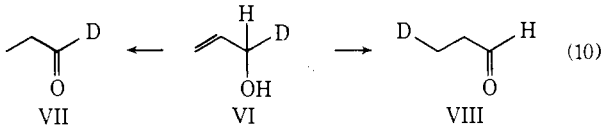
eliminate a hydrogen–deuterium isotope effect as the cause of the observed specificity, the reaction of 1-buten-3-ol-*O-d* (IV) was investigated; the product isolated was 2-butanone-*3-d* (V) about 80% specifically labeled (eq 9).[11] In light of these observations then, the reaction mechanism cannot involve the intermediacy of molecular hydrogen.

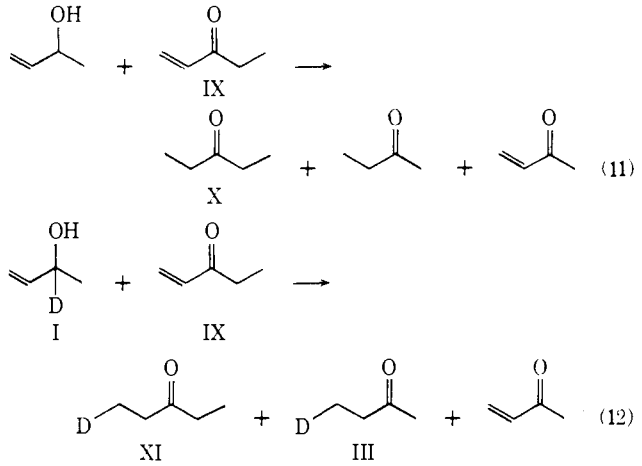
The rate determining step in the reaction involves cleavage of the C-3–H bond. This was suggested by the observation that 1-buten-3-ol-*3-d* was markedly less reactive than the unlabeled alcohol. To arrive at a quantitative value for the isotope effect, 1-propen-3-ol-*3-d* (VI) was prepared. Hydrogen migration produces propanol-*1-d* (VII) while deuterium migration produces propanal-*3-d* (VIII) (eq 10); nmr measurements



(10)

established that, at 180°, hydrogen migration predominates roughly four to one. No isotope effect was observed in the reaction of 1-buten-3-ol-*O-d;* the transfer of the hydroxy proton and the C-3 hydrogen must therefore occur in separate steps.

The unsaturated carbonyl compound is apparently an intermediate in the reaction. Coreaction of 1-buten-3-ol and 1-penten-3-one (IX) produces 3-pentanone (X) as well as 2-butanone and a large amount of 1-buten-3-one (eq 11). Further, reaction of 1-buten-3-ol-*3-d* (I) and

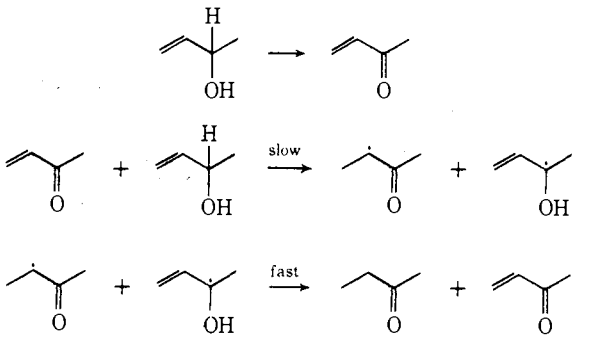


(11)

(12)

1-penten-3-one (IX) produces 3-pentanone-*1-d* (XI) in high isotopic purity (eq 12). Reaction of 1-buten-3-ol, 1-buten-3-ol-*3-d* (I), and 1-penten-3-one (IX) produces 2-butanone and 3-pentanone with virtually identical deuterium content. Since control experiments demonstrated that the unreacted allylic alcohols had not undergone extensive hydrogen–deuterium exchange, this observation suggests that both products are formed *via* a mechanism with the same rate-limiting step, *i.e.*, one involving the unsaturated carbonyl compound as an intermediate. The report of Satake and Akabori[12] that an α,β-unsaturated aldehyde can be converted to the saturated aldehyde in the gas phase over metallic copper using saturated alcohols as reducing agents lends credence to this proposal.

A mechanism consistent with these observations is depicted below. Initially, dehydrogenation of the allylic alcohol generates a small amount of α,β-unsaturated ketone. In the rate limiting step, the C-3 hydrogen of the alcohol is transferred to the terminus of the ketone carbon–carbon double bond.[13] A rapid abstraction of the hydroxyl hydrogen generates a new molecule of α,β-unsaturated ketone and a molecule of saturated ketone.



Efforts are underway to ascertain the generality of these mechanistic observations over other metal and metal oxide surfaces.

Acknowledgment. Support of this work by Research Corporation is gratefully acknowledged.

(11) Mass spectral analysis indicated that the ketone was 36% do, apparently, as a result of incomplete deuteration of the starting alcohol and the presence of adventitious water. After correcting for the undeuterated material, the nmr (CCl_4) consists of a pair of triplets (J_{vic} = 1.1 and 7.5 Hz) at 1.00, a singlet at 2.05, and a very broad quartet ($J \cong 8$ Hz) at 2.35 ppm. After integration and correction for unlabeled material, the ketone was calculated to be about 80% specifically labeled at C-3.

(12) K. Satake and S. Akabori, *Nippon Kagaku Zasshi*, **70**, 84 (1949).

(13) W. R. Patterson and R. L. Burwell, Jr., *J. Amer. Chem. Soc.*, **93**, 833 (1971), have suggested an analogous rate limiting step for the reduction of saturated ketones with alcohols over impure silica. "Hydride" transfer from the alcohol to the carbonyl group is catalyzed by a surface aluminum atom.

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Conformational Flexibility of the Neurohypophyseal Hormones Oxytocin and Lysine-vasopressin. A Carbon-13 Spin–Lattice Relaxation Study of Backbone and Side Chains

Sir:

Spin–lattice relaxation times (T_1) of carbon-13 nuclei are proving useful in determining rates of overall molecular reorientation as well as in monitoring segmental motion of amino acid side chains in peptides.[1–5] We

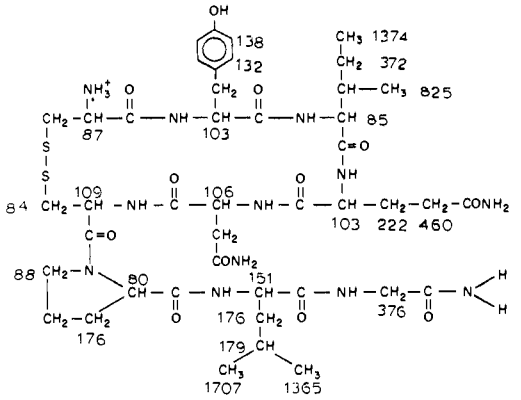
(1) P. Keim, R. A. Vigna, R. C. Marshall, and F. R. N. Gurd, *J. Biol. Chem.*, **248**, 6104 (1973).

(2) R. Deslauriers, R. Walter, and I. C. P. Smith, *Fed. Eur. Biochem. Soc., Lett.*, 37, 27 (1973).

(3) R. Deslauriers, R. Walter, and I. C. P. Smith, *Biochem. Biophys. Res. Commun.*, **53**, 244 (1973).

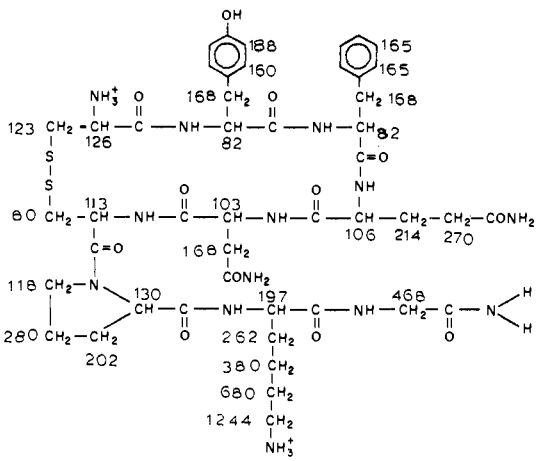
(4) A. Allerhand, D. Doddrell, V. Glushko, D. W. Cochran, E. Wenkert, P. J. Lawson, and F. R. N. Gurd, *J. Amer. Chem. Soc.*, **93**, 544 (1971).

(5) (a) H. Saitô and I. C. P. Smith, *Arch. Biochem. Biophys.*, **158**, 154 (1973); (b) I. C. P. Smith, R. Deslauriers, H. Saitô, R. Walter, C. Garrigou-Lagrange, H. McGregor, and D. Sarantakis, *Ann. N.Y. Acad. Sci.*, **222**, 597 (1974); (c) H. Saitô and I. C. P. Smith, *Arch. Biochem. Biophys.*, in press.



OXYTOCIN

Figure 1. NT_1 values in milliseconds observed in oxytocin (100 mg/ml D_2O, pH meter reading 3.5, 32°, 10,000 scans/spectrum). Spectra were obtained on a Varian XL-100-15 spectrometer operating at 25.16 MHz. T_1 data (±15%) were acquired using a 180°–τ–90° pulse sequence. Fifteen values of τ from 10 to 1600 msec were chosen, and T_1 values were obtained by least-squares fit to the best straight line. A 2-sec interval was allowed between each pulse sequence.



LYSINE VASOPRESSIN

Figure 2. NT_1 values in milliseconds observed in LVP (100 mg/ml D_2O, pH meter reading 4.2, 10,000 scans/spectrum). Other conditions were identical with those described in legend to Figure 1.

have determined T_1 values of the individual carbon atoms of the nonapeptide hormones oxytocin (Figure 1) and lysine-vasopressin (LVP, Figure 2) in aqueous medium. The results of this study give a *complete* picture of the conformational flexibility of these hormone molecules and thus provide a comparison with models previously proposed for oxytocin and LVP.[6]

The individual T_1 values, given in Figures 1 and 2, are multiplied by N, the number of directly attached hydrogen atoms. In the extreme narrowing limit, the longer NT_1 is, the greater is the mobility of the carbon atom.[7] Recently a detailed discussion of the principles of the method and their application to peptides of this size has been presented by Allerhand and Komoroski.[8] The assignments of the ^{13}C resonances of oxytocin and LVP in aqueous solution were reported previously.[9,10] The T_1 values of the unresolved resonances of the β-carbons of Tyr, Asn, and Ile of oxytocin are not given nor is that of the γ-carbon of Pro (a shoulder on the resonance of the γ-carbon of Leu) and that of the β-carbon of Cys-1 (a shoulder on the β-carbon peak of Leu). This in no way affects the following conclusions.

In oxytocin the T_1 values of the α-carbons in the ring portion are all approximately 95 msec (Figure 1), indicating a similar degree of rigidity.[7] The T_1 values of the α-carbons in the terminal tripeptide of oxytocin[11] increase toward the C-terminus as a consequence of increased mobility. This is the first experimental evidence which gives a residue-by-residue account of the intramolecular mobility of the total backbone structure of oxytocin. The results are in excellent agreement with the contention that the "acyclic tripeptide exhibits a much greater conformational freedom than the . . . ring component"[12] of neurohypophyseal hormones. This has also been implied from enzymatic studies; *e.g.*, chymotrypsin fails to hydrolyze the Tyr–Ile peptide bond in the 20-membered ring of oxytocin but cleaves the Leu–Gly bond in the linear portion.[13] The elegant ^2H nmr work recently reported by Glasel, *et al.*,[14] also reveals considerable flexibility of the tail with respect to Cys in position 1 of the cyclic portion. The side chains show varying degrees of freedom. That of Gln undergoes segmental motion as judged by the increase of a factor of 2 in T_1 on going from the α- to the β-carbon and from the β- to the γ-carbon. This argues against one of the models of neurohypophyseal hormones proposed by Honig, *et al.*,[6h] which includes a hydrogen bond between the carbonyl oxygen of the Gln side chain and the backbone NH of Asn. A similar proposal has also been made for oxytocin in dimethyl sulfoxide.[6i] As may be expected for the hydrophobic side chain of a residue at the corner of a β-turn,[6a] the sec-butyl moiety of Ile appears to be unimpeded by the other residues in the hormone, because the ratios of T_1 values for the various carbon atoms are similar to those measured in the free

(6) (a) D. W. Urry and R. Walter, *Proc. Nat. Acad. Sci. U.S.*, **68**, 956 (1971); (b) P. H. von Dreele, A. I. Brewster, H. A. Scheraga, M. F. Ferger, and V. du Vigneaud, *ibid.*, **68**, 1028 (1971); (c) P. H. von Dreele, A. I. Brewster, F. A. Bovey, H. A. Scheraga, M. F. Ferger, and V. du Vigneaud, *ibid.*, **68**, 3088 (1971); (d) R. Walter, J. D. Glickson, I. L. Schwartz, R. T. Havran, J. Meienhofer, and D. W. Urry, *ibid.*, **69**, 1920 (1972); (e) J. D. Glickson, D. W. Urry, and R. Walter, *ibid.*, **69**, 2566 (1972); (f) P. H. von Dreele, A. I. Brewster, J. Dadok, H. A. Scheraga, F. A. Bovey, M. F. Ferger, and V. du Vigneaud, *ibid.*, **69**, 2169 (1972); (g) D. Kotelchuck, H. A. Scheraga, and R. Walter, *ibid.*, **69**, 3629 (1972); (h) B. Honig, E. A. Kabat, L. Katz, C. Levinthal, and T. T. Wu, *J. Mol. Biol.*, **80**, 277 (1973); (i) A. I. Brewster and V. J. Hruby, *Proc. Nat. Acad. Sci. U.S.*, **70**, 3806 (1973); (j) A. I. Brewster, V. J. Hruby, J. A. Glasel, and A. E. Tonelli, *Biochemistry*, **12**, 5294 (1973).

(7) A. Allerhand, D. Doddrell, and R. Komoroski, *J. Chem. Phys.*, **55**, 189 (1971).

(8) A. Allerhand and R. A. Komoroski, *J. Amer. Chem. Soc.*, **95**, 8228 (1973).

(9) R. Walter, K. U. M. Prasad, R. Deslauriers, and I. C. P. Smith, *Proc. Nat. Acad. Sci. U.S.*, 70, 2086 (1973).

(10) We wish to reverse the assignments of the β- and δ-carbon resonances of Lys in LVP reported in ref 9. These assignments were based on those for Lys by W. Horsley, H. Sternlicht, and J. S. Cohen, *J. Amer. Chem. Soc.*, **92**, 680 (1970). The T_1 values reported herein as well as in ref 5 justify the reversal.

(11) We have examined the isolated tripeptide Pro-Leu-Gly-NH_2 (MSH-R-IF) (see ref 2) and find that the T_1 values for the Leu and Gly residues in this compound and in oxytocin behave similarly. However, the Pro residues differ; the T_1 of the Pro α-carbon is considerably shorter than those of Leu and Gly in oxytocin, whereas all are comparable in Pro-Leu-Gly-NH_2. This is undoubtedly due to anchoring of the Pro residue to the cyclic portion of oxytocin.

(12) R. Walter, "Structure-Activity Relationships of Protein and Polypeptide Hormones," Vol. 1, M. Margoulies and F. C. Greenwood, Ed., Excerpta Medica Foundation, Amsterdam, 1971, pp 181–193.

(13) P. L. Hoffman, and R. Walter, *Biophys. J.*, **13**, 202a (1973); R. Walter and P. L. Hoffman, *Biochim. Biophys. Acta*, in press.

(14) J. A. Glasel, V. J. Hruby, J. F. McKelvy, and A. F. Spatola, *J. Mol. Biol.*, **79**, 555 (1973).

amino acid.[2] The Tyr residue does not rotate rapidly relative to the peptide backbone as indicated by the only slightly greater T_1 values of the aromatic carbons relative to those of the α-carbons of the cyclic moiety. The T_1 values of the Pro residue reflect the attachment of the Cys-6 residue to the imino nitrogen.[11] The relative T_1 values of the various CH_2 moieties of Pro provide insight into the rate and nature of interconversions between various ring-puckered forms. In TRH (<Glu-His-Pro-NH_2) the relative mobilities are $\gamma > \beta > \delta$,[3] whereas in Pro-Leu-Gly-NH_2 they are $\gamma > \beta \simeq \delta$[2]. In oxytocin the β-carbon has roughly twice the mobility of the δ-carbon. We attribute the difference between the behavior of oxytocin and Pro-Leu-Gly-NH_2 to the influence of the Cys–Pro peptide bond in oxytocin on the time-averaged conformation of the Pro ring; in particular formation of this peptide bond inhibits the possibility of rapid endo–exo interconversion at the δ-carbon. A similar influence is obvious in the data for LVP (Figure 2, *vide infra*).

The α-carbons in the ring portion of LVP have T_1 values (Figure 2) very similar to those found in the ring portion of oxytocin. However, the relaxation times of the α-carbons in the linear tripeptide are all longer than those of the corresponding residues in oxytocin, indicating greater freedom for this moiety in LVP than in oxytocin. This correlates well with the conformation differences proposed by Walter for these two hormones.[12] The side chains of the Tyr and Phe residues in LVP have comparable mobilities, perhaps due to the proposed stacking of the two aromatic rings in aqueous medium.[15] The T_1 values of corresponding carbon atoms are equal within experimental error (because of bad resolution no values are given for the para carbon of the aromatic ring of Phe). The NT_1 values for the ortho and meta carbons are essentially equal to those of the β-carbons. There is a difference of a factor of 2 in the NT_1 values of the α- and β-carbons of the aromatic residues. This leads us to conclude that the side chains are less restricted than the backbone but that rotation about the aryl–βCH_2 is not significantly faster than that about the α–β axis, in contrast to the behavior observed in substituted benzenes.[16] The side chain of Lys provides an example of segmental motion with adjacent CH_2 carbons having *ca.* 1.7-fold T_1 differentials, as found recently in oligo- and poly-L-lysine.[5] The Pro residue in LVP behaves qualitatively in the same manner as that in oxytocin, and we believe that the ring conformations of Pro are similar to that found in TRH,[3] the γ-carbon being by far the most mobile in the Pro ring. The Asn side chain shows more mobility than the backbone of the ring, whereas the Gln γ-carbon has a considerably lower mobility than in oxytocin, suggesting the involvement of this side chain in some type of secondary structure.[6h]

We calculate an effective overall correlation time (τ_c) for both compounds of 5×10^{-10} sec based on an average T_1 value of $\simeq$95 msec for the α-carbons of the ring. This is in agreement with the value calculated for a monomeric peptide in this molecular weight range[14] and corresponds to an approximate diameter of 16 Å for neurohypophyseal hormones; from thin-film dialysis studies diameters of 14–15 and 16 Å were calculated for oxytocin and lysine vasopressin, respectively.[17]

These studies serve as a basis to relate conformational characteristics of neurohypophyseal hormones and synthetic analogs to their agonistic and antagonistic properties. Measurements on ^{13}C-enriched neurohypophyseal peptides should allow monitoring of conformational changes and dynamic processes which occur on binding to their intracellular carrier proteins, the neurophysins.[18]

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(17) L. C. Craig, personal communication.
(18) Issued as N.R.C.C. Publication No. 33750.

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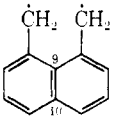
Synthesis and Characterization of a 1,8-Naphthoquinodimethane

Sir:

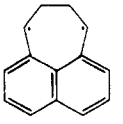
Although Hückel MO theory is naive by the standards of today's sophisticated calculations, it has been eminently successful at predicting the aromaticity of cyclic systems containing an array of $(4n + 2)\pi$ electrons. One of its other outstanding achievements, although less well known and appreciated, is its success in predicting triplet ground states for organic molecules such as the cyclopentadienyl cation,[1] trimethylenemethane,[2] tetramethyleneethane,[3] and *m*-xylylene.[4]

Hückel theory also predicts that 1,8-naphthoquinodimethane (**1**) should possess a triplet ground state.



1

$E = 0 + S + A$



2

In addition to the five bonding molecular orbitals (MO's), which contain a total of ten π electrons, this species has a pair of degenerate nonbonding MO's at $E = 0$, one of which is symmetric (S) to the plane bisecting the molecule and going through the C-9–C-10 bond and the other which is antisymmetric (A) to this

(15) R. Deslauriers and I. C. P. Smith, *Biochem. Biophys. Res. Commun.*, **40**, 179 (1970).
(16) (a) G. C. Levy and G. L. Nelson, "Carbon-13 Nuclear Magnetic Resonance for Organic Chemists," Wiley-Interscience, Wiley, New York, N. Y., 1972; (b) D. Doddrell and A. Allerhand, *J. Amer. Chem. Soc.*, **93**, 1558 (1971); (c) G. C. Levy, *J. Magn. Resonance*, **8**, 122 (1972); (d) G. C. Levy, J. D. Cargioli, and F. A. L. Anet, *J. Amer. Chem. Soc.*, **95**, 1527 (1973).

(1) M. Saunders, *et al.*, *J. Amer. Chem. Soc.*, **95**, 3017 (1973), and references cited therein.
(2) (a) P. Dowd, *J. Amer. Chem. Soc.*, **88**, 2587 (1966); P. Dowd and K. Sachdev, *ibid.*, **89**, 715 (1967); P. Dowd, A. Gold, and K. Sachdev, *ibid.*, **90**, 2715 (1968).
(3) (a) P. Dowd, *J. Amer. Chem. Soc.*, **92**, 1066 (1970); (b) W. Roth and G. Erker, *Angew. Chem., Int. Ed. Eng.*, **12**, 503 (1973).
(4) (a) G. Kothe, K. H. Deukel, and W. Suemmermann, *Angew. Chem., Int. Ed. Engl.*, **9**, 906 (1967); (b) G. R. Luckhurst, G. F. Pedulli, and Tiecco, *J. Chem. Soc. B*, 329 (1971).