t = time; and K_a, K_b and V_a, V_b are the Michaelis constants and maximal velocities for A and B, respectively. By insertion of the E value and three sets of experimental data $[(t_1, A_1), (t_2, A_2), (t_3, A_3)]$ into eq 1, the values of V_a, V_b, and K_a may be determined; K_b may be calculated from eq 2. To obtain more reliable kinetic constants, additional sets of data are required.

For this competitive experiment, methyl (±)-2-(4-chlorophenoxy)propionate [(±)-**4a**] was used as the substrate. The E values for the crude lipase and the purified lipase (A-form) have been determined previously to be 17 and >100, respectively. These experiments were conducted at a substrate concentration of 0.1 M under identical reaction conditions. For each sample, (±)-**4a** was dissolved in hexane and appropriate aliquots were distributed to reaction vials. The hexane was then removed by evaporation using a stream of nitrogen. Crude lipase or lipase A-form ([Et] = 5 × 10^{-7} M, 22.5 units), dissolved in 1 mL of 0.2 M phosphate buffer, pH 7.0, was prewarmed in a 25 °C water bath for 10 min. It was then carefully added to each vial containing the substrate. The reaction mixture was stirred vigorously at 24 °C with a Corning PC-351 magnetic stirrer. The reaction was terminated by acidification (1 N HCl) to pH 2.0 and the time was recorded. The reaction mixture was then extracted with four 1-mL portions of ethyl acetate. The organic layer was dried over Na_2SO_4, filtered, and then evaporated to dryness. The residue was dissolved carefully in 1 mL of ethyl acetate and an aliquot was used for the subsequent HPLC analysis. The extent of conversion was determined by HPLC analysis using a 4.6 × 500 mm (Alltech porasil, 10 μm) column, which was eluted with a solvent system consisting of hexane–ethyl acetate–acetic acid (500:100:6) at a rate of 4 mL/min. The absorbance at 254 nm was monitored with a Waters Model 440 detector and a Hewlett-Packard 3390A integrator. A standard mixture of (±)-**4a** and its carboxylic acid at varying ratios gave equal molar responses of integral areas. The concentration of the fast-reacting enantiomer, (*R*)-(+)-**4a**, can be calculated from the value of conversion, c, by solving eq 3 when E is known. The data were fitted to

$$E = \frac{\ln (A/A_0)}{\ln \{[(A_0 + B_0)(1 - c) - A]/B_0\}} \qquad (3)$$

eq 1 on an IBM PC using the BASIC program of Duggleby,[20] prepared by Dr. Dexter B. Northrop, to obtain the kinetic constants listed in Table VIII.

Registry No. 1a, 122623-82-9; **1b**, 114315-58-1; **1c**, 114315-57-0; **1d**, 105052-64-0; **1e**, 124604-52-0; **1f**, 64382-51-0; **2a**, 7782-24-3; **2b**, 22161-81-5; **2c**, 51543-39-6; **2d**, 22204-53-1; **2e**, 95976-43-5; **2f**, 52780-12-8; **3a**, 122674-97-9; **3b**, 122674-99-1; **3c**, 122674-98-0; **3d**, 124649-62-3; **3e**, 124604-53-1; **3f**, 81601-94-7; **4a**, 95262-84-3; **4b**, 122623-80-7; **4c**, 122623-81-8; **4d**, 23844-57-7; **4e**, 23844-56-6; **5a**, 20421-34-5; **5b**, 31460-41-0; **5c**, 31460-39-6; **5d**, 15165-67-0; **5e**, 16484-77-8; **6a**, 95342-42-0; **6b**, 122674-93-5; **6c**, 122674-94-6; **6d**, 122674-95-7; **6e**, 115304-96-6; lipase, 9001-62-1.

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Communications to the Editor

$Os_2(CO)_8$: Transient Existence in Solution As Observed by Time-Resolved Infrared Spectroscopy upon Flash Photolysis of Octacarbonyl-1,2-diosmacyclobutane

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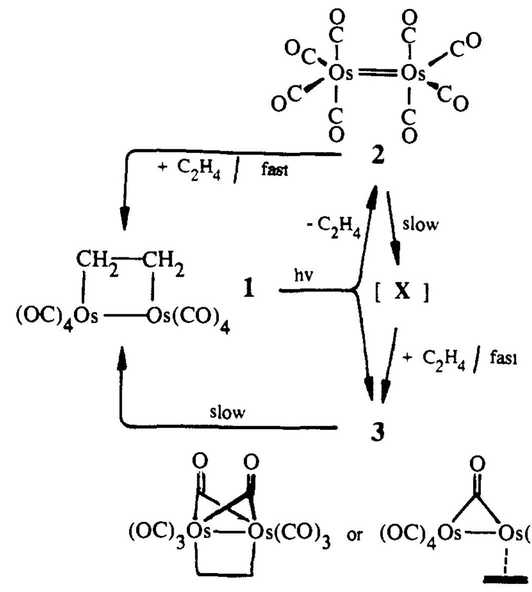
The dinuclear $Os_2(CO)_8$ unit has been invoked as a key intermediate in the photochemical synthesis of octacarbonyl-1,2-diosmacyclobutane complexes from $Os_3(CO)_{12}$[1] and in reactions involving exchange of the bridging organic moiety for other olefins[2,3] or alkyne[3,4] ligands.

Herein we report on the first observation of this species in solution at ambient temperature by means of fast time-resolved infrared spectroscopy[5] in the CO stretching vibrational region. Flash photolysis (λ = 308 nm, XeCl excimer laser) of $Os_2(CO)_8(\mu\text{-}\eta^1,\eta^1\text{-}H_2CCH_2)$ (**1**, 8 × 10^{-4} M in cyclohexane) at ambient temperature generates $Os_2(CO)_8$ (**2**) as a short-lived species (microsecond time domain) together with a second transient (**3**), apparently a CO-bridged isomer of **1**, which decays much more slowly (millisecond time domain), Scheme I. The ν_{CO} bands associated with **2** (2058, 2018, 2003 cm^{-1}) and **3** (2068, 2027, 2016, 2000, 1992, 1780 cm^{-1}) have been extracted from series of decay curves monitored at different wavenumbers and in the presence of varying amounts of ethene in the solution.

Scheme I



In detail, the initially observed (≤3 μs after the flash) transient spectrum under neat argon atmosphere is dominated by the ν_{CO} bands of **2** (Figure 1A), but the weak band in the bridging CO region indicates that **3** is also present. Under these conditions, the decay of **2** ($\tau_{1/2} \leq 40$ μs), apart from minor side reactions, involves re-formation of **1** as well as secondary formation of **3**. The former process (but apparently not the latter one) is greatly accelerated by added ethene (ca. 10^{-2} M) such that **2** decays much more rapidly ($\tau_{1/2} \leq 5$ μs) and cleanly with re-formation of **1**, leaving behind the ν_{CO} pattern of **3** (Figure 1B). In ethene-saturated cyclohexane solution, the spectrum of **3** is observed im-

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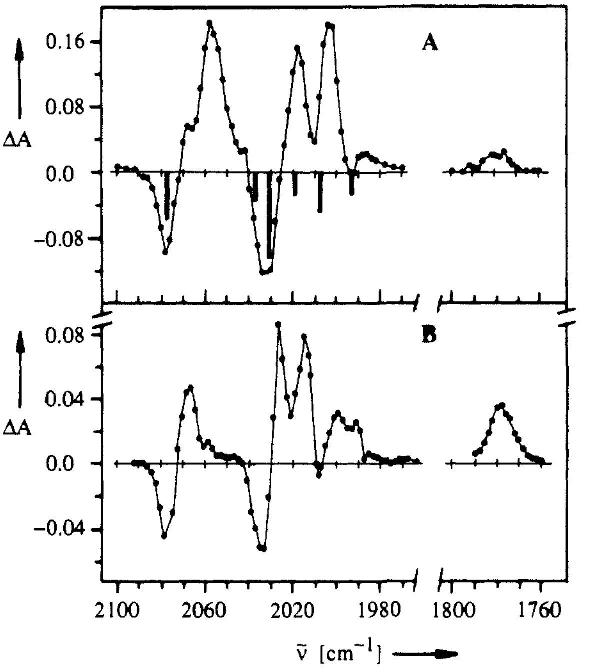

Figure 1. Transient ν_{CO} infrared difference spectra from flash photolysis (λ = 308 nm) of **1** (8×10^{-4} M) in cyclohexane at ambient temperature: (A) after ca. 3 μs under neat argon atmosphere (the vertical bars represent the ν_{CO} pattern of **1**); (B) after 100 μs in the presence of added ethene (ca. 10^{-2} M); note the difference in the scales.

mediately after the flash, **2** being too short-lived for detection. The decay of **3**, with nearly complete re-formation of **1**, occurs by first-order kinetics ($k = 8 \pm 3$ s^{-1}) independent of the amount of added ethene. From this we conclude that **3** already contains the ethene unit and thus represents an isomer of **1**, whereas **2** logically is identified as $Os_2(CO)_8$. Apparently, the (partial) conversion of **2** into **3** does not involve ethene in the rate-determining step, which leads us to postulate an intermediate X (Scheme I), presumably a CO-bridged isomer of **2**.

The ν_{CO} band pattern of **3** (Figure 1B) is in accord with a C_{2v} $(OC)_3Os(\mu\text{-}CO)_2Os(CO)_3$ skeleton, which requires five IR-active CO stretching vibrations in the terminal CO region (two A_1, two B_1, B_2)[6] and two bridging CO vibrations, one of which may be very low in intensity.[7] However, by analogy with the structure of $Os_2(CO)_9$[8] (and in view of the reluctance of third-row transition metals to support CO bridges), an alternative structure such as $(OC)_4Os(\mu\text{-}CO)Os(CO)_3(\eta^2\text{-ethene})$, with only one bridging CO, is equally plausible, if not even more likely. This structure would require more than five terminal CO bands, but it seems possible that weak bands are not observed or overlapping absorptions are not resolved.

For $Os_2(CO)_8$ (**2**), structures with D_{4h} or D_{4d} symmetry (two IR-active ν_{CO} modes: A_{2u}, E_u or B_2, E_1) can be excluded simply by the fact that three ν_{CO} bands are observed (Figure 1A), i.e., one band more than predicted. Although structures with D_{3h} or D_{3d} symmetry (three IR-active ν_{CO} modes: two A_2'', E' or two A_{2u}, E_u) would be in accord with the observed number of bands, they are unlikely because three bands with distinctly different intensities should be expected: the very strong E' or E_u mode, one A_2'' or A_{2u} mode of medium intensity (mainly involving the two axial CO groups), and one A_2'' or A_{2u} mode of low intensity (mainly involving the six equatorial CO groups). The D_{2h} structure shown in Scheme I, analogous to that assigned to the unbridged isomer of $Fe_2(CO)_8$,[7] is logical on the basis of the isolobal analogy between a d^8 ML_4 dimer and ethene.[9] Four IR-active CO stretching vibrations are expected for this geometry (two B_{1u}, B_{2u}, B_{3u}), which at first sight seems difficult to reconcile with the observed spectrum (Figure 1A). However, the one B_{1u} vibration mainly involving the axial CO groups and presumably located at high frequency should be distinctly weaker than the other modes and may be too weak to be observed. But we must not fail to note that the same reasoning would also apply to other structures composed of two C_{2v} $M(CO)_4$ subunits, such as D_{2d} (IR-active ν_{CO}: two B_2, two E) or C_{2h} (IR-active ν_{CO}: A_u, three B_u). Thus at present the latter two geometries cannot be excluded.

Low-temperature matrix experiments[10] complement the above studies in solution. Irradiation of **1** in an ethene-argon (1:5) matrix at 10-12 K with λ = 365 nm results in the exclusive formation of **2**, as indicated by the appearance of three terminal ν_{CO} bands at 2057, 2023, and 2006 cm^{-1} and no absorption in the bridging ν_{CO} region. Carbon monoxide is not detectable even after 50% conversion of **1**. It is noteworthy, in view of the fast re-formation of **1** in solution, that annealing of the matrix to 30 K leaves the ν_{CO} pattern of **2** essentially unchanged, apart from a small red shift of the maxima and some broadening of the band shapes. Photochemical reactions occurring upon irradiation into the electronic absorption of **2** (λ_{max} = 525 nm) are currently under investigation, which, in combination with a more detailed knowledge of the kinetics of **2** in solution, may provide a promising basis for the understanding of dinuclear reductive elimination and oxidative addition reactions[9b] in this and related species.

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Registry No. 1, 83705-05-9; **2**, 124442-24-6; **3**, 124602-64-8.

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Direct Determination of the Partitioning of an Enzyme-Bound Intermediate

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In order to fully understand the basic principles by which enzymes function, it is important to be able to elucidate detailed free energy profiles for a variety of enzymatic reactions.[1-4] The nature of the partitioning of enzyme-bound intermediates is a particularly difficult problem, which generally must be addressed by indirect means. We now report the *direct* determination of the partitioning ratio of the dienol intermediate (**2**) in the 3-oxo-Δ^5-steroid isomerase (also called ketosteroid isomerase, KSI, EC 5.3.3.1)[5] catalyzed isomerization of 5-androstene-3,17-dione

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