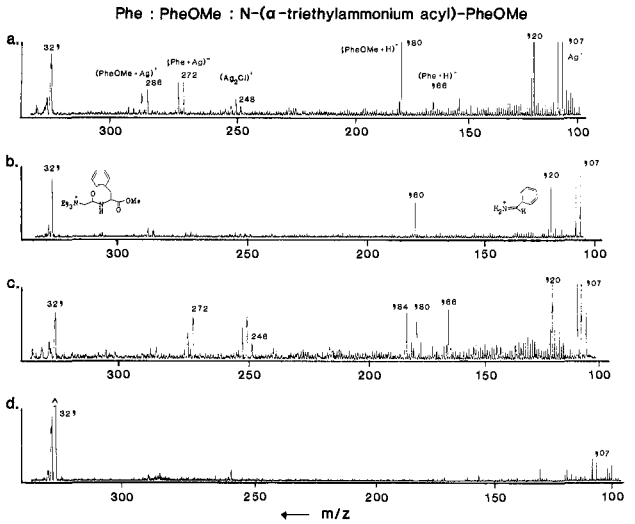

Figure 2. Static SIMS spectra of an equal molar mixture of phenylalanine (Phe), phenylalanine methyl ester (PheOMe), and *N*-((triethylammonio)acetyl)phenylalanine methyl ester at various pH's. Spectra are recorded from acid-etched silver surfaces using 3-keV Xe^0 2×10^{-9} A/cm^2. Spectrum a was obtained at a slightly acidic pH, b was obtained at a more basic pH, c was obtained at pH <2, and d was the charged acyl derivative alone.

rivative.[9] A better approach to labeling the N-terminus is by acylation with chloroacetyl chloride. This is best accomplished by heating the peptide and the reagent in an inert solvent[10] or by the Scotten-Baumann technique if the peptide is water soluble.[11] After acylation, the peptide is cleaved by acid, esterified with 3 M HCl in methanol, and treated with triethylamine to form the ammonium salt.[12]

These derivatization reactions have two principal objectives: (1) to label the peptide with a charged group and (2) to eliminate any potential sites for charge production by protonation of components in the mixture. This second objective is accomplished by esterification and acylation reactions. If these precautions are not taken, the static SIMS spectrum of all cleavage products would be observed, depending on the pH. As an example, the SIMS spectra of an equal molar mixture of phenylalanine, phenylalanine methyl ester, and *N*-((triethylammonio)acetyl)phenylalanine methyl ester deposited on a silver surface are shown in Figure 2. Spectrum 2a was obtained when the mixture was deposited at a slightly acidic pH. At this pH, the phenylalanine is in the zwitter ionic form and has no net charge. Consequently, ions from it are observed at only low intensity compared to the protonated methyl ester and the charged acyl derivative. Spectrum 2b shows the same mixture deposited at a more basic pH. No phenylalanine molecular ions are present and the methyl ester molecular ions are reduced in intensity. Spectrum 2c was taken at pH <2 where ions from all three components are observed. In all three spectra, silver-cationized ions of phenylalanine and its methyl ester, but not the charged acyl derivative, are also observed. The charged acyl derivative is seemingly insensitive to the chemical environment from which it is deposited and when examined alone (as in the spectrum in Figure 2d) shows little, if any, fragmentation.

The derivatization methods suffer unfortunately from being nonspecific. Any basic internal amino acid would also be derivatized, and these extra ions would complicate the mass spectrum. This problem can be overcome by protecting any internal lysines as thioureas with one cycle of an Edman degradation and then performing the next cycle with a charged isocyanate rather than an isothiocyanate. Unfortunately, the commercially available 2-chloroethyl isocyanate undergoes ring formation[13] in triethylamine solution faster than formation of the ammonium salt. Attempts to prepare the ammonium salt of the isocyanate before reaction with the peptide were also unsuccessful. Derivatization with other charged isocyanates is in progress and will be reported shortly.

Although FABMS has been used to examine the peptide derivatives, static SIMS gives a better signal-to-noise ratio and lower intensities of matrix ions. Also, the relative signal intensities of the ions in the static SIMS spectra are more representative of the components in the sample than with FABMS. For example, the FABMS spectra of equal molar mixtures of similar quaternary salts can show very different intensities for the molecular ions[14] due to the different ways the molecules reside on the surface of the liquid matrix.

In summary, we have developed a general peptide sequencing technique that relies upon the enhanced detection of charged groups in SIMS or FABMS. The peptide may be sequenced from the *n*-terminus. The current level of detection is in the low nanogram range.

Acknowledgment. D.A.K. and M.M.R. thank the National Research Council for Resident Research Associateships and Maria B. Barnes for preparing some of the figures.

Registry No. Me_3^+GlyGlyPheOMe, 89178-06-3; Et_3^+-NCH_2COProGlyGhyOMe, 89196-36-1; phenylalanine, 63-91-2; phenylalanine methyl ester, 2577-90-4; *N*-(triethylammonio)acetylphenylalanine methyl ester, 89178-07-4.

(9) Typical conditions for quaternization with methyl iodide: 1 mg of the peptide is dissolved in 2 mL of 50% methanol/water; a 10-fold excess of methyl iodide is added in portions, while keeping the pH at 10 using lithium or potassium hydroxide in methanol as base.

(10) Ronwin, E. J. *J. Org. Chem.* **1953**, *18*, 127-142.

(11) Birnbaum, S. M.; Levintow, L.; Kingsley, R. B.; Greestein, J. P. *J. Biol. Chem.* **1952**, *194*, 455-470.

(12) Peptide cleavage is done in 6 N HCl at 110 °C for 5-10 min. The reaction with triethylamine is performed in methanol at 60 °C, overnight.

(13) Wheeler, G. P.; Bowdon, B. J.; Struck. R. F. *Cancer Res.* **1975**, *35*, 2974-2984, 1750-1761.

(14) Keuogh, T.; DeStefano, A. J. Presented at the 31st Annual Conference on Mass Spectrometry and Allied Topics, Boston, MA, May 8, 1983.

Activation of Methane by Supported Rhodium Complexes

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Alkane "activation" is a current theme in organometallic chemistry that commands considerable attention derived in part from interest in selective catalytic conversion of alkanes to organic products. In this context, two disparate approaches can be considered for developing catalysts for selective alkane activation: These involve "electron-deficient" species or "electron-rich" ones. In the former category fall alkane isomerization catalysts and other species associated with conversion of alkanes to products via carbonium ions.[1] The latter category includes complexes of metals such as those of Ir(I) containing strong donor ligands which "oxidatively add" simple alkanes stoichiometrically.[2] Of these two approaches, the former one seems most auspicious for seeking *catalytic* systems for alkane activation: many of the most interesting of these processes will be oxidative in nature, and reagents to accomplish these transformations likely would be incompatible with strongly reduced transition-metal centers. Our recent investigations concerning supported rhodium complexes show that it is possible to use an oxide to help stabilize the metal in a high

(1) (a) Pine, H. "The Chemistry of Catalytic Hydrocarbon Conversions"; Academic Press: New York, 1981. (b) Kochi, J. A. "Organometallic Mechanism and Catalysis"; Academic Press: New York, 1978; p 89. (c) Olah, G. A. "Friedel-Crafts Chemistry"; Wiley-Interscience: New York, 1973.

(2) (a) Janowicz, A. H.; Bergman, R. G. *J. Am. Chem. Soc.* **1982**, *104*, 352. (b) Hoyano, J. K.; Graham, W. A. G. *Ibid.* **1982**, *104*, 3723.

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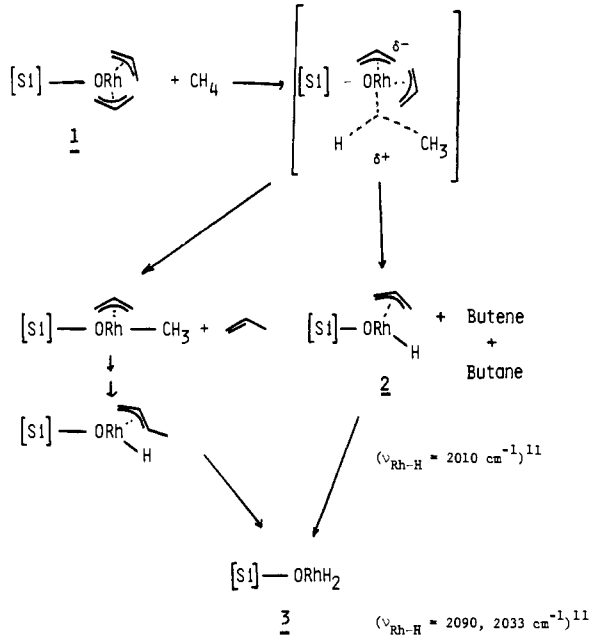
Scheme I. Methanation of 1

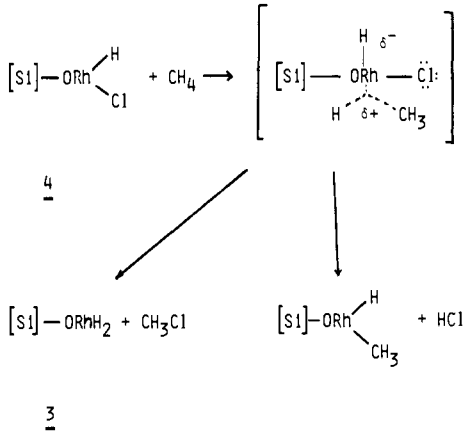


oxidation state,[3] and we have demonstrated general electrophilic character of several of these oxide-bound complexes.[4] We had noted activation of H_2 by electrophilic rhodium complexes and have described this reaction in terms of a general acid–base pathway;[4a] we have proposed that alkanes could interact with such complexes in much the same way.[4] Accordingly, we have now found that methane can be used to effect stoichiometric transformations of oxide-bound Rh(III) complexes which have already been accomplished by using H_2 and which provide a basis for catalytic activation of that hydrocarbon.

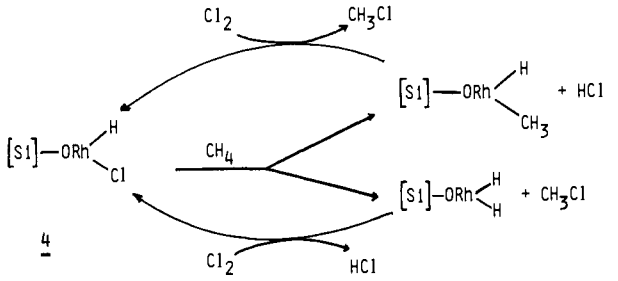
When allylic rhodium species **1** is treated with H_2, hydridorhodium species **2** results (ν_{Rh-H} = 2010 cm^{-1}, strong);[3,5] similarly we now find that when **1** (0.115 mmol of Rh) is treated with methane (1.20 mmol) for 2 days at 100 °C, a mixture of hydride complexes **2** and **3** are obtained (ν_{Rh-H} = 2091, 2012 (s, broad) cm^{-1}).[3] Propylene (0.017 mmol) and a small amount of butane and butene were detected. Protonolysis of an aliquot of the resulting organorhodium species (0.065 mmol Rh) with MeO^-/MeOH yielded propane (0.031 mmol), butane (0.012 mmol), and *n*-hexane (0.058 mmol). Since protonolysis of **1** is known to yield propane and hexane,[3] these observations can be explained by the reactions in Scheme I. That methane activation and incorporation were indeed responsible for these observations was proven by use of $^{13}CH_4$. Here, complex **1** (0.09 mmol) was treated with $^{13}CH_4$ (1.2 mmol, 90% ^{13}C) under similar conditions; propylene (0.03 mmol) was evolved, suggesting that this amount of methane had reacted with **1**. Hydrolysis of the resulting rhodium species yielded propane and hexane containing no significant incorporation of ^{13}C above natural abundance. Butane was formed (approximately 0.03 mmol) containing 70% incorporation of ^{13}C above natural abundance.

Hydrogen reacts with hydrido chloride complex **4** to give compound **3**, and, therefore, the reaction between **4** and methane was examined. Complex **4** (0.105 mmol of Rh) was treated with methane (1.20 mmol) for 2 days at 100 °C. Methyl chloride was

Scheme II. Methanation of 4



Scheme III. Metal-Catalyzed Chlorination of Methane



obtained (0.09 mmol, 0.85 equiv per Rh). Reaction of **4** with $^{13}CH_4$ gave $^{13}CH_3Cl$: **4** (0.105 mmol) was treated with $^{13}CH_4$ (1.2 mmol, 90% ^{13}C enrichment) for 12 h at 100 °C to give 0.046 mmol of CH_3Cl (80% ^{13}C, 89% incorporation) above natural abundance. Infrared analysis was performed on materials resulting from studies using ordinary methane; these data (2137 w, 2096 s, 2040 br s cm^{-1}) showed the presence of dihydride species **3**. Broadened absorption centered at 2040 cm^{-1} suggested that this species could be contaminated with another hydride species, here, likely a methylrhodium hydride complex (for [Si]ORh(H)Bu, ν_{Rh-H} = 2010 cm^{-1}). Indeed when this material was treated with chlorine, methyl chloride (approximately 0.20 equiv based on starting Rh) was obtained. These observations can be explained by the sequences shown in Scheme II.

The stoichiometric reactions shown in Scheme II suggest the possibility for a cycle for methane chlorination catalyzed by **4**, or in which **2** or **3** are converted to **4** by Cl_2. Indeed, we find this to be the case: reaction between methane and Cl_2 catalyzed by **2**, **3**, or **4** yields HCl and chloromethanes; CH_3Cl predominates. For example, methane (1.2 mmol) and Cl_2 (4 mmol) were placed in a flask containing 100 mg of **4** (0.024 mmol Rh). The contents were heated to 100 °C for 11 h in the dark and the following product distribution was obtained: CH_4 86.3%, CH_3Cl 12.6%, CH_2Cl_2 1.1%, $CHCl_3$ trace, CCl_4 trace. Under comparable conditions, complex **2** (0.024 mmol of Rh) gave after 14 h, CH_4 88.1%, CH_3Cl 9.8%, CH_2Cl_2 1.4%, $CHCl_3$ 0.7%, and CCl_4 trace, and complex **3** (0.024 mmol of Rh) gave, after 12 h, CH_4 85.5%, CH_3Cl 12.6%, CH_2Cl_2 1.1%, $CHCl_3$ 0.8%, and CCl_4 3.3%. In all cases HCl was formed but was not quantified, **4** was the only Rh product detected by infrared analysis, and by use of each of the catalysts approximate rates for C–H to C–Cl conversion were 0.4–0.6 turnovers per h. With complex **2** trichloropropane was also obtained. Neither thermolyzed **2** (at 400 °C for 4 h under 1 atm H_2; no observable ν_{Rh-H}) nor conventionally prepared Rh/SiO_2 (from $RhCl_3 \cdot 3H_2O$(aq)) show activity for methane chlorination.

Although its mechanism has not yet been proven, we believe that the sequence for metal-catalyzed chlorination parallels those steps noted for the stoichiometric cases described above (Scheme III) and derives from the electrophilic nature of the metal systems involved:[6,7] according to this hypothesis, as for the stoichiometric

(3) Ward, M. D.; Schwartz, J. *J. Mol. Catal.* **1981**, *11*, 397.
(4) (a) Ward, M. D.; Schwartz, J. *Organometallics* **1982**, *1*, 1030. (b) Kitajima, N.; Schwartz, J. *J. Mol. Catal.*, in press.
(5) (a) The reaction between silica-supported (allyl)rhodium species and H_2 has been reported[5b] to give silica-supported metallic rhodium. In striking contrast to **2** (which has a strong IR absorption at 2010 cm^{-1} (ν_{Rh-H})), this other material[5b] shows no IR absorption in the region 2000–2050 cm^{-1} in the absence of excess H_2; therefore this material[5b] is fundamentally different from **2**, and conclusions of that study[5b] are not germane to a discussion of the chemistry of **2**. (b) Foley, H. C.; DeCanio, S. J.; Tau, K. D.; Chao, K. J.; Onuferko, J. H.; Dybowski, C.; Gates, B. C. *J. Am. Chem. Soc.* **1983**, *105*, 3074.

reactions shown in Scheme II, coordinatively unsaturated Rh(III) electrophilically attacks a C-H bond and competitive migration of either proton or the alkyl fragment to a ligand in the coordination sphere of the metal generates the mixture of products observed (see Scheme III).[8,10] Elucidation of this catalytic alkane activation is now in progress.

Acknowledgment. We acknowledge support for this work given by the National Science Foundation

(6) Conversion of alkanes to alkyl chlorides has been accomplished using electrophilic Pt(II) as a catalyst and Pt(IV) salts as reoxidants. Gol'dschleyer, N. F.; Eskova, V. V.; Shilov, A. E.; Shteinman, A. A. *Russ. J. Phys. Chem. (Engl. Transl.)* **1972**, *46*, 785.

(7) Several iridium complexes have been proposed to activate alkanes by an electrophilic mechanism: Crabtree, R. H.; Mellea, M. I.; Mihelcic, J. M.; Quirk, J. M. *J. Am. Chem. Soc.* **1982**, *104*, 107.

(8) Relative rates for chlorination of CH_4, CH_3Cl, CH_2Cl_2, and CH_3Cl were determined to be 1.0, 1.6, 0.5, 0.4. For free-radical chlorination, these relative rates are reported[9] as 1.0, 1.8, 1.3, 0.6. These relative rate observations, however, cannot conclusively prove a non-free-radical pathway.

(9) Goldfinger, P.; Huybrechts, G.; Martens, G. *Trans. Faraday Soc.* **1961**, *57*, 2210.

(10) Stoichiometric incorporation of hydrocarbons into an organolutetium complex has recently been noted and may proceed according to a pathway such as the one shown in Scheme I: Watson, P. L. *J. Am. Chem. Soc.* **1983**, *105*, 6491.

(11) These frequencies were measured by FT/IR and are slightly different from those obtained[3] by using conventional IR techniques for the same materials.

Acyl-Enzyme Exchange Detection by Intermolecular Oxygen Scrambling: An Application of the ^{18}O-Isotope Effect in ^{13}C NMR

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We report herein the use of ^{18}O-induced shifts on ^{13}C NMR signals[3] as a general method for detecting enzymic acyl-exchange processes by intermolecular oxygen scrambling. A key feature of this technique is the preparation of substrate that is doubly labeled in the carboxylate group involved in the putative exchange mechanism: ^{13}C enrichment to increase sensitivity and ^{18}O monolabeling to detect the scrambling phenomenon.

Bacterial citrate lyase (citrate oxaloacetate-lyase; EC 4.1.3.6) from *Enterobacter aerogenes* provides an ideal illustration. The "resting" enzyme has been shown to be a covalent acetyl-enzyme thioester via the sulfhydryl of the 4-phosphopantetheine cofactor bound to the enzyme.[4] The catalytic process may be described in two steps (Scheme I), an acyl exchange yielding free acetate and a citryl-enzyme complex and a Claisen cleavage releasing oxaloacetate with concomitant regeneration of the acetyl-enzyme.[5] The acetyl group has been shown to originate from the *pro-S* carboxymethylene "arm" of citrate.[6] Evidence for this mechanism has rested on the detection and regeneration of the acetyl-enzyme, approaches that require reasonable amounts of pure enzyme and the fortuitous reactivation of nonfunctional enzyme by acetic anhydride.[7] While the existence of the acetyl-enzyme strongly implies acyl exchange, little has been accomplished toward the direct observation of this process independent of intermediate detection.[8]

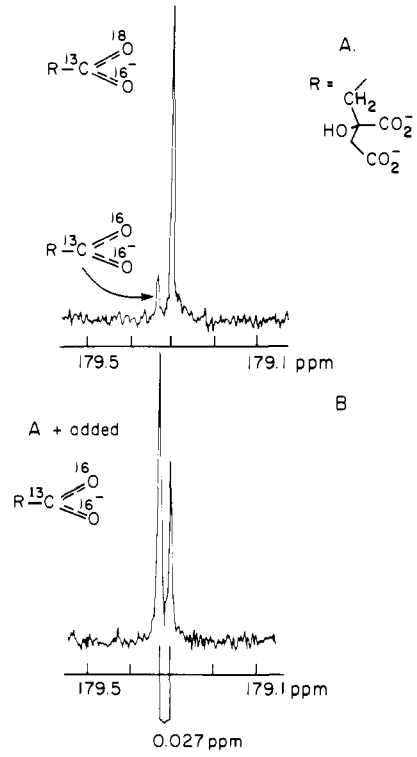
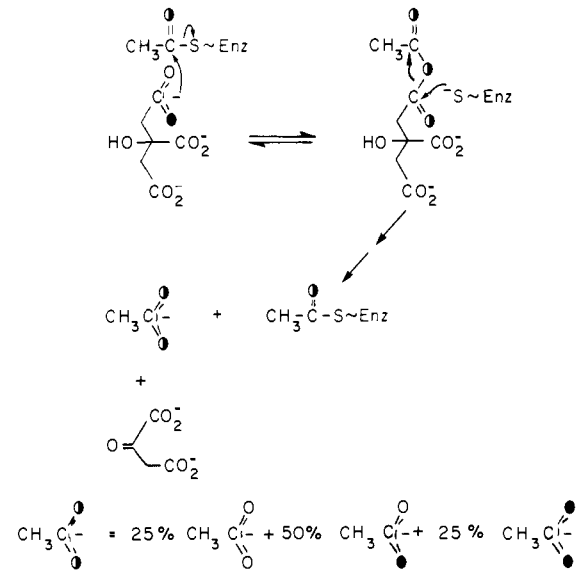


Figure 1. 125.8-MHz ^{13}C NMR spectra of [5-^{13}C,^{18}O]-(3*S*)-citrate (20 mM): (A) prepared by citrate synthase as discussed in text, (B) with added [5-^{13}C]-(3*S*)-citrate. Spectra were obtained on a Bruker WM 500 spectrometer by using broad-band proton decoupling. Chemical shifts are referenced relative to tetramethylsilane. Samples required Chelex chromatography and the presence of EDTA (40 mM) to throughly remove citrate-metal complexes.

Scheme I



The basis of our analysis is outlined in Scheme I. If citrate labeled with a single ^{18}O in the *pro-S* carboxylate is acted upon by the lyase, the acetyl-enzyme generated after one turnover has

(1) National Research Service Postdoctoral Awardee (1983-1985).

(2) American Cancer Society Faculty Research Awardee (1983-1988).

(3) (a) Risley, J. M.; Van Etten, R. L. *J. Am. Chem. Soc.* **1979**, *101*, 252. (b) Vederas, J. C. *Ibid.* **1980**, *102*, 374. (c) Risley, J. M.; Van Etten, R. L. *Ibid.* **1980**, *102*, 4609; (d) *Ibid.* **1980**, *102*, 6699.

(4) Srere, P. A. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1975**, *43*, 57 and references cited therein.

(5) Buckel, W.; Buschmeier, V.; Eggerer, H. *Hoppe-Seyler's Z. Physiol. Chem.* **1971**, *352*, 1195.

(6) Wheat, R. W.; Ajl. S. L. *J. Biol. Chem.* **1955**, *217*, 909.

(7) Buckel, W.; Buschmeier, V.; Eggerer, H. *Hoppe-Seyler's S. Physiol. Chem.* **1969**, *350*, 1367.

(8) A mass spectral approach to this problem utilizing a mixture of individually ^{13}C- and ^{18}O-labeled substrate has been briefly described for citramalate lyase from *Clostridium tetanomorphum* (Martinoni, B.; Arigoni, D. *Chimia* **1975**, *29*, 26).

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