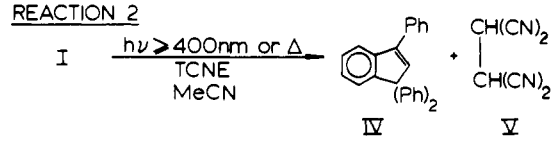
tron-transfer process involving the first excited singlets of both IIIa and IIIb as acceptors and I as donor should be favorable ($\Delta G = -8.87$ and -21.09 kcal mol^{-1}, respectively).

It seemed likely that irradiation, or thermal activation of the charge-transfer complex between I and TCNE in polar solvents, would also give products from the radical ions. We found that irradiation of the charge-transfer transition of the complex between I and TCNE in acetonitrile solution results in the formation of 1,3,3-triphenylindene[3] (IV) and dihydrotetracyanoethylene[3] (V) in essentially quantitative yield (reaction 2). These same products were also obtained upon heating solutions of I and TCNE. The rate of this thermal reaction is

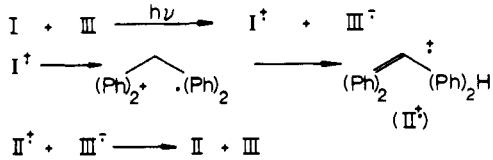
REACTION 2



remarkably dependent upon solvent polarity; reaction was complete after 24 h at 125 °C when acetonitrile was the solvent, but required several days at this temperature when benzene was used.

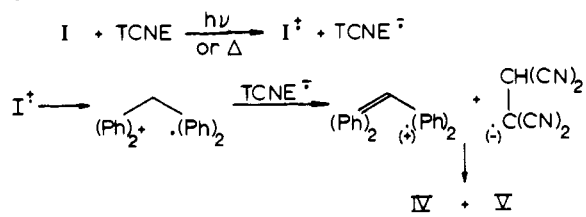
A possible mechanism for reaction 1 is shown in Scheme I. Irradiation of the sensitizer leads to the formation of the radical cation of I and the sensitizer radical anion. Cleavage of the radical cation of I, followed by proton (or hydrogen atom) migration, will give the radical cation of 1,1,3,3-tetraphenylpropene. Back electron transfer from the sensitizer radical

Scheme I



anion completes the sequence. Although reaction 2 presumably also involves the radical cation of I, the fate of this intermediate is different from that in reaction 1. This difference may be the result of a reluctance of the TCNE radical anion to back-transfer an electron to the ring-opened radical cation. It is possible that proton or hydrogen atom transfer to the TCNE radical anion from the intermediate ring-opened radical cation leads to the propenyl radical or carbonium ion, either of which could cyclize to eventually give the indene IV (Scheme II). The mechanistic possibilities are being investigated.

Scheme II



Some control experiments should be mentioned. The possibility that triplet–triplet energy transfer from IIIa ($E_T = 57.4$ kcal mol^{-1}) or IIIb ($E_T = 55.5$ kcal mol^{-1}) causes reaction 1 was ruled out by showing that I is essentially stable to irradiation using 4-methoxyacetophenone ($E_T = 71.7$ kcal mol^{-1}) or 2-acetylnaphthalene ($E_T = 59.3$ kcal mol^{-1}) as photosensitizers. Heating an acetonitrile solution of II and TCNE under conditions identical with those causing reaction 2 led to no reaction. Thus, the radical cation of II is not involved in the conversion of I to IV. The propene II does, however, give the indene IV and dihydro-TCNE at much higher temperatures (~180–190 °C). The acid-catalyzed rearrangement product of I,[8] 1,1,3-triphenylindan is likewise stable to TCNE under the conditions of reaction 2.

Martini and Kampmeier have reported the reaction of 1,1-diphenylcyclopropane (VI) with TCNE in benzene at 125 °C.[9] Product formation (1,1,2,2-tetracyano-3,3-diphenylcyclopentane and 1,1,2,2-tetracyano-5,5-diphenyl-4-pentene) was rationalized in terms of the addition of TCNE to VI with ring cleavage, to give the most stable 1,5 dipole, followed by closure or proton transfer. Our results point to the importance of considering electron transfer from VI to TCNE preceding the addition and ring opening. We found no evidence for the formation of an adduct between I and TCNE.

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References and Notes

(1) Contribution No. 193 from the Photochemistry Unit, Department of Chemistry, The University of Western Ontario.
(2) H. Kristinsson, K. N. Mehrotra, and G. W. Griffin, *Chem. Ind. (London)*, 1562 (1966).
(3) Positively identified by comparison of spectra (IR, NMR) with those of an authentic sample.
(4) A. Weller, *Nobel Symp. n5, Fast React. Primary Processes Chem. Kinet.*, 413 (1967).
(5) $E^{1/2}_{red\ IIIa} = 2.33$ V, $E^{1/2}_{red\ IIIb} = 1.67$ V, $E_{S(IIIa)} = 89.4$ kcal mol^{-1}, $E_{S(IIIb)} = 86.4$ kcal mol^{-1}, coulombic attraction term = 1.3 kcal mol^{-1}: D. R. Arnold and A. J. Maroulis, *J. Am. Chem. Soc.*, **98**, 5931 (1976).
(6) D. A. Labranca, G. N. Taylor, and G. S. Hammond, *J. Am. Chem. Soc.*, **94**, 3679 (1972).
(7) (a) R. Foster, "Organic Charge-Transfer Complexes", Academic Press, New York, 1969, Chapter 3 and references cited therein. (b) $E^{1/2}_{ox} = 0.095\Delta E_{CT} - 0.455$ V D. R. Arnold and P. C. Wong, *Can. J. Chem.*, in press.
(8) W. J. Bailey and R. A. Baylouny, *J. Org. Chem.*, **27**, 3476 (1962).
(9) Th. Martini and J. A. Kampmeier, *Angew. Chem., Int. Ed. Engl.*, **9**, 236 (1970).
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Deuterium Nuclear Magnetic Resonance Measurements of Glycoproteins Which Have Been Specifically Deuterated Either at Selected Carbohydrate or at Lysine Residues: Determination of Motional Correlation Times

Sir:

There is currently much interest[1] in the properties and biological functions of the carbohydrate residues of glycoproteins, including those of isolated substances (such as mucin and serum glycoproteins) as well as of intact cell-surface glycoconjugates. This interest has recently prompted us,[2] and others,[3] to develop methods for attaching stable nitroxide free radicals (spin labels[4]) to selected carbohydrates and amino acid residues of a number of glycoproteins. Concerned with the possibility[5] that such studies might be compromised by the substantial steric bulk of the spin label itself, we have evaluated alternative spectroscopic probes which do not suffer from that same limitation. In the present communication we demonstrate, for the first time in the glycoprotein area, that the combination of highly selective deuteration and ^{2}H NMR studies[6] using a pulse Fourier transform instrument operating at 61.4 MHz (B_0 9.4 T) can lead to potentially valuable motional information.

Deuterium labeling at C-7 of the sialic acid residues[7] of glycoproteins was easily achieved[8] (Scheme I) by using limited

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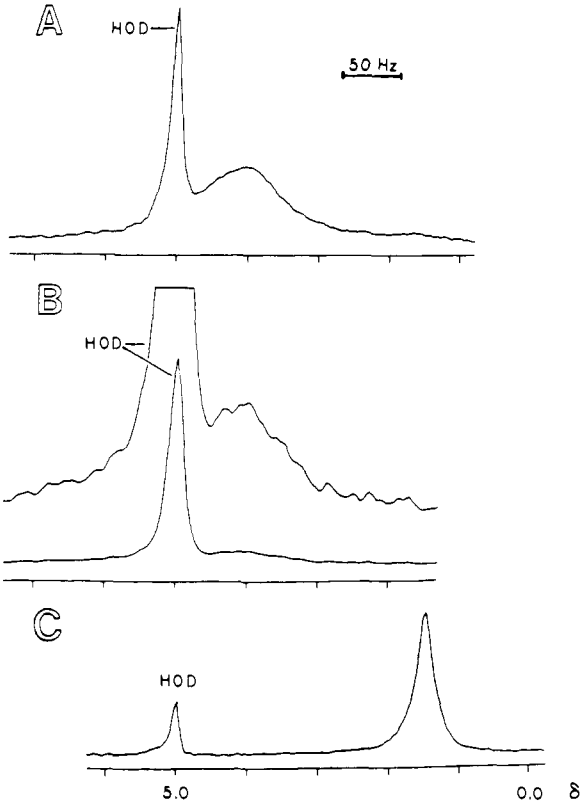
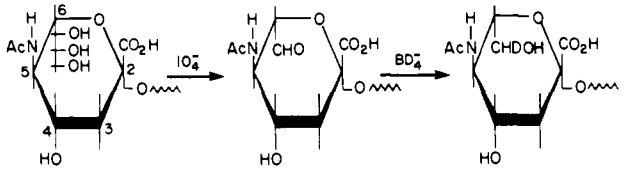


Figure 1. NMR spectra were measured using a Bruker WH-400 instrument at 61.4 MHz (B_o 9.4 T) using a fixed-tuned probehead for 10-mm-diameter NMR tubes and without field-frequency lock. Samples were dissolved in ~1.5 mL of distilled H_2O and the transients were sampled using a 25-μs pulse (acquisition time, 0.85 s). (A) BSM deuterated at the sialic acid residues (145 mg; 2700 transients). (B) Fetuin deuterated at terminal galactose positions (190 mg). (C) Fetuin reductively aminated with hexadeuterioacetone on lysine residues (240 mg; 550 transients). The HOD resonance was arbitrarily assigned a shift of 5 ppm.

Scheme I

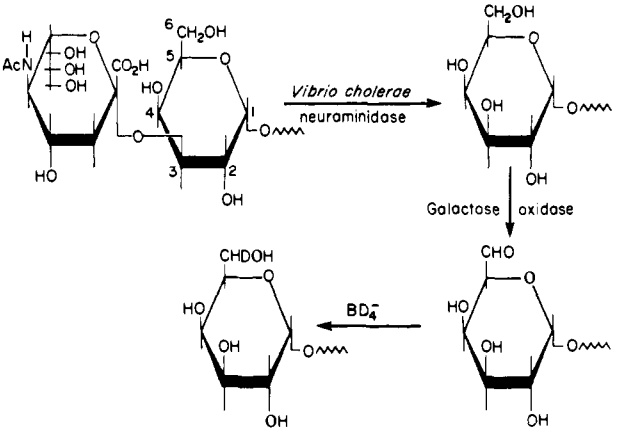


quantities of aqueous sodium metaperiodate to cleave selectively the C-7, C-8, C-9 triol moiety followed by reduction using sodium borodeuteride as the deuteration reagent. The ^2H NMR spectrum in Figure 1A shows the excellent sensitivity which can be obtained using a 61.4-MHz spectrometer; in this case, the substrate is bovine submaxillary mucin (BSM,[9] 145 mg, mol wt 1.3×10^6 dalton), which has potentially one deuterium substituent attached to each of the ~300 sialic acid residues per molecule.

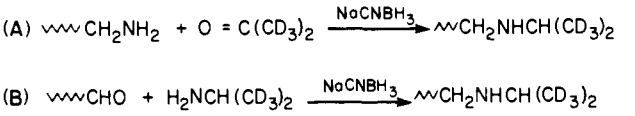
Selected monodeuteration at C-6 of the terminal galactose residues of suitable asialo glycoproteins was readily effected by a variant on the galactose oxidase procedure (Scheme II) previously used by Morell et al.[10] for the equivalent tritiation reaction, but with $NaBD_4$ as the reductant. The deuterium resonance (Figure 1B) for the asialofetuin[11] product (mol wt 4.8×10^4 dalton) shows essentially the same line width as that of the sialic acid labeled material. Terminal galactose units (along with fucose or mannose, if suitably substituted) may also be derivatized by periodate oxidation.[12]

In some cases it may be of interest to attach other classes and sizes of deuterium probes and this can be readily accomplished using the well known reductive amination[13] procedure, summarized in Scheme IIIA, for the attachment of a carbonyl-containing substituent to a lysine residue, and in IIIB for an amine-containing subsitutent. The former application is exemplified[14] by the attachment of hexadeuterioacetone to the lysine groups of fetuin (Figure 1C).

Scheme II



Scheme III



Although overlap from the resonance of the natural abundance of deuterium oxide in water was not a problem here, it could complicate studies of smaller quantities of glycoproteins; this problem could be minimized either by using one of the several pulse techniques well known for nulling water resonances[15] or by using deuterium depleted water (or by a combination of both methods).

An approximate estimate of the upper limit for the overall motional correlation times (effective τ_c) can be easily obtained[16] from the half-height line width of the deuterium resonances. Figure 1 affords the following effective τ_c values: A, $5.0 \pm 0.3 \times 10^{-10}$; B, $5.7 \pm 0.7 \times 10^{-10}$; C, $1.2 \pm 0.1 \times 10^{-10}$ s. The relative narrowness of the resonances of the labeled lysine residues is probably due to the well-known segmental motion common to this amino acid in proteins and peptide hormones.[17] The effective τ_c values for the labeled sugar indicate that there is also substantial segmental motion in the sugar chains. Without giving details here, it important to note that these data have the same relative ordering as the effective τ_c values derived by the spin-labeling method for analogously derivatized glycoproteins.[2]

Clearly the approaches to the selective labeling of sugar and amino acid residues of glycoproteins, summarized above, are flexible ones, with the wide variety of specifically or fully deuterated reagents being commercially (and inexpensively) available, further adding to the general potential of this approach. The reductive amination procedure can be used with Schemes I and II to incorporate either deuterium labels, or those of other "magnetic" nuclides such as ^{13}C. That the line widths of the deuterium resonances are as narrow as they are leaves open the possibility that substantial decreases in molecular motion can be sustained before the deuterium resonances become prohibitively broadened; this augurs well for studies of associative processes such as the binding of lectins[18] and of antibodies[19] to the carbohydrate moieties of glycoconjugates, both free and on cell surfaces.

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References and Notes

(1) V. Ginsburg, Ed., *Methods Enzymol.*, 50 (1978), and others in the series; N. Sharon, "Complex Carbohydrates—Their Chemistry, Biosynthesis and Functions", Addison-Wesley Reading, Mass., 1975; J. L. Fox, *Chem. Eng. News*, **56**, 25 (1978); R. C. Hughes and N. Sharon, *Nature (London)*, **247**, 637 (1978).
(2) J. D. Aplin, M. A. Bernstein, C. F. A. Culling, L. D. Hall, and P. E. Reid, *Carbohydr. Res.*, in press. J. D. Aplin, D. E. Brooks, C. F. A. Culling, L. D. Hall, and P. E. Reid, *ibid.*, in press.
(3) K. J. Willan, B. Golding, D. Givol, and R. A. Dwek, *FEBS Lett.*, **80**, 133 (1977); R. S. Nezlin, V. P. Timofeev, Yu. K. Sykulev, and S. F. Zurabyan, *Immunochem.*, **15**, 143 (1978); V. P. Timofeev, I. V. Dudich, Yu. K. Sykulev, and R. S. Nezlin, *FEBS Lett.*, **89**, 191 (1978).
(4) L. J. Berliner, Ed., "Spin Labelling, Theory and Applications", Academic Press, New York, 1976.
(5) Y. K. Levine, P. Parkington, G. C. K. Roberts, N. J. M. Birdsall, A. G. Lee, and J. C. Metcalfe, *FEBS Lett.*, **23**, 203 (1972).
(6) H. H. Mantsch, H. Saitō, and I. C. P. Smith, *Prog. Nuclear Magn. Reson. Spectrosc.*, **11**, 211 (1977), and references therein; for a specific example, see J. A. Glasel, V. J. Hruby, J. F. McKelvy, and A. F. Spatola, *J. Mol. Biol.*, **79**, 555 (1973).
(7) In the case of bovine submaxillary mucin, whose sialic acid residues are frequently O-acylated at the C-7, C-8, or C-9 positions, periodate oxidation was preceded by saponification.
(8) L. Van Lenten and G. Ashwell, *J. Biol. Chem.*, **246**, 1889 (1971).
(9) A. Gottschalk and A. S. Bhargava in "Glycoproteins. Their Composition, Structure and Function", Vol. 5B, A. Gottschalk, Ed., Elsevier, Amsterdam, 1972, p 810.
(10) A. G. Morell, C. J. A. Van Den Hamer, I. H. Scheinberg, and G. Ashwell, *J. Biol. Chem.*, **241**, 3745 (1966).
(11) E. R. B. Graham in "Glycoproteins. Their Composition, Structure and Function", Vol. 5A, A. Gottschalk, Ed., Elsevier, Amsterdam, 1972, p 717.
(12) R. D. Guthrie, *Adv. Carbohydr. Chem.*, **16**, 105 (1961).
(13) G. E. Means and R. E. Feeney, *Biochemistry*, **7**, 2192 (1968); R. F. Borch, M. D. Bernstein, and H. D. Durst, *J. Am. Chem. Soc.*, **93**, 2879 (1971).
(14) Although we illustrate this concept with a derivatized glycoprotein, the procedure is general to all lysine-containing proteins; bovine serum albumin has been labeled in this way (effective $\tau_c = 1.3 \pm 0.1 \times 10^{-10}$ s) (M. A. Bernstein, unpublished results).
(15) For example, S. L. Patt and B. D. Sykes, *J. Chem. Phys.*, **56**, 3182 (1972).
(16) See ref 6 for a discussion. It is assumed that there is a single chemical shift, the motion is isotropic, and the deuterium quadrupole coupling constant is 165 KHz. The quoted "errors" are derived from the error in determining an accurate peak half-height width for a broad, overlapping resonance.
(17) D. Wallach, *J. Chem. Phys.*, **47**, 5258 (1967).
(18) I. J. Goldstein and C. E. Hayes, *Adv. Carbohydr. Chem.*, **35**, 127 (1978).
(19) W. M. Watkins in "The Red Blood Cell", Vol. 1, D. Mac N. Surgenor, Ed., Academic Press, New York, 1974, p 293; R. U. Lemieux, D. R. Bundle, and D. A. Baker, *J. Am. Chem. Soc.*, **97**, 4076 (1975).

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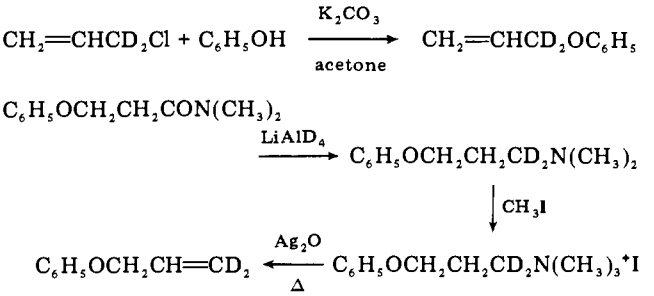
Secondary Deuterium Isotope Effects and Transition State Structure in the Aromatic Claisen Rearrangement

Sir:

The Claisen rearrangement of allyl aryl ethers is a classic example of the now familiar [3,3]-sigmatropic shift.[1] The intervention of the cyclohexadienone intermediate and its rapid enolization have been long established.[1] A concerted process leading to a transition state for the formation of the intermediate is supported by volume of activation measurements and on orbital symmetry grounds,[2] and a chair-like picture of this transition state provides a satisfactory rationalization of the observed stereoselectivity.[3,4] On the other hand, the relative insensitivity of reaction rates to polar substituent and solvent effects has been interpreted in terms of significant contributions of radical-pair-like structures to the transition state structure.[5] Unfortunately, the interpretation of such experiments is clouded by the fact that they depend upon alterations in transition state structure in order to produce observable effects. To clarify this picture it is important to understand the details of the bonding between the phenoxy and allyl moieties in the transition state, using an experimental approach which does not in itself alter the transition state structure. We report measurements of the secondary deuterium kinetic isotope effect attending deuterium substitution at the α and γ carbons of the allyl moiety and their interpretation in terms of transition state structure.

Scheme I



The deuterated allyl phenyl ethers required for this study were prepared as described in Scheme I, modeled on literature precedent.[6] All compounds exhibited NMR and IR spectra consistent with the structure shown. NMR analysis indicated >99% deuterium incorporation in the positions shown.

Kinetic experiments were carried out simultaneously on separate methyl salicylate solutions of allyl phenyl ether and its deuterated analogues at temperatures between 170 and 195 °C. Gas chromatographic analysis for allyl phenyl ether using an internal standard (anisole) and mechanical integration afforded concentration/time data which were fitted to the exponential form of the first-order rate equation by a standard nonlinear least-squares program.[7] At least 15 points, each the average of measurements on three ampules, were obtained for each run, covering 10–85% reaction. The derived isotope effects show no trend with temperature; hence averages for six runs with each compound are $k_H/k_{\alpha\text{-}D_2} = 1.18 \pm 0.02$ and $k_H/k_{\gamma\text{-}D_2} = 0.95 \pm 0.02$.[8]

Interpretation of these results in terms of transition state structure requires an estimate of the equilibrium isotope effects associated with the $O\text{-}CH_2(D_2) \rightarrow =CH_2(D_2)$ (α effect) and $=CH_2(D_2) \rightarrow C_6H_5CH_2(D_2)\text{-}C$ (γ effect) conversions. Using the spectroscopically based calculations of Hartshorn and Shiner,[9] one may obtain an equilibrium α effect of 1.30 and a γ effect of 0.87, both calculated for two deuterium atoms at 185 °C. Gajewski and Conrad have recently provided experimental support for these calculations, observing an equilibrium α effect of 1.27 at 160 °C and a γ effect of 0.88 at 185 °C.[10] Thus the simplest interpretation of these results is that the C–H vibration frequencies at the α carbon for the transition state are approximately (1.18–1)/(1.27–1) or 57–77% of the way from those of allyl phenyl ether to those of the cyclohexadienone intermediate. In the same way, the C–H frequencies of the γ carbon in the transition state are about (0.95–1)/(0.88–1) = 22–62% of the way to those of the intermediate.

The structure of the transition state, at least insofar as it is reflected in C–H bonding frequencies at the α and γ carbons of the allyl moiety, is entirely consistent with the long-held concerted description of the mechanism of the Claisen rearrangement.[1] Alternatives such as a fragmentation–recombination (diradical) mechanism would be expected to show little or no γ effect if fragmentation were rate determining and an essentially equilibrium α effect if recombination were rate determining. A mechanism involving rate-determining formation or decomposition of a diyl species would be consistent with the stereochemical results[3,4] but should show a nearly equilibrium γ effect and little or no α effect. Clearly, the ob-

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