lowed to stand in a capped amber vial for one week at room temperature. The resulting semisolid was dissolved in methanol, applied to a preparative silica gel plate (1000 μm, PreAdsorbant Uniplate, Analtech), and the plate was developed with EtOAc. The band which corresponded to that of coeluted authentic *N*-acetyl-*N'*-cyanoguanidine (**5b**) prepared above was scraped off and extracted with THF. The pale yellow liquid obtained by evaporation of the solvent in vacuo was further purified by additional preparative TLC using CH_2Cl_2/EtOH (15:1): TLC R_f = 0.49, 0.47, and 0.72 in EtOAc/hexane/AcOH (200:100:1), CH_2Cl_2/EtOH (15:1), and EtOAc, respectively; the product was UV quenching and gave a pale pink color with FCNP spray; IR (neat, cm^{-1}) 2180 (C≡N), 1720 (C=O), 1640 and 1570 (C=N); HPLC (t_R = 7.0 min), eluent MeOH/AcOH/10 mM aqueous ammonium acetate (60:20:920); FAB-MS (glycerol) (MH^+) calcd 127, found 127.

Colorimetric Assay for Cyanamide (1). All experiments were conducted in triplicate except as otherwise noted. A standard stock solution (10 mM) of **1** was prepared either from **1** or from sodium cyanamide. A standard curve was prepared as follows: 10 μL of the stock solution was mixed with 100 μL of FCNP spray solution[3] [10% NaOH/10% sodium nitroprusside/10% potassium ferricyanide/distilled water (1:1:1:3)] and 890 μL of distilled water. Serial dilutions were made and after 30 min their maximum absorption at 535 nm was measured against the reagent blank. The lower limit of sensitivity was about 1 μg, which is essentially the same as that of a procedure[9] using pentacyanoamine ferroate. *N*-Acetylcyanamide (**2b**), urea, cyanoguanidine, or melamine did not give any detectable absorbance at this wavelength when present in concentrations equal to that of **1**.

Typically, stock solutions of **2b** were made by dissolving 40 mg of **2b** in 50 mL of THF. Twenty-five sealed test tubes, each containing 2 mL of stock solution, were allowed to stand at room temperature for up to 1 week. At varying time intervals, the presence of cyanamide was measured colorimetrically: 100 μL of the test solution was mixed with 100 μL of FCNP solution and 800 μL of distilled water, and the absorbance was measured at 535 nm. There were no detectable levels of cyanamide observed at any time. Cyanamide was also not detected by TLC analysis using the FCNP reagent.

Acknowledgment. We thank Susie Kim and Soyoun Han (St. John's University) for technical assistance with the colorimetric assay.

Registry No. **1**, 420-04-2; **2a**, 86554-53-2; **2b**, 5634-51-5; **3a**, 19245-33-1; **3b**, 126297-12-9; **5a**, 126297-13-0; **5b**, 63071-29-4; **6**, 15150-25-1; **8**, 965-04-8; (benzyloxy)carbonylchloride, 501-53-1; cyanoguanidine, 461-58-5; sodium cyanamide, 19981-17-0.

(9) Buyske, D. A.; Downing, V. *Anal. Chem.* **1960**, *32*, 1798.

Hydrolysis of Adenosine Triphosphate by Conventional or Microwave Heating

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Sun et al.[1] recently reported that the hydrolysis rate of adenosine-5'-triphosphate (ATP) was 25 times greater during microwave heating than during conventional heating at comparable temperatures (100–105 °C). This remarkable rate increase was both attributed to and cited as evidence for a nonthermal microwave effect. Attempts to replicate those findings, however, lead to the conclusion that the hydrolysis rate is instead related solely to temperature. There is no need to postulate a nonthermal microwave effect. Accurate temperature measurement within the microwave-heated sample has been found to be crucial and not trivial.

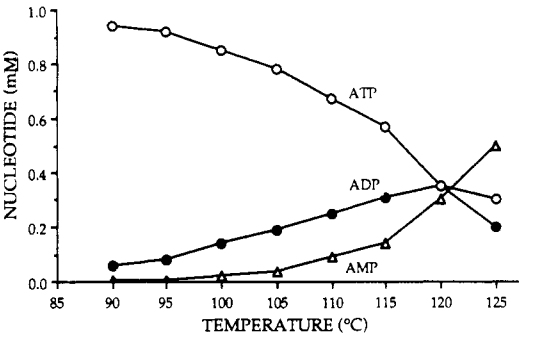

Figure 1. HPLC-determined nucleotide concentrations following 10-min conventional heating.

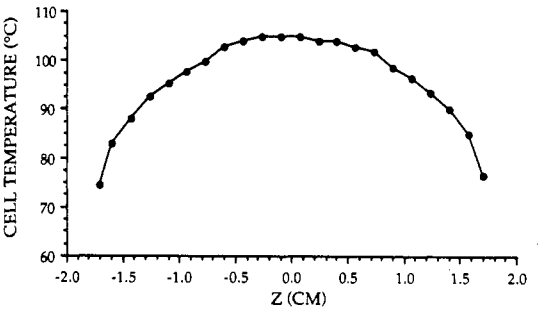

Figure 2. Sample temperature during microwave heating as a function of axial distance from middle of exposed sample.

In the present paper, ATP hydrolysis reaction kinetics determined from conventionally heated samples have been used in conjunction with temperatures measured in microwave-heated samples to calculate predicted final concentrations of ATP in the microwave-heated samples. These calculations, based only on temperature, accurately predicted the measured ATP concentrations in microwave-heated samples.

Conventional reaction kinetics were determined by exposing a series of samples for 10 min to fixed oil bath temperatures between 90 and 125 °C at 5 °C intervals. As the temperature increased, a regular decrease in ATP was seen along with a gradual increase in adenosine-5'-diphosphate (ADP) with an eventual plateau and dropoff, and a steady increase of adenosine-5'-monophosphate (AMP) concentration (Figure 1). Attention has been mainly confined to the decay of ATP to ADP by first-order reaction kinetics.

Using the oil bath data, the ATP hydrolysis rate k_1 was found to follow the Arrhenius law with regard to temperature T:

$$k_1(T) = A \exp\left(-\frac{E_a}{RT}\right)$$

with

$$E_a = 107.774 \times 10^3 \text{ J/mol}$$

$$A = 3.18217 \times 10^{11} \text{ s}^{-1}$$

$$R = 8.314 \text{ J/(K mol)}$$

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(1) Sun, W.-C.; Guy, P. M.; Jahngen, J. H.; Rossomondo, E. F.; Jahngen, E. G. E. *J. Org. Chem.* **1988**, *53*, 4414-4416.

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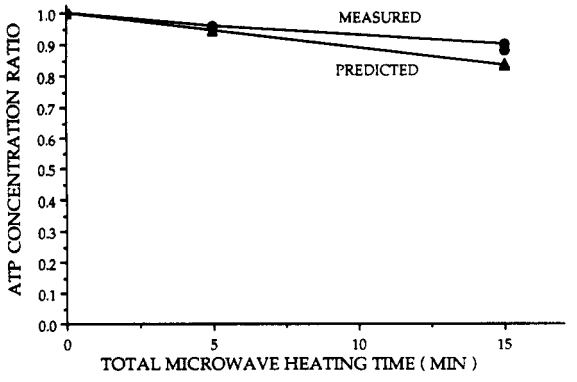

Figure 3. Measured and predicted ATP concentration ratios after microwave heating at 105 °C. The measured values were determined by HPLC; the predicted values were computed from eq 2.

The temperature of the sample during microwave heating was both more difficult to determine and more difficult to account for mathematically than was the temperature of the conventionally heated sample. The microwave-heated samples experienced a significant thermal gradient along the sample tube axis (Figure 2). This gradient must be accounted for in the mathematical analysis of the microwave heating process.

To predict the final concentration in the microwave-heated samples, the sample of length L was axially partitioned into a stack of differential volume elements. The temperature, T, within the sample depends on time, t, and axial position, z, measured from the center of the waveguide. The final ATP concentration within each volume element was computed by integrating the first-order reaction rate equation governing conventional ATP hydrolysis over the measured time–temperature profile of that element. The final ATP concentration within the sample was then computed by averaging over the ATP concentrations in all of the differential volume elements. The ratio of the final ATP concentration after t seconds of microwave heating to the initial concentration was thus found to be

$$\frac{[\mathrm{ATP}]}{[\mathrm{ATP}]_0} = \frac{1}{L}\int_{-L/2}^{L/2} \exp\left[-A\int_0^t \exp\left[-\frac{E_a}{RT(t,z)}\right]dt\right]dz \quad (2)$$

Using temperature data recorded during microwave exposure, the integrations in eq 2 were carried out numerically for samples that were heated to 105 °C. The predicted and measured final ATP concentrations are shown in Figure 3. The data at 15 min are replicates. The computed and measured ATP concentration ratios differ by 3% after 5 min of heating and by 8% after 15 min of heating. Conventionally determined kinetics accurately predicted microwave heating results.

Numerical studies of possible error sources revealed that the observed errors may have been caused by variation in sample length, errors in temperature measurement, and in the HPLC analysis of the samples. After 15 min of microwave heating of the sample, the measured concentration ratio would have increased by 0.55% had the sample been 1 mm shorter than the nominal 3.65 cm while a 2 °C increase in sample temperature over the nominal 105 °C would have resulted in a 3.4% decrease in the measured concentration ratio.

Although the reaction kinetics for the ADP and AMP hydrolysis products were not analyzed, the measured results shown in Table I for microwave-heated samples were comparable to those found in conventional heating in that the ADP appeared in increasing concentration with time and the AMP concentration increased just slightly over the time period examined.

Table I. Measured ATP Hydrolysis Products after Microwave Heating at 105 °C

	concentration, mM		
microwave exposure	AMP	ADP	ATP
5 min	0.01	0.03	0.96
0 min, control I	0.01	0.01	0.98
15 min	0.01	0.09	0.90
15 min	0.02	0.10	0.88
0 min, control II	0.00	0.01	0.99

An additional microwave trial was made for a sample heated to 100 °C for 10 min. The measured ATP concentration ratio was 0.91 while the calculated ratio was 0.92. Again, conventional kinetics accurately predicted the result of microwave heating.

The data analysis program was rerun with A equal to 7.526×10^{12}/s to mimic the special microwave ATP hydrolysis rate reported by Sun et al.[1] The activation energy, E_a, was maintained at the value given in eq 1. For the 15-min microwave heating time, the calculated ATP concentration ratio was 0.085, about a factor of 10 lower than the 0.89 observed here. For the 5-min microwave heating time, the calculated ratio was 0.32 compared to the 0.96 observed in this work. The reported[1] special microwave hydrolysis rate for ATP would thus have been clearly observable.

Microwave heating of a sample in a stirred multimode cavity, i.e., a microwave oven, typically results in very uneven heating; see, for example, the infrared images in Lentz.[2] The heating pattern is strongly dependent upon the details of the oven construction as well as the shape, size, orientation, and dielectric properties of the sample. In an attempt to reconstruct the thermal profile of the samples used in Sun et al.,[1] an infrared image of a capillary tube heated in a microwave oven was made. The temperature rise was not symmetrical about the center of the sample, and a 25% variation in temperature rise occurred along the sample. The results in the previous report[1] may well have been influenced by erroneous temperature readings or by the assignment of one temperature to a sample which in fact had a strong temperature gradient.

In conclusion, no special microwave heating effects on the hydrolysis of ATP were observed. Microwave-heating results were correctly accounted for by conventional reaction kinetics determined from oil bath heated samples when accurate temperature data were used for the microwave-heated samples. This example underscores the delicacy and importance of accurate temperature measurements. Researchers interested in examining the differences between conventional and other methods of heating need to pay careful attention to thermometry and its ramifications in their experimental designs. In particular, microwave exposure in multimode cavities like consumer ovens is fraught with difficulties in the measurement of the temperature of the sample.

Experimental Section

Sample Preparation. Adenosine-5′-triphosphate disodium salt (ATP, Sigma Chemical Co., St. Louis, MO) and adenosine-5′-diphosphate sodium salt hydrate (ADP, Aldrich Chemical Co., Milwaukee, WI) were used as standards as received. ATP was approximately 99% pure by peak area percent, while ADP was found to contain 30% adenosine-5′-monophosphate (AMP) both by HPLC peak area percent at 254 nm and by comparison to pure

(2) Lentz, R. R. *J. Microwave Power* **1980**, *15*(2), 107–111.

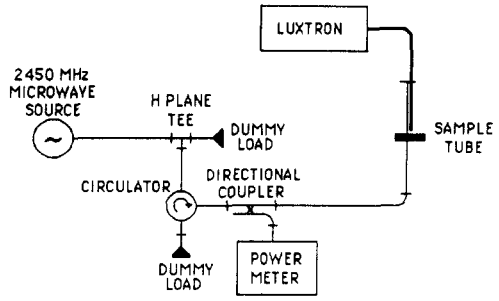

Figure 4. Schematic diagram of the microwave exposure system.

AMP (Aldrich). A single weighing of ADP was used to calculate both ADP and AMP concentrations in samples; 1 mM solutions of ATP in 0.01 M KH_2PO_4 (Mallinckrodt, Paris, KY), adjusted to pH 6.8 with base, were used as standards and for exposure to heat as samples. Millipore water was used to prepare the buffer, but no special precautions were taken to minimize cations such as Mg^{2+} or Mn^{2+}.

Samples were heated in borosilicate glass capillaries, cut 10-cm long from 3.9 mm o.d. (2.3 mm i.d.) glass and sealed at one end with a MAPP gas torch (Whirlwind Model PR-3600P, Cleanweld Turner, Sycamore, IL). Samples were sparged 15 min with helium and added to fill the tubes to 3.4–3.9 cm (0.14–0.16 mL). The open end was then sealed, and tubes were refrigerated before and after exposure to minimize the ambient hydrolysis.

Conventional Heating. Conventionally heated samples were immersed to the sample meniscus in a hot oil bath (Blue M Model MW-1445A-1, Blue M Electric Co., Blue Island, IL) equipped with a blade stirrer (type RZR1, Caframo, Wharton, Ontario). Temperature was maintained manually within ±0.5 °C and monitored with a Fluke 41 K/J thermometer and thermocouple (John Fluke Mfg. Co., Inc., Everett, WA). The temperature at the center of samples immersed in the oil bath rose to within 10 °C of the bath temperature in 15 s and to within 2 °C in 30 s.

Microwave Heating. Microwave heating was provided by an in-house 2450-MHz microwave generator constructed from the power supply taken from a Kenmore 566-8868510 microwave oven (Sears, Roebuck and Co., Chicago, IL). The microwave power output was made adjustable by the addition of a separate magnetron filament transformer (Model 17789, Basler Electric Co., Highland, IL) and a variable transformer (Powerstat type 3PNB6B, The Superior Electric Co., Bristol, CT) to the primary of the magnetron high voltage supply transformer. The magnetron to waveguide mount was fashioned after the mounting found in the oven. The balance of the sample exposure system shown in Figure 4 was assembled from the following standard WR-284 waveguide components: a GL401A circulator and a GL206 directional coupler (Gerling Labs, Modesto, CA) along with two WE0025 dummy loads and an H-plane tee junction (Micro-Lab/FXR, Livingston, NJ). The iris opening in a metal plate across the circulator arm of the H-plane tee junction was adjusted to give 100 W maximum incident power at the sample tube. The samples absorbed about 1 W while maintaining 100 °C steady-state center temperature.

Sample tube temperature was monitored with a Luxtron 750 thermometer with remote phosphor probe RPM-750 (Luxtron Corp, Mountain View, CA) as shown in Figure 5. To prevent false temperature readings caused by relative motion between the cable and the phosphor paint on the sample tube, the supple fiber optic cable of the remote phosphor probe was passed through a rigid glass tube (1.5 mm i.d., 2.8 mm o.d.) firmly clamped to the waveguide. A small amount of stopcock grease (Dow Corning Co., Midland, MI) was used as a vibration-dampening caulking between the cable and the end of the glass stabilizing tube inside the waveguide. Ambient light was subdued to prevent erroneous temperature readings with the remote phosphor probe.

The temperature of the outside wall of the sample tube at the center of the heated section of the sample was measured by the Luxtron thermometer as shown in Figure 5. Samples were heated to the target temperature within 60 s and maintained at the target temperature ± 3 °C by manual adjustment of the variable transformer. This center temperature was recorded at 1-s intervals by computer (IBM XT-PC, IBM, Valhalla, NY).

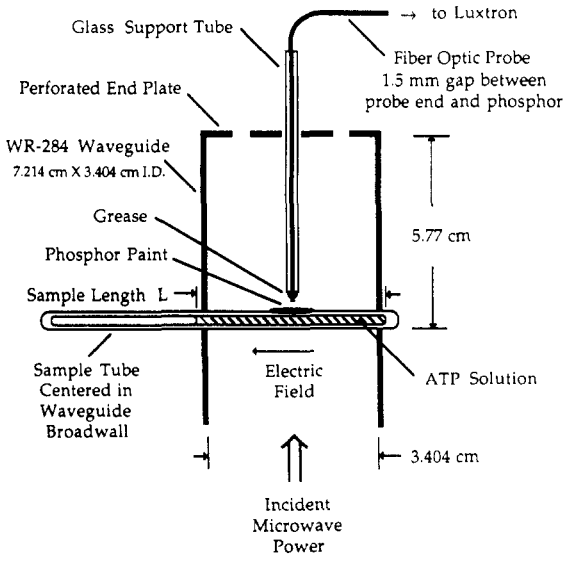

Figure 5. Detailed view of a sample tube positioned in the microwave exposure system of Figure 4.

Detailed electromagnetic field strength and power dissipation calculations coupled with heat transfer computations predicted a 1.6 °C drop between the center of the sample and the interior glass wall. A total temperature drop of 3.3 °C was predicted between the center and outside glass wall. The electromagnetic and heat-transfer calculations were made via the methods of Weeks[3] and Janna,[4] respectively. The complex relative dielectric constant of the ATP solution at 2.45 GHz, required for the electromagnetic calculations, was found to be $\epsilon_r = 50 - j6$ at 105 °C. A simple criterion for uniform electric field and hence uniform power dissipation within a microwave heated cylinder of radius a is given in Lewin.[5] The electrical radius of the liquid sample $|(2\pi a/\lambda_o)\sqrt{\epsilon_r}|$ should be as small as possible and certainly < 1. These experiments were conducted at 2.45 GHz so the free-space wavelength, denoted by λ_o, was 122.4 mm; hence, the electrical radius of the samples was 0.42. A second criterion for uniform internal temperature is given by Janna.[6] The Biot number should be < 0.1. For the samples used, the Biot number was 0.007. The samples met both the simple criteria. A measurement of the radial dependence of the temperature was made with an MIW-04 Luxtron probe placed inside an open but otherwise normally filled and positioned sample tube. The internal temperature was less than 4 °C warmer than the external surface temperature reported by the remote phosphor probe during the warm up period and less than 0.6 °C warmer during the hold period of sample exposure at 95 °C. Radial temperature variation was thus neglected in the data analysis.

The far more severe temperature gradient along the tube axis shown in Figure 2 was measured with a Thermovision 870 infrared imaging system (Agema Infrared Systems, Inc, Secaucus, NJ) and verified with the remote sensing phosphor probe. During sample exposure, the temperatures indicated by the Luxtron thermometer were verified by the infrared system. Sample temperature as a function of time and position, required for the numerical integration of eq 2, was determined from the measured center temperature and normalized temperature rise data derived from Figure 2. Simple rectangular rule numerical integration was used to evaluate eq 2. The z axis stepsize was 0.85 mm and the time stepsize was 1 s. Integration results were verified by using Romberg integration.[7]

(3) Weeks, W. L. *Electromagnetic Theory for Engineering Applications*; John Wiley & Sons: New York, 1964; pp 518–523.
(4) Janna, W. S. *Engineering Heat Transfer*; PWS Engineering: Boston, 1986.
(5) Lewin, L. *Theory of Waveguides*; Butterworth: London, 1975; p 122.
(6) Janna, W. S. *Engineering Heat Transfer*; PWS Engineering: Boston, 1986; pp 250–251.
(7) Gerald, C. F.; Wheatly, P. O. *Applied Numerical Analysis*; Addison-Wesley: Reading, MA, 1984; pp 250–252.

Nucleotide Analysis. Mobile phase consisted of a 90:10 mixture of 1 mM *tert*-butylammonium dihydrogen phosphate (Aldrich) in 0.065 M KH_2PO_4 + methanol (OmniSolv, EM Science, Gibbstown, NJ). Glass-distilled water (OmniSolv, EM Science) was used for mobile-phase buffer preparation. Instrumentation consisted of a Waters M6000 pump, WISP 710B autosampler, M-490 variable-wavelength detector at 254 nm, and 840 data handling systems (Waters Chromatography Division, Milford, MA) connected to a DEC LA50 printer (Digital Equipment Corp., Marlboro, MA). A 5 μm Spherisorb S5ODS2 column, 150 × 4.6 mm (Regis Chemical, Morton Grove, IL), was used in series with a PP-18 Spheri-10 guard column, 30 × 4.6 mm (Brownlee Labs, Santa Clara, CA). Mobile-phase flow rate was 1 mL/min, and 20 μL of standards and samples were injected. Peak heights were used for quantitation and all results were normalized to [ATP] + [ADP] + [AMP] = 1 mM.

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Selective Deuteriation of Arylalkanes with Mixed Metal Reagents

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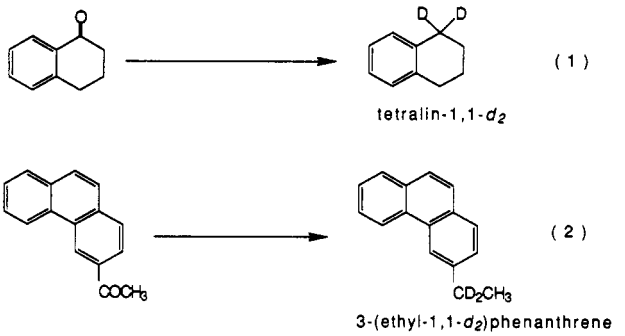
There are several procedures for introducing deuterium atoms into the benzylic positions of arylalkanes.[1-6] The most common procedures are base- and palladium-catalyzed deuterium–hydrogen exchange,[2,3] Clemmensen and palladium-catalyzed reduction of aromatic carbonyl compounds,[4,5] and lithium aluminum deuteride reduction of carbonyl groups to alcohols, tosylation of the alcohols, and the reduction of the tosylates to the hydrocarbons.[6] The preparative procedures have certain shortcomings. The base- and palladium-catalyzed exchange reactions, though selective, are rather slow and often require repetition of the reactions to attain a high degree of deuterium atom incorporation.[2,3] The Clemmensen reduction of carbonyl compounds is nonselective.[4,5] The reduction of a carbonyl compound to an alcohol, tosylation, and reduction produces both alkanes and alkenes.[6] In this paper, we report another facile, selective deuteriation approach that is based upon a mixed metal reagent. These reagents react rapidly and produce highly enriched isotopically pure products.

During the synthesis of some deuterium labeled tetrahydronaphthalene derivatives, it was observed that the use of aluminum chloride and lithium aluminum hydride at various stages of the synthesis had no effect on the deuterium atom content in the starting hydrocarbons. This observation and the fact that Brown and co-workers and Nystrom and Berger had used a mixture of aluminum chloride and lithium aluminum hydride to reduce diaryl ketones and aryl alkyl ketones[7-9] to the corresponding hydrocarbons, prompted us to adopt their method to introduce deuterium selectively in the benzylic positions in arylalkanes as illustrated in eqs 1 and 2.



In their study, Nystrom and Berger observed that the ease and extent of reduction of the carbonyl group depended on the order of mixing of reactants and on the nature of the substituent present in the organic compound.[9] They established that optimal results were usually obtained when the mixture of the ketone and aluminum chloride in ether was added to a solution of equimolar quantities of aluminum chloride and lithium aluminum hydride in ether,[9] and the reaction mixture was stirred under dinitrogen at 35 °C.

The reactions of several ketones were carried out in accord with the recommendations of Nystrom and Berger.[9] The crude products were easily isolated by vacuum distillation or flash column chromatography. Although Nystrom and Berger did not report the formation of alkenes, we found that their method gave 6–17% alkene. Accordingly, we obtained the pure labeled alkanes by careful column chromatography. The experimental results for several representative ketones are reported in Table I.

The mixed metal reduction technique selectively introduced two atoms of deuterium in place of the oxygen atom in 35–45 min. The products were characterized by ^{1}H NMR, ^{2}H NMR, IR, and GC–MS, and the product distributions were calculated from gas chromatographic data. The deuterium content exceeded 96% of the expected value in every case with the monodeuterium derivative as the principal contaminant. The possibility of deuterium incorporation into the carbon adjacent to the original carbonyl group and into the aromatic position was investigated by IR and ^{2}H NMR spectroscopies. Both techniques showed that the arylalkanes were deuteriated selectively in the benzylic position. In each case, the IR spectrum revealed a new intense band at 2100–2200 cm^{-1} due to C_{Al-D} stretch. ^{2}H NMR spectroscopy revealed that the position adjacent to the former keto group was free of deuterium atoms, but that the aromatic positions contained about 2% deuterium atoms. The Clemmensen reduction, unlike the mixed metal and the palladium-catalyzed reductions, incorporates deuterium at the original carbonyl carbon, in the aromatic position, and in the adjacent carbon atom. These side reactions are minimized in the mixed metal procedure by the short reaction times.

A more striking disparity was observed between the reaction of 1-tetralone in the presence of palladium catalyst under dideuterium gas and in the presence of mixed metal deuteride. The former gave tetralin-*1,1,4,4*-d_4 and the latter gave tetralin-*1,1*-d_2, as shown in Figure 1A. The formation of the tetralin-*1,1,4,4*-d_4 in the palladium-cata-

(1) Thomas, A. F. *Deuterium Labeling in Organic Chemistry*; Appleton Century Crofts: New York, 1971; p 283.
(2) (a) Chan, T. S.; Wolinska Mocydlarz, J.; Leitch, L. C. *J. Labeled Compd.* **1970**, VI(3), 284.
(3) Ofosu Asante, K.; Stock, L. M. *J. Org. Chem.* **1986**, *51*, 5452.
(4) (a) Enzell, C. R. *Arkiv. Kemi* **1966**, *26*, 87. (b) Enzell, C. R. *Tetrahedron Lett.* **1966**, 1285.
(5) Ofosu Asante, K.; Stock, L. M. *J. Org. Chem.* **1987**, *52*, 2938.
(6) Djerassi, C.; Shapiro, R. H.; Vandewalle, M. *J. Am. Chem. Soc.* **1965**, *87*, 4892.
(7) Broodme, J.; Brown, B. R. *Chem. Industry* **1956**, 1307.
(8) Brown, B. R.; White, A. M. S. *J. Chem. Soc.* **1957**, 3755.
(9) Nystrom, R. F.; Berger, C. R. A. *J. Am. Chem. Soc.* **1958**, *80*, 2896.

0022-3263/90/1955-3409$02.50/0