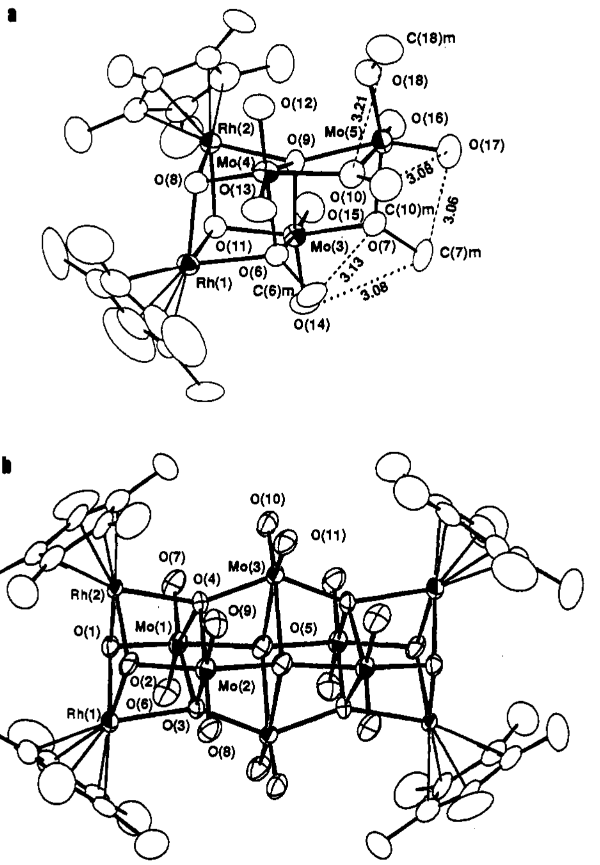
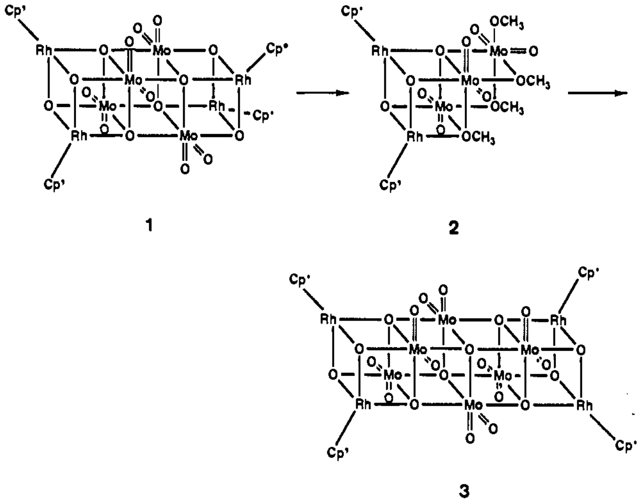


Figure 1. Perspective drawings of (a) $[(RhCp^*)_2Mo_3O_9(OMe)_4]$ (**2**) and (b) $[(RhCp^*)_4Mo_6O_{22}]$ (**3**). Selected interatomic distances and angles have been deposited as supplementary material.

Scheme I



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