for **4c** and C-1 for **4d**), it is clear from the cyanamide resonance that the cyanamide carbon is specifically enriched (2.7%) by **6c**, comparable to enrichments of the relevant ring carbons.

If the cyanamide carbon were biosynthetically added to a preformed benz[*b*]carbazole, any of the known independent pathways of acetate metabolism would have led through one of the compounds already tested. In view of the relatively uniform levels of enrichment from **6c**, we propose that the cyanamide carbon is derived from C-5 of **5** via oxidation, nitrogen insertion, and ring contraction/rearrangement as shown in Scheme III.[16] Additional, albeit circumstantial, support is provided by two additional benz[*b*]carbazole metabolites of *S. murayamaensis* that contain the cyanamide functionality; one of these is **9**.[17] No benz[*b*]carbazole metabolites have been found that lack the cyanamide unit. Efforts to synthesize **5** specifically labeled with ^{13}C at C-5 in order to test this hypothesis are in progress.

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(16) In order to test the proposed ring contraction and the intermediacy of **9**, we have attempted to prepare **5** and **9** sufficiently biosynthetically enriched in ^{13}C for subsequent feeding to *S. murayamaensis*. While we have obtained as much as 10% enrichment this has been insufficient—in part due to the insolubility of these compounds—to yield reliable enrichment in derived kinamycin D even with the known intermediate **5**.[6]

(17) These will be reported in a separate communication.

Crossed-Beam Study of HX Elimination Reactions

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Most studies of organic reactions are carried out in solution, where the role of the solvent is often complicated and very difficult to elucidate. Recently, there have been an increasing number of studies carried out in the gas phase or in beams where the states of the reactants and products can be better controlled and characterized. Studies of gas-phase acidities and basicities using ICR[1] have shown large deviations from the aqueous values. We have previously used crossed supersonic nozzle beams to study several aspects of three types of organic chemiionization reactions.[2]

We report here a preliminary study of the reactions of a strong organic base with an alkyl halide. The base abstracts a proton β to the halogen, and the halide negative ion is eliminated to give the protonated amine, the halide ion, and an olefin. In the present study we used all four isomers of butyl iodide reacting with tetrakis(dimethylamino)ethylene (TDMAE) $[(CH_3)_2N]_2C{=}C[N(CH_3)_2]_2$. Previous papers[2] have given detailed descriptions of the apparatus, the techniques of beam formation, and the data analysis. Briefly, each reactant beam is prepared by bubbling H_2 carrier gas through the liquid. The bubbler temperature determines the vapor pressure of the reagent in the beam. The mixture is passed through a Pyrex nozzle into the main vacuum chamber. The product ions are extracted by a grid, mass analyzed by a quadrupole mass analyzer, and detected on a continuous dynode electron multiplier. Each nozzle can be heated by passing a current through a coil of wire surrounding it. The kinetic energy of the beam can be calculated by using well-known formulas[3] and is varied by changing the temperature of the TDMAE nozzle. The temperature of the butyl iodide beam is kept at 26 °C to prevent decomposition.

Figure 1 shows the reactive cross section versus relative translational energy for the reaction of all four butyl iodides with TDMAE. The only ions that we observe are I^- and the protonated TDMAE. We cannot see the neutral product, but it would almost have to be some isomer of butene. The reactions of the four isomers have similar thresholds of 7.9 ± 1.0 eV. The cross sections were scaled in magnitude by a single multiplying factor but otherwise have a similar energy dependence. This is not surprising since the thermodynamic ΔH for each reaction is roughly the same. The heats of formation of the four butyl iodides and of the four butenes are close to each other. We cannot obtain the thermodynamic ΔH because the absolute proton affinity of TDMAE has never been measured. We have tentative evidence[4] that it is higher than the proton affinity of tri-*n*-butylamine. If this is so, then the thermodynamic threshold is less than 4.3 eV. The difference in threshold energies can be explained in several ways. There can be a barrier in the potential energy surface. The reaction may go at the thermodynamic threshold but requires that much of the energy be put into vibrational energy, or our extrapolation to obtain the threshold is in error. For example, the cross section might actually have a slowly rising exponential dependence at the threshold rather than the linear dependence which we assume.

One obvious difference between the four isomers is the number of β hydrogens. The tert isomer has nine, the sec five, the normal two, and the iso only one. While one would not expect that the cross section would be exactly proportional to the number of hydrogens, one would expect to see a trend, and this is exactly what is observed. The scaling factor for the cross sections plotted in the figure are *tert*-butyl 1.00, *sec*-butyl 0.44, *n*-butyl 0.31, and isobutyl 0.31. The relative cross section is given by the ordinate of the figure times the scaling factor. All four systems were run back to back while attempting to hold the experimental conditions constant. However, there is inevitably some drift in beam intensity and detector sensitivity. We estimate an error of 20% in the ratios.

Finally, we must exclude other possible processes which could give the same products. It is well-known that *tert*-butyl iodide decomposes at high temperatures to give isobutene and HI.[5] HI can then react with TDMAE to give the protonated amine and I^-. Indeed, we can clearly see this happening. As the temperature of the nozzle for butyl iodide is raised above 210 °C, the product intensity rises dramatically, the threshold energy drops from 7.9 to 3.9 eV, and we see cations produced by the elimination of $(CH_3)_2NH$ from the protonated amine. Evidently, the reaction of HI with TDMAE has a larger cross section than the reaction of butyl iodide, it has a lower threshold, and it produces a cation with appreciable vibrational energy. The behavior at high temperature is so different from that at low temperature that two very different reactions must be taking place. Furthermore, the other isomers of butyl iodide are very much more stable, yet they react readily with TDMAE at low temperatures.

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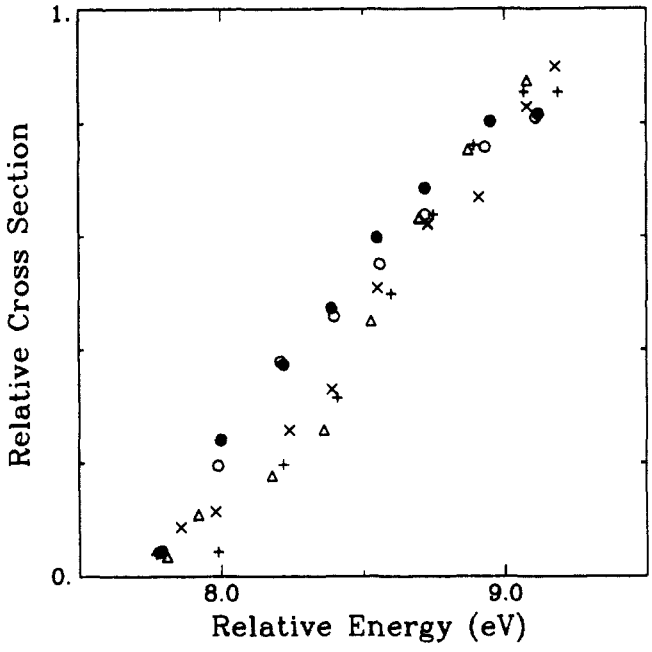

Figure 1. The relative cross section for butyl iodide reacting with tetrakis(dimethylamino)ethylene is plotted against the relative translational energy: (○) *tert*-butyl iodide, (△) *sec*-butyl iodide, (+) *n*-butyl iodide, (×) isobutyl iodide. The open and closed circles are two separate runs. The cross sections were scaled by the following factors: *tert*-butyl iodide, 1.00; *sec*-butyl iodide, 0.44; *n*-butyl iodide, 0.31; and isobutyl iodide, 0.31. The relative cross section is given by the ordinate of the figure times the scaling factor. The butyl iodide beam was held at 26 °C, while the temperature of the TDMAE beam was varied over a range of 40–300 °C to obtain the range of translational energies.

In summary, we have seen the reaction of alkyl iodides with TDMAE, a very strong organic base. The TDMAE abstracts a proton from the alkyl halide which then eliminates I^-. The reaction takes place in a molecular beam and therefore must occur on a single molecular collision. Beyond this, it is not clear what the time scale of the reaction is or whether the elimination occurs immediately during the collision or after the protonated amine has left the vicinity of the reaction. We have seen reaction with isopropyl iodide and with *tert*-butyl bromide with a smaller cross section, so the reaction appears to be more general than with butyl iodides.

The Necessity of an Intact Polyene for the Biological Isomerization of Vitamin A

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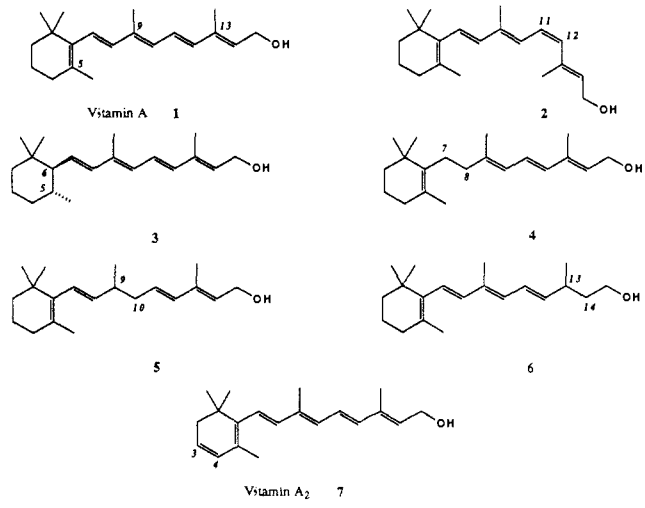
The vertebrate visual cycle is completed by an enzyme system which converts added *all-trans*-retinol (**1**) (vitamin A) into 11-*cis*-retinol.[1-3] The retinol dehydrogenase mediated oxidation of 11-*cis*-retinol to 11-*cis*-retinal provides the chromophore for rhodopsin.[4]

Chart I



The isomerization process is of interest both biologically and chemically—chemically because the isomerization is endothermic, with the product 11-*cis*-retinol being approximately 4.0 kcal/mol less stable than its all-trans congener.[5] Possible mechanisms for the energy requiring isomerization are shown in Scheme I. Here the isomerization event is coupled to the hydrolysis of a putative ester (OX), formed from retinol, which could provide the necessary energy to drive the thermodynamically uphill isomerization.[2] The recent observation that inversion of configuration of the prochiral methylene hydroxyl group of *all-trans*-retinol accompanies isomerization is consistent with the mechanisms shown in Scheme I.[6] Mechanisms of the type shown in Scheme I would predict an important role for the double bonds of the polyene backbone of the substrate in the isomerization process. In this communication, the specificity of the isomerase is probed with respect to dihydro and dehydro-retinol substrates in order to elucidate further the mechanism of action of the enzyme. The experiments show that the polyene system must remain intact in order for appreciable isomerization to occur.

The four dihydroretinols studied are shown below in Chart I. The 5,6-dihydro- (**3**), 7,8-dihydro- (**4**), and 9,10-dihydro-*all-trans*-retinols (**5**) were prepared by $NaBH_4$ reduction of previously reported *trans*-dihydroretinals.[7a] The corresponding 11-cis isomers were obtained similarly, from the 11-cis-dihydro series.[7b] The 13,14-dihydro-*all-trans*-retinol (**6**) was readily prepared by the Wittig reaction between [3-methyl-5-(2,6,6-trimethyl-1-cyclohexen-1-yl)-2,4-pentadienyl]triphenylphosphonium bromide and 2-methyl-4-[(*tert*-butyldimethylsilyl)oxy]butanal, followed by deprotection of the silyl ether with tetrabutylammonium fluoride. The 15-^{3}H-labeled dihydro and dehydro *all-trans*-retinols were prepared by the reduction of the corresponding aldehydes with sodium boro[^{3}H]hydride. The label in the 15-position of *all-trans*-retinol is not lost during its isomerization, when subjected to the membrane-bound amphibian or bovine pigment epithelium derived isomerase systems.[3] This membrane fraction also contains retinyl ester synthetase activity, and the latter appears in all membrane fractions containing the isomerase.[2] In addition, unwashed membranes also contain substantial retinol redox activity.[1]

When the retinoids discussed above were subjected to the amphibian isomerase containing membranes (washed and unwashed), they were readily enzymatically esterified to their corresponding palmitate esters, but none were substantially isomerized to their 11-cis congeners (Table I). Small amounts of the 11-*cis*-retinols

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