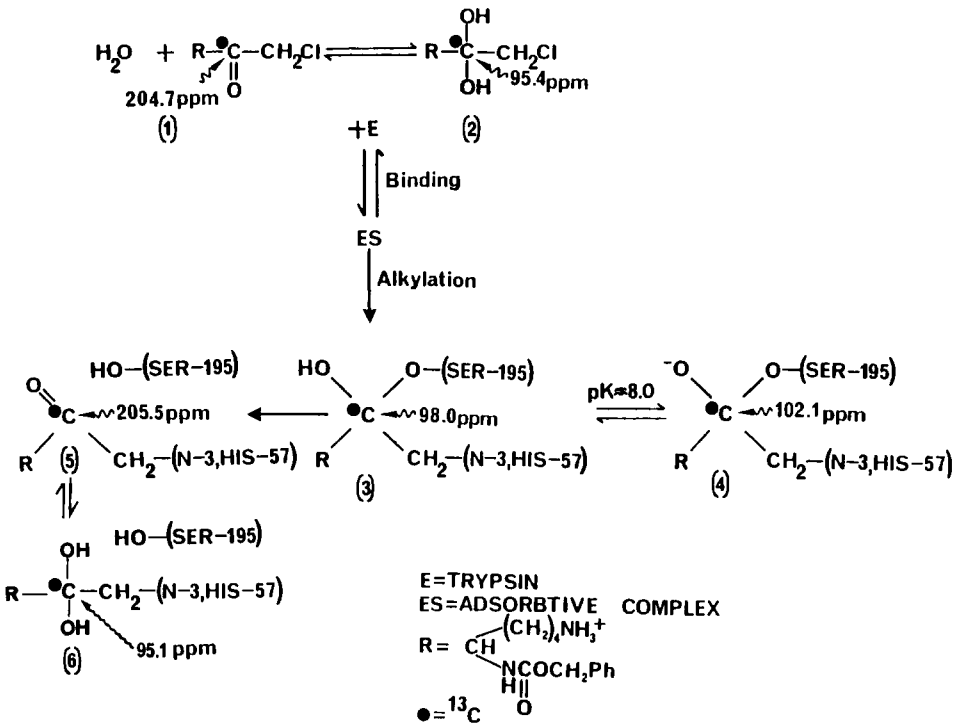
Scheme I



98.0 ppm and an additional resonance at 205.1 ppm appeared (not shown).

It is clear that native trypsin stabilizes[19] the tetrahedral adduct (**3**;[20] 98.0 ppm) as only nucleophilic attack by the hydroxyl group of serine-195 and covalent bond formation would cause the upfield shift of >100 ppm. A shift of this magnitude cannot be rationalized by a simple perturbation of the carbonyl trigonal hybridization. It is unlikely that tetrahedral geometry results from aqueous hydration of the enzyme-bound label for the following reasons. First, such a species should persist on denaturation, whereas new signals at 205.5 and 95.1 ppm (Figure 1h), observed on denaturation, provide clear evidence for the free ketone (**5**) and its hydrate (**6**). Secondly, with increasing pH the peak at 98.0 ppm was gradually shifted downfield ($pK_a \approx 8.0$) to a limiting value of 102.1 ppm, suggesting that there is stabilization of the *ionized* tetrahedral intermediate (**4**) by hydrogen bonding to the backbone NH groups of serine-195 and glycine-193 (Scheme I). No evidence of a carbonyl resonance (~205 ppm) was found over the pH range 3-11 prior to denaturation. The un-ionized tetrahedral intermediate (**3**) may also be stabilized by hydrogen bonding to one of the backbone carbonyl groups and/or electrostatic interaction with the protonated imidazole of histidine-57.[22] At pH 3.2 there is no evidence (Figure 1c) for the formation of a reversible tetrahedral adduct prior to alkylation of histidine-57, but this does not exclude the possibility of prior addition at pH >3.2.

Recently it has been claimed that a tetrahedral intermediate (99 ppm) has been detected by ^{13}C NMR with pepsin and a ketone inhibitor.[23] This result appears to be similar to those reported herein and taken together with the previous ^{13}C NMR studies indicate that the NMR method is an excellent probe for the diagnosis of carbon hybridization in complexes of inhibited proteases. The stage is now set for detailed studies on the enzyme–substrate complexes where tetrahedral intermediates can, in principle, be intercepted at subzero temperature and clearly recognized by ^{13}C NMR spectroscopy.

Acknowledgment. We thank the Science & Engineering Research Council (U.K.) for generous support of this work and for the provision of a Bruker WB 300-MHz spectrometer.

Registry No. CMK, 52780-79-7; trypsin, 9002-07-7.

(19) On the basis of peak heights (Figure 1g) at least 90% of the labeled carbon is sp^3 hybridized.

(20) Analogous chemical shifts are found in the anomeric carbon of many sugars.[21]

(21) Rosenthal, S. N.; Fendler, J. H. *Adv. Phys. Org. Chem.* **1976**, *13*, 279-424.

(22) Kossiakoff, A. A.; Spencer, S. A. *Biochemistry* **1981**, *20*, 6462-6474.

(23) Rich, D. H.; Bernatowicz, M. S.; Schmidt, P. G. *J. Am. Chem. Soc.* **1982**, *104*, 3535-3536.

Stereospecific Synthesis of Racemic Daunosamine. Diastereofacial Selectivity in a Nitrone Cycloaddition[1]

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There are several recent reports of stereoselective cycloadditions involving nitrones and nitrile oxides. Uskoković,[2] Belzecki,[3] and Vasella[4] have reported that nitrones carrying a chiral substituent on nitrogen (R^1 in **1**) displayed high diastereoselectivity in cycloadditions with a variety of achiral dipolarophiles (Scheme I). Koizumi[5] and Kozikowski[6] have found that the reaction of chiral dipolarophiles with nitrones and nitrile oxides, respectively, leads to the diastereoselective formation of cycloadducts. Since the N,O bonds of these cycloadducts are readily cleaved to produce acyclic molecules, the diastereoselectivity displayed in the cycloadditions serves as a means of controlling acyclic stereochemistry.[7] We

(1) Presented in part at the 183rd National Meeting of the American Chemical Society in Las Vegas, NV, Ap 1982.

(2) Wovkulich, P. M.; Uskoković, M. R. *J. Am. Chem. Soc.* **1981**, *103*, 3956 and references cited therein.

(3) Belzecki, C.; Panfil, C. *J. Org. Chem.* **1979**, *44*, 1212.

(4) Vasella, A.; Voeffray, R. *Helv. Chim. Acta* **1982**, *65*, 1134 and references cited therein.

(5) Koizumi, T.; Hirai, H.; Yoshii, E. *J. Org. Chem.* **1982**, *47*, 4005.

(6) Kozikowski, A. P.; Chen. Y. Y. *Tetrahedron Lett.* **1982**, *23*, 2081. Kozikowski, A. P.; Ghosh, A. K. *J. Am. Chem. Soc.* **1982**, *104*, 5788.

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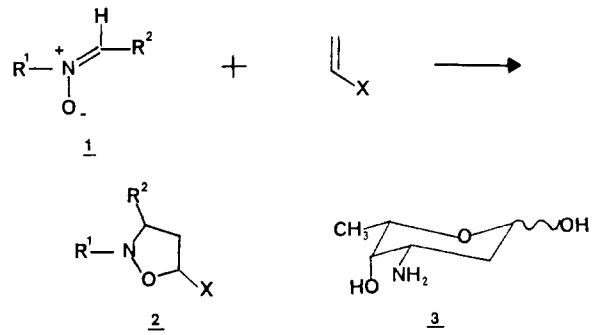
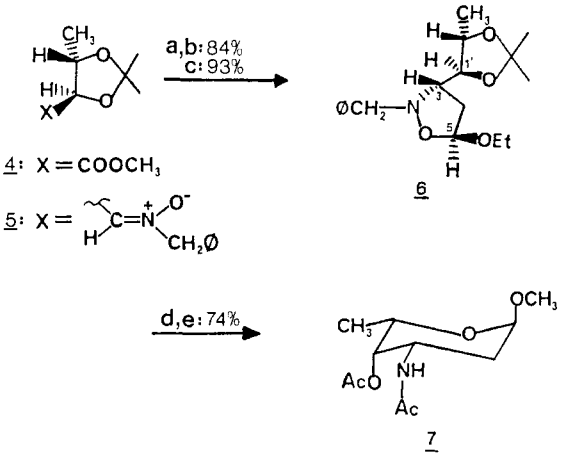
Scheme I



Scheme II



[a] Dibal, Et_2O, −78 °C. [b] Benzylhydroxylamine, Et_2O, 0 °C. [c] Ethyl vinyl ether, 35 °C, 72 h. [d] 10% HCl/MeOH, $Pd(OH)_2$, 50 psi, 48 h. [e] Ac_2O, pyridine, DIMAP, 25 °C, 24 h.

now report that nitrones which bear a chiral center on the carbon substituent of a nitrone (R^1 in **1**) undergo highly diastereoselective cycloadditions with achiral dipolarophiles, and we have employed the resulting cycloadducts in a short, efficient synthesis of the important amino sugar daunosamine.[8]

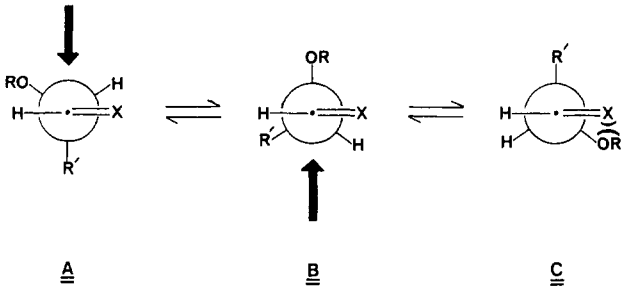
Racemic ester **4**[9] was converted to nitrone **5**[10] (84%) by Dibal reduction followed by treatment with benzylhydroxylamine. A single nitrone isomer was obtained, which was assigned the *Z* configuration on the basis of nuclear Overhauser effect difference spectroscopy (NOEDS).[11,12] Cycloaddition of nitrone **5** with excess ethyl vinyl ether (35 °C, 72 h, 93%) gave a *single* isoxazolidine isomer, **6**[10] (Scheme II).

The relative stereochemistry of **6** at C-3/C-5 was determined from its ^1H NMR spectrum. As we have shown in a series of similar isoxazolidines,[11] the spin multiplicity of the proton at C-5 is diagnostic for the relative stereochemistry between C-3 and C-5. The C-5 proton of **6** was observed as a doublet of doublets with J = 1.5 and 5.8 Hz, and this coupling pattern is only observed when the protons at C-3 and C-5 are anti. The relative stereochemistry of C-1′ and C-3 could not be determined by physical methods but was proven by conversion of **6** to daunosamine (vide infra).

Attempts to reductively cleave the N,O bond of **6** proved unexpectedly difficult. The remarkably stable **6** resisted all of the traditional reagents for N,O bond reduction.[13] However, hydrogenation of **6** over Pearlman's catalyst[14] in methanolic HCl at 50 psi for 2 days directly gave the methyl glycoside of daunosamine (**3**) in quantitative yield. The structure of the synthetic material was confirmed by acetylation to give **7**[11] which was identical with a sample prepared from authentic daunosamine[15] by standard procedures.

Using this methodology, it is possible to prepare daunosamine *in a stereospecific fashion in four steps from* **4** *in an overall yield of 58%*. Also, since the necessary enantiomer of ester **4** is readily available, the synthesis of the natural enantiomer of daunosamine by this method should be straightforward and is being pursued.

The observed diastereofacial selectivity can be rationalized by assuming that a Felkin model transition state[17] is adopted during cycloaddition (see A–C). Nitrone **5** (x = N(O)Bz) can adopt



either of two conformations A or B depending upon whether R′ (A) or OR (B) is considered the large substituent. Any other conformation, for instance C, should be disfavored due to steric repulsion between OR (or R′) and X. For A, the preferred direction of dipolarophile approach is onto the face of the nitrone anti to R′.[18,19] Anti attack onto conformation B would result in the selective approach onto the opposite face of the nitrone. Houk[18] and Anh[19] have concluded that anti approach, as in A and B, is favored due to the lack of unfavorable nonbonded orbital interactions in the transition state. Assuming anti attack, *nitrone* **5** *reacts exclusively via transition state A to produce isoxazolidine* **6**. Cycloaddition as conformation B would have resulted in the formation of an isoxazolidine which would have been diastereomeric with **6**.

Franck[20] and Kozikowski[6] have recently reported additional examples of diastereofacially selective reactions in which the anti-approach hypothesis rationalizes their results.

We have observed that the diastereofacial selectivity of nitrone cycloadditions is a general phenomenon and are currently in-

(7) For reviews of methods to control acyclic stereochemistry see: Bartlett, P. A. *Tetrahedron* **1980**, *36*, 2. Evans, D. A.; Nelson, J. V.; Taber, T. R. *Top. Stereochem.* **1982**, *13*, 1–115.

(8) For recent syntheses of daunosamine (3-amino-2,3,6-trideoxy-L-lyxo-hexose) and its derivatives, see: Wovkulich, P. M.; Uskoković, M. R. *J. Am. Chem. Soc.* **1981**, *103*, 3656 and references cited therein. Hauser, F. M.; Rhee, R. P. *J. Org. Chem.* **1981**, *46*, 227. Dyong, I.; Wiemann, R. *Chem. Ber.* **1980**, *113*, 2666. Fronza, G.; Fuganti, C.; Grasselli, P. *J. Chem. Soc., Chem. Commun.* **1980**, 442. Iwataki, I.; Nakamura, Y.; Takahashi, K.; Matsumoto, T. *Bull Chem. Soc. Jpn.* **1979**, *52*, 2731. Overall yields range from <5% to a maximum of 42% with the number of reaction steps varying from 5 to >10.

(9) Hatch, R. P.; Shringarpure, J.; Weinreb, S. M. *J. Org. Chem.* **1978**, *43*, 4172. Although racemic **4** was utilized in the synthesis, optically active **4** (either enantiomer) can be prepared from tartaric acid by literature procedures: Fronza, G.; Fuganti, C.; Grasselli, P.; Marinoni, G. *Tetrahedron Lett.* **1979**, 3883.

(10) Spectral data for all compounds are reported in the supplemental material.

(11) DeShong, P.; Dicken, C. M.; Staib, R. R.; Freyer, A. J.; Weinreb, S. M. *J. Org. Chem.* **1982**, *47*, 4397.

(12) DeShong, P.; Leginus, J. M.; Dicken, C. M.; Stauffer, D. A., unpublished results.

(13) House, H. O.; Wichman, P. P.; Müller, H. C. *J. Am. Chem. Soc.* **1962**, *84*, 3139. Adams, R.; Marshall, J. R. *Ibid.* **1928**, *50*, 1970. Keck, G. E. *Synth. Commun.* **1979**, *9*, 281.

(14) Pearlman, W. M. *Tetrahedron Lett.* **1967**, *17*, 1663.

(15) Authentic daunosamine was purchased from Pfanstiehl Laboratories, Inc., Waukegan, IL.

(16) We have observed similar diastereofacial selectivities in the addition of nitrones to various dipolarophiles. For instance, cycloaddition of nitrone **5** and vinyl acetate yields a cycloadduct that has the same C-1′/C-3 relative stereochemistry as **6**.

(17) Chêrest, M.; Felkin, H.; Prudent, N. *Tetrahedron Lett.* **1968**, 2199. Chêrest, M.; Felkin, H. *Ibid.* **1968**, 2205.

(18) Caramella, P.; Rondan, N. G.; Paddon-Row, M. N.; Houk, K. N. *J. Am. Chem. Soc.* **1981**, *103*, 2438. Rondan, N. G.; Paddon-Row, M. N.; Caramella, P.; Mareda, J.; Müeller, P. H.; Houk, K. N. *Ibid.* **1982**, *104*, 4974.

(19) Anh, N. T.; Eisenstein, O. *Nouv. J. Chim.* **1977**, *1*, 61.

(20) Franck, R. W.; John, T. V.; Olejniczak, K. *J. Am. Chem. Soc.* **1982**, *104*, 1106.

vestigating this methodology for the synthesis of other natural products.

Acknowledgment. We thank the Research Corp. and the donors of the Petroleum Research Fund, administered by the American Chemical Society, for generous financial support. We acknowledge valuable discussions with C. M. Dicken, O. Baralt, Dr. Richard Friary of Schering Corp. and Professors John Lowe of this department and Ken Houk of the University of Pittsburgh. Partial support for the purchase of a Bruker WP-200 NMR spectrometer from the National Science Foundation is also acknowledged.

Supplementary Material Available: Spectral data for **5–7** (1 page). Ordering information is given on any current masthead page.

Mechanistic Studies of Unimolecular Ionic Decompositions by Deuterium and Heavy-Atom Isotope Effects. Concerted Elimination of Acetaldehyde from the Benzyl Ethyl Ether Radical Cation

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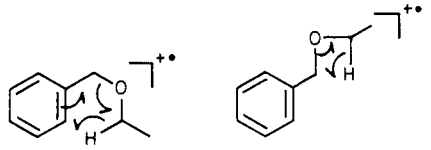
Received December 3, 1982

The question of whether mechanisms of the McLafferty[1,2] and related rearrangements[2] are stepwise or concerted has long been a matter of debate. Of late, the balance of opinion has swung toward the former—the prevailing view being that low-energy barriers arising as a consequence of concerted making and breaking of bonds do not occur.[2,3] The evidence in favor of stepwise mechanisms, however, is not extensive. A theoretical analysis has favored a stepwise mechanism,[4] and for one specific reaction there are experimental results clearly supporting a stepwise mechanism.[5] We present evidence that the loss of acetaldehyde from the benzyl ethyl ether radical cation proceeds *via* the six-centered γ-hydrogen rearrangement as shown in I in a concerted fashion.



I

II

It has been reported[6] that the loss of acetaldehyde from the benzyl ethyl ether radical cation proceeds in either a concerted or stepwise fashion via a six-membered transition state and that the deuterium isotope effect on the ion abundances in the electron impact mass spectrum is about 2. In principle, the elimination may be either six-centered (I) or four-centered (II). The product ions formed initially through I and II would be the methylenecyclohexadiene and toluene radical cations respectively. We have measured[7] the collisional activation spectra of the toluene molecular ion and the m/z 92 product ion from benzyl ethyl ether and observed significant differences in the m/z 75–78 region of the two spectra.[8] Either the m/z 92 ion formed is not the toluene radical cation or the toluene radical cation is formed initially and rearranges at least partially prior to collision.

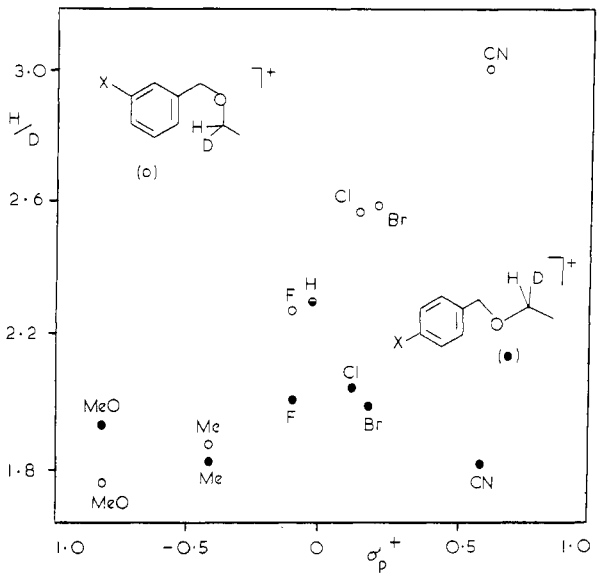


Figure 1. Plots of H/D isotope effects on metastable ion abundances for losses of CH_3CHO and CH_3CDO from the molecular ions of 3(5)-substituted benzyl ethyl-1-$^2H_1$ ethers and of 4-substituted benzyl ethyl-1-$^2H_1$ ethers vs. the σ^+ value of the substituent.

Deuterium isotope effects on metastable ion abundances for losses of CH_3CHO and CH_3CDO from the molecular ions of 3(5)-substituted benzyl ethyl-1-$^2H_1$ ethers ($XC_6H_4CH_2$–O–$CHDCH_3$) have been determined[9,10] and are plotted in Figure 1 against the σ^+ value of X.[11] The corresponding plot of the 4-substituted isomers is also shown. On the basis of mechanism I, the substituent X at 3(5) is conjugated to the hydrogen acceptor site, in which case the isotope effect would be expected to increase with an increase in the electron-withdrawing capacity of X. This is what has been observed (Figure 1). On the basis of mechanism II, the conjugative effect of a 4-substituent on the electron density of the acceptor site (C-1 in the side chain) might be expected to be manifested in a trend in the isotope effects. No such trend is detected (Figure 1). These results (Figure 1) are consistent with a concerted mechanism I. The magnitude of the isotope effects rules out the possibility of a stepwise mechanism in which the second step (C–O bond cleavage) is rate determining.[12,13]

(1) G. P. Hall and D. W. Stewart, *J. Am. Chem. Soc.*, **74**, 4404 (1952); A. J. C. Nicholson, *Trans. Faraday Soc.*, **50**, 1067 (1954); F. W. McLafferty, *Anal. Chem.*, **28**, 306 (1956); **31**, 82 (1959).

(2) S. Meyersen and J. D. McCollum, *Adv. Anal. Chem. Instrum.*, **2**, 179 (1963); D. G. I. Kingston, J. T. Bursey, and M. M. Bursey, *Chem. Rev.*, **2**, 215 (1974); J. L. Holmes, in "Isotopes in Organic Chemistry", Vol. 1, E. Buncel and C. C. Lee Eds., Elsevier, Amsterdam, 1975, p 61.

(3) J. S. Splitter and M. Calvin, *J. Am. Chem. Soc.*, **101**, 7329 (1979).

(4) F. P. Boer, T. W. Shannon, and F. W. McLafferty, *J. Am. Chem. Soc.*, **90**, 7239 (1968).

(5) J. S. Smith and F. W. McLafferty, *Org. Mass Spectrom.*, **5**, 483 (1971); D. J. McAdoo, D. N. Witiak, and F. W. McLafferty, *J. Am. Chem. Soc.*, **99**, 7265 (1977).

(6) J. K. MacLeod and C. Djerassi, *J. Am. Chem. Soc.*, **89**, 5182 (1967).

(7) Measurements were made on a reversed-sector instrument at the University of New South Wales. See P. G. Cullis, G. M. Neumann, D. E. Rogers, and P. J. Derrick, *Adv. Mass Spectrom.*, **8**, 1729 (1980).

(8) Our results are in agreement with those reported for $[C_7H_8]^{+\cdot}$ isomers by F. W. McLafferty, R. Kornfield, W. F. Haddon, K. Levsen, I. Sakai, P. E. Bente, S.-C. Tsai, and H. D. R. Schuddemage, *J. Am. Chem. Soc.*, **95**, 3886 (1973). Loss of $CH_3\cdot$ is more pronounced from the toluene molecular ion than from the methylenecyclohexadiene radical cation. See also P. C. Burgers, J. K. Terlouw, and K. Levsen, *Org. Mass Spectrom.*, **17**, 295 (1982).

(9) All new compounds prepared for this investigation gave correct analytical results. Incorporation of deuterium: $^2H_1$ = 100% for all compounds shown in Figure 1; $^2H_2$ = 100% for compound **2**. Deuterium isotope effects were measured for decompositions in the first field-free region of an Hitachi Perkin-Elmer RMU 7D instrument. Precision of measurements were ±0.1.

(10) G. Klass, D. J. Underwood, and J. H. Bowie, *Aust. J. Chem.*, **34**, 507 (1981).

(11) The H transfer in the system is site specific as evidenced by the losses of CH_3CDO and CH_3CHO from the molecular ions of $C_6H_5CH_2OCD_2CH_3$ and $C_6D_5CD_2OCH_2CH_3$, respectively. No H/D equilibration occurs for decompositions in field-free regions.

(12) Quasi-equilibrium theory calculations have been performed. For the method employed see P. J. Derrick and K. F. Donchi in "Comprehensive Chemical Kinetics", Suppl. Vol. 1, C. H. Bamford and C. F. H. Tipper, Eds. Elsevier, Amsterdam, 1983.

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