anisatin triacetate,[2] and noranisatin[19] and in particular established the integrity of the tertiary hydroxyls at C-3a and C-5 (singlets at δ 5.18, 4.73) and the lone secondary hydroxyl at C-3 (δ 2.41, d, J = 5.3 Hz); the latter collapses to a singlet upon irradiation of C-3 H at δ 4.63. The above sequence thus completes a stereocontrolled 18-step route to (±)-8-deoxyanisatin from 2-allyl-2-cyclopentenone and represents the first synthesis of any member of this intricate tetracyclic series.[20]

Supplementary Material Available: Tables of atomic coordinates, temperature factors, bond lengths, and bond angles for **13a** and NMR data for compounds **6a**, **9**, and **11** (9 pages). Ordering information is given on any current masthead page.

(19) Since 8-deoxyanisatin is a new compound not readily available from natural anisatin, diagnostic δ and J values were compiled from the 60-MHz spectra of Yamada for (a) anisatin in CF_3COOH,[3] (b) anisatin triacetate in $CDCl_3$[2] or (c) the closest analogue, the corresponding γ-lactone noranisatin in $CDCl_3$.[2] These data are tabulated by proton position and source below.

C-3	H	4.60	dd	J = 8.5, 5,5 Hz	(c)
C-6	H	4.31	d	J = 5.0 Hz	(c)
		[4.59	dd	J = 4.2 Hz	(a)]
C-12	CH_2	4.21, 4.13	ABq	J = 7.0 Hz	(b)
C-7β	H	2.74	d	J = 13.5 Hz	(c)
C-7α	H	2.21	dd	J = 13.5, 5.0 Hz	(c)
C-10	CH_3	1.50	s		(c)
C-13	CH_3	0.87	d	J = 7.0 Hz	(b)

(20) Partial support of this research by grant CA-18846, awarded by the National Cancer Institute (USPHS), is gratefully acknowledged.

NMR Isotope Shifts as a Probe of Electronic Structure

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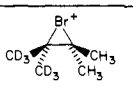
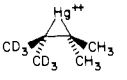
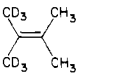
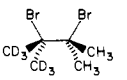
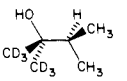
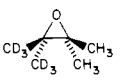
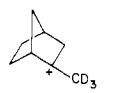
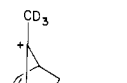
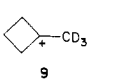
Deuterium isotope effects on carbon-13 chemical shifts have been used for the study of degenerate rearrangements,[1] for conformational analysis,[2] for spectral assignments,[3] and for probing charge distribution.[4] The two-bond isotope effects appear to be particularly revealing of the electronic distribution in the molecule.[4,5] The sign and magnitude of the two-bond deuterium isotope effect at the positively charged carbon atom in β-deuterated carbocations has been shown to depend on the electron demand and the charge delocalization mechanism.[4a]

For classical carbocations, the positive (downfield) β-isotope shifts are related to the demand for hyperconjugative stabilization by the C–H (or D) bonds.[4,5] In other cases in which the C–H (or D) bond is adjacent to an electron-deficient sp^2 hybridized carbon, the smaller positive shifts can be similarly explained.[6] For σ-delocalized carbocations, the negative (upfield) β-isotope shifts have been attributed to an isotopic perturbation of resonance.[4a] This perturbation results from averaging a vibrational motion over an anharmonic potential well and produces a change in the averaged electron distribution for different isotopomers.[7]

Table I. Deuterium Isotope Effects on the Carbon-13 NMR Chemical Shifts of 2,3-Dimethyl-2-butene Derivatives

compd	$^2\Delta C(D)^a$	$^3\Delta C(D)^a$
 1	+1.51	−0.30
 2	−1.59	+1.49
 3	−0.194	+0.032
 4	−0.404	−0.062
 5	−0.280	−0.089
 6	−0.253	−0.114
 7	−2.2	+0.4 (C1)[b]
 8	−0.8	0.0[b]
 9	−1.1	0.0[b]

[a] The two- and three-bond deuterium isotope effects (in ppm) at C2 and C3 of the 2,3-dimethyl-2-butene derivatives were determined at 67.9 MHz. [b] Reference 4a.

Although the energy surfaces for deuterated and nondeuterated materials should exactly coincide, small changes in bond lengths and angles which result from averaging a vibrational motion in an anharmonic potential well can be expected to occur. The increased electron density at an sp^3 hybridized carbon as a result of the lower zero-point vibrational energy of deuterium and a reduced C–D bond length is conveniently referred to as an inductive effect. For a C–D bond adjacent to an empty p orbital, the lower zero-point vibrational energy leads to a reduced electron delocalization to the p orbital and a reduced electron density at that carbon. This effect at a carbon β to the site of substitution is termed a hyperconjugative isotope effect.[7] Numerous examples of these effects are known, but in only a few cases have NMR isotope shifts been used to probe the mechanism of electron delocalization in moleules.[4a] We present here studies that suggest important new insights into the electronic structure of the bromonium ion **1** and the mercurinium ion **2** derived from 2,3-dimethylbutene.

The deuterium isotope effects on the carbon-13 chemical shifts were determined from the NMR spectra of samples containing a mixture of the nondeuterated and the gem-d_6 isotopomer of **1**[8,9]

(1) (a) Saunders, M.; Telkowski, L.; Kates, M. R.; *J. Am. Chem. Soc.* **1977**, *99*, 8070. (b) Saunders, M.; Kates, M. R. *J. Am. Chem. Soc.* **1977**, *99*, 8071. (c) Saunders, M.; Kates, M. R.; Walker, G. E. *J. Am. Chem. Soc.* **1981**, *103*, 4623.

(2) (a) Forsyth, D. A.; Botkin, J. H. *J. Am. Chem. Soc.* **1984**, *106*, 4296. (b) Aydin, R.; Gunther, H. *J. Am. Chem. Soc.* **1981**, *103*, 1301.

(3) Wehrli, F. W.; Wirthlin, T. "Interpretation of Carbon-13 NMR Spectra"; Heyden: London, 1978; pp 107–110.

(4) (a) Servis, K. L.; Shue, F. F. *J. Am. Chem. Soc.* **1980**, *102*, 7233. (b) Forsyth, D. A.; MacConnell, M. M.; *J. Am. Chem. Soc.* **1983**, *105*, 5920. (c) Ernst, L.; Eltamany, S.; Hopf, H. *J. Am. Chem. Soc.* **1982**, *104*, 299. (d) Ernst, L.; Hopf, H.; Wullbrandt, D. *J. Am. Chem. Soc.* **1983**, *105*, 4469.

(5) (a) Forsyth, D. A.; Lucas, P.; Burk, R. M. *J. Am. Chem. Soc.* **1982**, *104*, 240. (b) Wesener, J. R.; Gunther, H. *Tetrahedron Lett.* **1982**, *23*, 2845. (c) Forsyth, D. A.; Botkin, J. H.; Osterman, V. M. *J. Am. Chem. Soc.* **1984**, *106*, 7663.

(6) (a) Maciel, G. E.; Ellis, P. D.; Hofer, D. C. *J. Phys. Chem.* **1967**, *71*, 2160. (b) Morris, D. G.; Murray, A. M. *J. Chem. Soc., Perkins Trans. 2* **1976**, 1579. (c) Hansen, P. E.; Led, J. J. *Org. Magn. Reson.* **1981**, *15*, 288.

(7) Halevi, E. A. *Prog. Phys. Org. Chem.* **1963**, *1*, 109.

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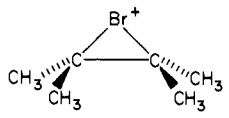
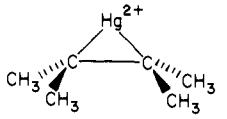


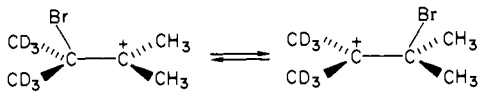
1



2

and **2**[10,11] and also for the related compounds **3**–**6**. The results are summarized in Table I.[12] The two-bond isotope effect, $^2\Delta C(D)$, for **3**–**6** are small and shielding and are in the normal range of values reported for simple aliphatic molecules.[13] For **1**, the positive β-isotope effect ($^2\Delta C(D) = +1.5$ ppm) is somewhat larger than the value ($^2\Delta C(D) = +0.8$ ppm) observed for the *tert*-butyl-d_6 cation.[4a] For **2**, the β-isotope shift ($^2\Delta C(D) = -1.6$ ppm) and the γ-isotope shift ($^3\Delta C(D) = +1.5$ ppm) are highly unusual.

The large positive value of $^2\Delta C(D)$ for **1** cannot be due to an equilibrium isotope effect of the type **1a** ⇄ **1b**. An equilibrium

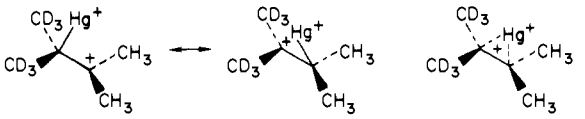


1a **1b**

isotope effect would be expected to be temperature-dependent;[1] the isotope effect for **1** is independent of temperature over the range −63 to −33 °C. Furthermore, the equilibrium isotope effect for **1** would be expected to be negative since isotopic substitution will selectively destabilize **1b**. The results indicate that **1** has an electronic structure that responds to deuterium substitution in the same way as does a classical carbocation. The value of $^2\Delta C(D)$ of 0.75 ppm per CD_3 group indicates a strong hyperconjugative interaction with an electron-deficient p orbital at the 2-carbon in **1**. The bromonium ion appears to be best represented by a three-membered cyclic structure in which all bonds are of the two-electron two-center type.[14]

The negative value of $^2\Delta C(D)$ in the mercurinium ion **2** suggests that this ion has an electronic structure that responds to deuterium substitution in an entirely different way. The β-isotope effects in the 2-methyl-2-norbornyl **7**, 7-methyl-2-norbornen-7-yl **8**, and 1-methyl-1-cyclobutyl **9** cations are also negative and of comparable magnitude to that in **2**. In these cases the isotope shifts were attributed to the redistribution of the bonding electrons in a delocalized three-center, two-electron bond. The bonding in the mercurinium ion can also be described by a three-center, two-electron bond composed of a vacant orbital of Hg^{2+} and the 2p orbitals of the C2 and C3 carbons.

The absence of a temperature dependence of the isotope effect over the range from −63 to −33 °C and the similarity to the values for **7**–**9** suggest an isotopic perturbation of resonance rather than a perturbation of equilibrium. Because of the reduced hyperconjugative ability of the C–D bond, the preferred contributor is **2a** and this form makes a greater contribution to the resonance hybrid in the deuterated compound. The negative isotope effect results from a shift of the positive charge toward C3. The observation of a large positive value of $^3\Delta C(D) = +1.5$ ppm for



2a **2b** **10**

carbon 3 is in agreement with this conclusion. The isotope effects suggest that a three-membered cyclic structure such as **2** is not appropriate for the mercurinium ion. If only the two π-electrons of the alkene and none of the electrons of Hg^{2+} (d^{10} configuration) are used in bonding then the mercurinium ion would have a bridging two-electron three-center bond as represented by **10**.

Acknowledgment. We are grateful to Dr. S. Prakash for advice on the preparation and for helpful discussion.

Registry No. 1 unlabeled, 25681-73-6; **2** unlabeled, 98778-45-1; **3** unlabeled, 563-79-1; **4** unlabeled, 594-81-0; **5** unlabeled, 594-60-5; **6** unlabeled, 5076-20-0; deuterium, 7782-39-0.

Selenium-77 Nuclear Magnetic Resonance Investigation of a Protein–Selenoligand Complex: Interaction of α-Chymotrypsin with (Phenylselenyl)acetate

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Of current interest in our laboratory is the development of selenium-77 NMR spectroscopy[2-7] and its application to biochemical investigations.[8] We have previously demonstrated the feasibility of observing selenium-77 resonances for selenium covalently attached to proteins.[8] We now demonstrate the first application of selenium-77 NMR spectroscopy to a protein–selenoligand complex, namely, a selenium-77 NMR investigation of the binding of a selenium-containing substrate analogue, (phenylselenyl)acetate, $PhSeCH_2COO^-Na^+$, to the enzyme α-chymotrypsin. (Phenylselenyl)acetate is the second product in the α-chymotrypsin-catalyzed hydrolysis of the substrate *p*-(nitrophenyl)(phenylselenyl) acetate and acts as an inhibitor for α-chymotrypsin.[9] The use of selenium-77 NMR for the investigation of biochemical systems is attractive for two reasons: (1) selenium can mimic oxygen, sulfur, and methylene functionalities in biomolecules[10] and (2) selenium-77 has a wide chemical shift range (2800 ppm).[11] This investigation was designed to address the question: Is selenium-77 chemical shift sensitivity sufficient to reflect the mechanism of binding of a selenium-containing inhibitor to α-chymotrypsin?

(8) The 2,3-dimethyl-2,3-butanediylbromonium ion was prepared by ionization of 2,3-dibromo-2,3-dimethylbutane by the previously reported procedure.[9] The ^{13}C chemial shifts are in satisfactory agreement with those previously reported[9]: $^{13}C\{H\}$ NMR (67.9 MHz, $CDCl_3$, 240 K) δ 26.6 (Cl, C4), 140.0 (C2, C3).

(9) Olah, G. A.; Bollinger, J. M. *J. Am. Chem. Soc.* **1967**, *89*, 4744.

(10) The 2,3-dimethyl-2,3-butanediylmercurinium ion was prepared by mercuration of 2,3-dimethyl-2-butene by the procedure of Olah et al.[11] The ^{13}C chemial shifts of the ion were similar to those reported of related derivatives.[11b]

(11) (a) Olah, G. A.; Yu, S. H. *J. Org. Chem.* **1975**, *40*, 3638. (b) Olah, G. A.; Garcia-Luna, A. *Proc. Natl. Acad. Sci., Phys. Sci.* **1980**, *77*, 5036. (c) Olah, G. A.; Clifford, P. R., *J. Am. Chem. Soc.* **1973**, *95*, 6067.

(12) In 2-(trideuteriomethyl)-3-methyl-2-butene-*1,1,1-d*$_3$ (**3**) and its derivatives, C2 has a longer relaxation time and lower intensity then C3.

(13) Hansen, P. E. *Annu. Rep. NMR Spectrosc.* **1983**, *15*, 105.

(14) The single-crystal X-ray structure of the bromonium ion from adamantylideneadamantane reveals it to be a three-membered cyclic bromonium ion tribromide: Slebocka-Tilk, H.; Ball, R. G.; Brown, R. S. *J. Am. Chem. Soc.* **1985**, *107*, 4504.

(1) Taken in part from the thesis of G. P. Mullen, to be submitted to the Department of Chemistry in partial fulfillment of the requirements of the Ph.D. degree.

(2) Mullen, G. P.; Luthra, N. P.; Dunlap, R. B.; Odom, J. D. *J. Org. Chem.* **1985**, *50*, 811–816.

(3) Anderson, J. A.; Odom, J. D.; Zozulin, A. J. *Organometallics* **1984**, *3*, 1458–1465.

(4) Luthra, N. P.; Dunlap, R. B.; Odom, J. D. *J. Magn. Reson.* **1983**, *52*, 318–322.

(5) Luthra, N. P.; Dunlap, R. B.; Odom, J. D. *J. Magn. Reson.* **1982**, *46*, 152–157.

(6) Odom, J. D.; Dawson, W. H.; Ellis, P. D. *J. Am. Chem. Soc.* **1979**, *101*, 5815–5822.

(7) Dawson, W. H.; Odom, J. D. *J. Am. Chem. Soc.* **1977**, *99*, 8352–8354.

(8) Luthra, N. P.; Costello, R. C.; Odom, J. D.; Dunlap, R. B *J. Biol. Chem.* **1982**, 1142–1144.

(9) The kinetic results associated with this substrate and inhibitor will be presented at a later time.

(10) Klayman, D. L.; Günther, W. H. H. "Organic Selenium Compounds: Their Chemistry and Biology"; Wiley: New York, 1973.

(11) Luthra, N. P.; Odom, J. D. In "The Chemistry of the Functional Groups"; Patai, S.; Rappaport, Z., Eds.; Wiley: New York, in press.

(12) Schonbaum, G. R.; Zerner, B.; Bender, M. L. *J. Biol. Chem.* **1961**, *236*, 2930–2935.

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