Synthesis of "Acetone-oxytocin" from an Isopropylidene Derivative of S-Benzyl-L-cysteinyl-L-tyrosine[1]

Donald Yamashiro and Vincent du Vigneaud[2]

Contribution from the Department of Biochemistry, Cornell University Medical College, New York, New York 10021, and the Department of Chemistry, Cornell University, Ithaca, New York 14850.
Received August 21, 1967

Abstract: "Acetone-oxytocin," an isopropylidene derivative resulting from the inactivation of the hormone by acetone, has been synthesized from the acetone derivative of S-benzyl-L-cysteinyl-L-tyrosine. This isopropylidene dipeptide was coupled with L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide by the mixed anhydride procedure and the resulting nonapeptide derivative was converted to acetone-oxytocin by cleavage of the benzyl groups with sodium in liquid ammonia and subsequent oxidation of the resulting disulfhydryl compound with potassium ferricyanide. The synthetic acetone-oxytocin was isolated and purified by partition chromatography on Sephadex. The identity of this synthetic material with authentic acetone-oxytocin was demonstrated by extensive comparison of their physical and chemical properties, including conversion to oxytocin possessing full avian vasodepressor activity.

The inactivation of oxytocin (Figure 1) by acetone with the formation of a new compound, referred to as "acetone-oxytocin," has recently been reported.[3] The compound was isolated by partition chromatography on Sephadex with a biological activity $^1/_{1000}$ that of oxytocin as measured in the avian vasodepressor assay, the official method of the U. S. Phaimacopeia for the assay of oxytocin.[4] Treatment of the acetone-oxytocin with 0.25% acetic acid at 90° for 30 min regenerates the hormone in good yield with full biological activity. Acetone is liberated from the compound, mole for mole, when the latter is boiled in 0.1 *N* acetic acid for 10 min. The analytical data obtained in the studies are in agreement with the elemental composition calculated for a monoisopropylidene derivative of oxytocin. Involvement of the amino group of oxytocin in the formation of the derivative is indicated by the fact that deamino-oxytocin,[5,6] a highly potent analog of oxytocin in which the free amino group is replaced by hydrogen, is not inactivated by acetone under the same conditions. Treatment of the acetone-oxytocin with sodium in liquid ammonia resulted in the liberation of approximately 83% of the sulfhydryl groups theoretically obtainable. Upon oxidation of this reduced material in neutral aqueous solution followed by partition chromatography on Sephadex, acetone-oxytocin was recovered in 33% yield (based on the amount of acetone-oxytocin used for the reduction). These results indicate that the isopropylidene group in acetone-oxytocin survives treatment with sodium in liquid ammonia and that the reduced material is the isopropylidene derivative of oxytoceine, the disulfhydryl form of oxytocin.

In the course of studies on the reaction of acetone with various peptides we recently found that S-benzyl-L-cysteinyl-L-tyrosine, which is the N-terminal dipeptide segment of S,S′-dibenzyl-oxytoceine, gives a crystalline monoisopropylidene derivative with acetone that has chemical properties similar to those of the acetone derivative of oxytocin. It then occurred to us that if the structural moiety containing the isopropylidene group in the acetone derivative of the dipeptide were the same as that in acetone-oxytocin, it should be possible to synthesize acetone-oxytocin from this dipeptide derivative. Condensation of the isopropylidene dipeptide with the heptapeptide L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide[7] should give the acetone derivative of S,S′-dibenzyl-oxytoceine, which after cleavage of the benzyl groups in liquid ammonia with sodium[8] and oxidation of the resulting disulfhydryl intermediate should yield acetone-oxytocin. This synthesis has now been accomplished and is presented here.

For the synthesis of the isopropylidene dipeptide, a 1% solution of S-benzyl-L-cysteinyl-L-tyrosine in acetone was allowed to stand at 25° for 26 hr. After removal of the acetone the product was obtained in crystalline form from isopropyl alcohol–hexane, The material is highly soluble in either acetone or isopropyl alcohol, in contrast to the limited solubility of S-benzyl-L-cysteinyl-L-tyrosine in these solvents. When a sample is heated in water at 90° for 10 min acetone is liberated, as is the case with acetone-oxytocin. Elemental analysis of the compound is in agreement with values calculated for an isopropylidene hemihydrate derivative of S-benzyl-cysteinyl-tyrosine. When a sample was boiled for 6 min in 0.1 *N* acetic acid, 98% of the theoretical amount of acetone was liberated. In another experiment the compound was heated at 87° for 3 min in 80% aqueous acetic acid, and crystalline S-benzyl-L-cysteinyl-L-tyrosine was isolated in 78% yield. The isopropylidene-S-benzyl-L-cysteinyl-L-tyrosine migrates toward the anode on paper electrophoresis at pH 5.6 while S-benzyl-L-cysteinyl-L-tyrosine travels toward the cathode, which indicates the absence of a free amino group in the isopropylidene derivative. The isopropylidene derivative

(1) This work was supported in part by Grants HE-01675 and HE-11680 from the National Heart Institute, U. S. Public Health Service.
(2) To whom correspondence should be addressed at the Department of Chemistry, Cornell University, Ithaca, N. Y. 14850.
(3) D. Yamashiro, H. L. Aanning, and V. du Vigneaud, *Proc. Natl. Acad. Sci. U. S.*, **54**, 166 (1965).
(4) "The Pharmacopeia of the United States of America," 17th Revision, Mack Publishing Co., Easton, Pa., 1965, p 475.
(5) V. du Vigneaud, G. Winestock, V. V. S. Murti, D. B. Hope, and R. D. Kimbrough, Jr., *J. Biol. Chem.*, **235**, PC64 (1960); D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *ibid.*, **237**, 1563 (1962).
(6) D. Jarvis and V. du Vigneaud, *Science*, **143**, 545 (1964).
(7) D. B. Hope and V. du Vigneaud, *J. Biol. Chem.*, **237**, 3146 (1962).
(8) R. H. Sifferd and V. du Vigneaud, *ibid.*, **108**, 753 (1935).

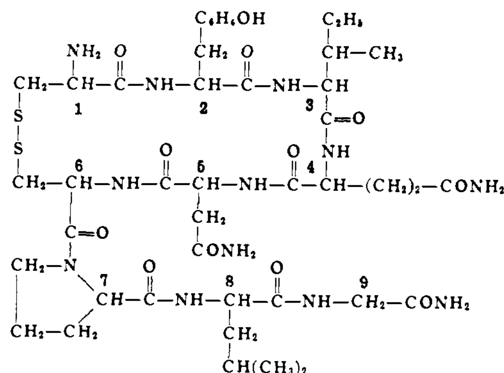

Figure 1. Structure of oxytocin with numbers indicating the position of the individual amino acid residues.

travels well ahead of the free dipeptide on partition chromatography on Sephadex G-25 in 1-butanol–3.5% acetic acid in 1.5% aqueous pyridine (1:1), the R_f values at 4° being approximately 0.82 and 0.43, respectively.

The isopropylidene derivative of S-benzyl-L-cysteinyl-L-tyrosine served as starting material for the synthesis of acetone-oxytocin. In the various steps involved in the synthesis and isolation of acetone-oxytocin the use of acetone was avoided to prevent a spurious synthesis of acetone-oxytocin. The isopropylidene dipeptide was treated with isobutyl chlorocarbonate[9] and then coupled with L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide. The resulting product, presumably the isopropylidene derivative of S,S′-dibenzyl-oxytoceine, was treated with sodium in liquid ammonia for removal of S-benzyl groups. The disulfhydryl intermediate so obtained was titrated in neutral aqueous solution with potassium ferricyanide[5] until the thiol content was negligible. The resulting solution was lyophilized, and the crude product was subjected to partition chromatography on Sephadex G-25 at 4° in 1-butanol–benzene–3.5% acetic acid in 1.5% aqueous pyridine (2:1:3) according to the procedure described previously for the isolation of acetone-oxytocin.[3] Analysis of the eluates by the Folin–Lowry method[10] gave a chromatogram containing a peak with an R_f of 0.34. The material represented by this peak was isolated and rechromatographed in the same solvent system on another column also used under the same conditions for the chromatography of an authentic sample of acetone-oxytocin. The R_f values for the two preparations were the same (0.36).

The synthetic material liberates acetone when it is heated in aqueous solution at 90° for 20 min as was also observed with acetone-oxytocin.[3] When a sample of the material is boiled in 0.1 N acetic acid for 10 min with simultaneous distillation, a quantitative determination of acetone is the distillate shows that the amount present is the same as that released by acetone-oxytocin under these conditions.

The synthetic material and authentic acetone-oxytocin show identical behavior when they are subjected to either gel filtration on Sephadex G-25 in 0.2 N acetic acid or partition chromatography on Sephadex G-25 in 1-butanol–benzene–pyridine–water (7:9:2:18) (R_f 0.29) at 25°. The synthetic and authentic materials migrate at the same rate on electrophoresis at pH 5.6, and their optical rotations in 95% ethanol are in agreement with each other.

The synthetic material exhibits the same low biological activity (about 0.5 unit/mg of avian vasodepressor activity) that was observed for acetone-oxytocin in the earlier study. Heating of a 0.1% solution of this synthetic material in 0.25% acetic acid at 90° for 30 min gives oxytocin in practically the same yield and with about the same avian vasodepressor potency (approximately 500 units/mg) as that obtained for the hormone regenerated from acetone-oxytocin under these conditions.

These comparisons demonstrate that the product obtained by total synthesis starting from the isopropylidene derivative of S-benzyl-L-cysteinyl-L-tyrosine is identical with acetone-oxytocin obtained by the reaction of oxytocin with acetone. Furthermore, the fact that acetone-oxytocin can be prepared from this isopropylidene dipeptide affords strong evidence that the isopropylidene group is part of the same structural moiety in both compounds. In the infrared spectrum[11] of the isopropylidene derivative of S-benzyl-L-cysteinyl-L-tyrosine the amide II band (1530–1560 cm^{-1}) is absent. Since a hydrogen on the nitrogen of the peptide linkage is required for the occurrence of this absorption band, this hydrogen obviously is not present in the derivative. Therefore a Schiff base structure where the isopropylidene group is attached only to the amino nitrogen is excluded. In other studies, not yet completed, evidence has been obtained which indicates the structure of the isopropylidene derivative of S-benzyl-L-cysteinyl-L-tyrosine to be that of a substituted 2,2-dimethyl-4-imidazolidinone. By analogy, acetone-oxytocin would then contain a 4-imidazolidinone ring structure in which the isopropylidene group forms the bridge between the nitrogen of the amino group of the half-cystine residue at position 1 and the nitrogen of the peptide bond between this half-cystine residue and the tyrosine residue at position 2.

Experimental Section[12]

Methods. Sephadex G-25 partition columns were prepared and operated as described previously.[13] Gel filtration was performed on Sephadex G-25 block polymerizate (200–270 mesh). Peptide materials in the column effluents were detected by the Folin–Lowry procedure.[10]

For qualitative detection of acetone by a modified Legal test,[14] solutions were spotted on "Acetest" reagent tablets (Ames Co., Inc., Elkhart, Ind.). A test was considered positive when a permanent purple color developed similar to that given by 0.1% aqueous acetone.

For the 2,4-dinitrophenylhydrazine test, the reagent was prepared by dissolving 0.25 g of 2,4-dinitrophenylhydrazine in a mixture of 0.6 ml of 6 N HCl and 25 ml of methanol. A sample (1 to 2 mg) of the peptide to be tested was heated with an aliquot (0.1 ml) of the reagent in a sealed tube at 90° for 20 min. Aliquots (0.1

(9) J. R. Vaughan, Jr., and J. A. Eichler, *J. Amer. Chem. Soc.*, **75**, 5556 (1953).

(10) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).

(11) L. J. Bellamy "The Infrared Spectra of Complex Molecules," 2nd ed, John Wiley and Son, Inc., New York, N. Y., 1958.

(12) All melting points were done in capillary tubes and are corrected.

(13) D. Yamashiro, *Nature*, **201**, 76 (1964); D. Yamashiro, D. Gillessen, and V. du Vigneaud, *J. Amer. Chem. Soc*, **88**, 1310 (1966).

(14) The test, used clinically for detection of ketone bodies, is described in "Documenta Geigy: Scientific Tables," 6th ed, K. Diem, Ed., Geigy Pharmaceuticals, Ardsley, N. Y., 1962, pp 532, 537.

μl) of the resulting solution and of the reagent, along with an authentic sample (0.5 μg) of acetone 2,4-dinitrophenylhydrazone, were subjected to paper chromatography.[15] The reagent remained at the starting line, and the presence of acetone 2,4-dinitrophenylhydrazone was detected under ultraviolet light by comparison of its R_f with that of the authentic hydrazone.

For the quantitative determination of acetone liberated by the isopropylidene derivatives, a sample (~1 mg) was heated in 5 ml of 0.1 *N* acetic acid in an apparatus where the distillate could be collected at 0° in a 5-ml volumetric flask containing 2 ml of water. The sample solution was heated near the boiling point for 5 min and then was boiled vigorously until almost 3 ml of distillate was collected. Appropriate aliquots of the distillate were analyzed by the salicylaldehyde method.[16]

Quantitative sulfhydryl determination was performed according to the procedure of Ellman.[17] Paper electrophoresis was carried out at 4° in pyridine acetate buffer of pH 5.6 at 300 V for 18 hr, and the Pauly reagent was used for color development. Infrared spectra were recorded on a Perkin-Elmer Model 237 spectrophotometer. Avian vasodepressor activity[18] was measured against the USP posterior pituitary reference standard.

Isopropylidene-S-benzyl-L-cysteinyl-L-tyrosine. S-Benzyl-L-cysteinyl-L-tyrosine[19] (1.00 g) was stirred in 100 ml of acetone (Spectranalyzed Reagent, Fisher Scientific Co., New York, N. Y.) at 25° for 27 hr. The resulting solution was evaporated *in vacuo* at 10° to an oil which was dissolved in 15 ml of isopropyl alcohol and then diluted with 3 ml of hexane.[20] After storage at 4° for 43 hr, a crystalline product separated which was collected on a filter at 4° and washed with four 5-ml portions of cold isopropyl alcohol-hexane (5:2); yield, 484 mg; mp 88-90°; $[\alpha]^{20}D$ −100° (*c* 1, acetone); infrared absorption (KBr) at 1720 (COOH), 1690 (amide I), 1370, and 1175 cm^{-1}.

Anal. Calcd for $C_{22}H_{26}N_2O_4S \cdot 0.5H_2O$: C, 62.4; H, 6.42; N, 6.62. Found: C, 62.4; H, 6.52; N, 6.32.

A sample (1.85 mg) was heated in 0.1 ml of water at 90° for 10 min. When the solution was cooled a gel formed, and the supernatant solution gave a positive Legal test. A heated solution of S-benzyl-L-cysteinyl-L-tyrosine of the same concentration gave a negative Legal test. Another sample (1.8 mg) of the isopropylidene derivative gave a positive 2,4-dinitrophenylhydrazine test under the conditions described in the Methods section.

A sample (2.99 mg) was heated in 5.0 ml of 0.1 *N* acetic acid at the boiling point for 6 min with simultaneous distillation, and a quantitative determination of acetone in the distillate showed that 98% of the theoretical amount of acetone had been liberated.

A sample (91 mg) was dissolved in 5 ml of 80% (v/v) aqueous acetic acid and heated at 87° for 3 min. The solution was evaporated *in vacuo*, and the residue was repeatedly evaporated *in vacuo* with benzene to remove traces of acetic acid. The material was dissolved in 5 ml of isopropyl alcohol at 90° within 2 min, and the cooled solution was diluted with 1 ml of hexane. The crystalline product (64 mg) had mp 103-107° and $[\alpha]^{18}D$ +26.5° (*c* 1, 80% acetic acid) as compared to a sample of S-benzyl-L-cysteinyl-L-tyrosine which had mp 103-107°, $[\alpha]^{18}D$ +26.5° (*c* 1, 80% acetic acid), and $[\alpha]^{20}D$ +25.3° (*c* 1, dimethylformamide) (lit. mp 114-115°,[19a] 117-119°,[19b] $[\alpha]^{27}D$ +26.5° (*c* 1, dimethylformamide)[19a]).

Acetone-oxytocin (Isopropylidene-oxytocin). Isopropylidene-S-benzyl-L-cysteinyl-L-tyrosine (124 mg) was dissolved in 2.0 ml of dry tetrahydrofuran and cooled to −25° before the addition of 0.042 ml of triethylamine followed by 0.040 ml of isobutyl chlorocarbonate. The mixture was warmed to −10° over a 15-min period and then cooled to −40°. A solution of 253 mg of crystalline L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide[7] in 2.5 ml of dimethylformamide was added, and the mixture was gradually warmed to 15° over a period of 5 hr. The thick mixture was diluted with 30 ml of ice-cold water and allowed to stand at 4° for 1 hr. The crude product was collected on a filter at 4°, washed with two 5-ml portions of ice-cold water, and dried *in vacuo* over P_2O_5 at 4°; yield, 261 mg.

This crude protected nonapeptide was dissolved in 150 ml of liquid ammonia (distilled from sodium) and treated with sodium until a blue color persisted throughout the solution for 1 min. The solution was evaporated *in vacuo* to low volume and lyophilized. The resulting powder was dissolved in 200 ml of deaerated water containing 0.1 ml of trifluoroacetic acid. The pH of the solution was adjusted to 7.5 with 1 *N* NH_4OH (0.5 ml). The sulfhydryl content of the solution was 352 μmol. The solution was treated with 35 ml of 0.01 *N* potassium ferricyanide while 1 *N* NH_4OH was added to maintain a pH of 7. The sulfhydryl content of the resulting solution was 3 μmol. The solution was stirred with 10 ml (wet volume) of the trifluoroacetate form of AG3X4 resin (Bio-Rad Laboratories, Richmond, Calif.) for 15 min after which time the resin was filtered off. These oxidation procedures were carried out within a period of 1 hr at 25°. The solution obtained after removal of the resin was lyophilized.

The crude product was subjected to partition chromatography on Sephadex G-25 at 4° under the following conditions: solvent, 1-butanol-benzene-3.5% acetic acid in 1.5% aqueous pyridine (2:1:3); column size, 2.85 × 52.8 cm; hold-up volume, 73.5 ml; flow rate, 22 cc/hr; fraction size, 5.25 ml; aliquots for Folin-Lowry determination, 0.10 ml; solvent for regeneration of column, pyridine-0.2 *N* aqueous acetic acid (5:4). Two peaks were detected in the chromatogram with R_f values of 0.70 and 0.34. The eluates represented by the peak with R_f 0.34 were mixed with 150 ml of ice-cold water, evaporated *in vacuo* to low volume in a bath at 15°, and lyophilized; yield, 62 mg. A portion (60 mg) of this product was rechromatographed at 4° in the same solvent system as follows: column size, 2.20 × 48.9 cm; hold-up volume, 46 ml; flow rate, 12 cc/hr; fraction size, 3.5 ml; aliquots for Folin-Lowry determination, 0.05 ml. One peak was detected with R_f 0.36, and the material isolated from the eluates represented by the central portion of the peak weighed 41 mg. Chromatography of an authentic sample (9.8 mg) of acetone-oxytocin on the same column gave a peak with R_f 0.36.

Comparisons of Synthetic and Authentic Acetone-oxytocin. The following tests for acetone were performed under the conditions noted in the Methods section. A sample (1 mg) of the synthetic material was dissolved in 0.05 ml of water. The solution gave a negative Legal test, but after it was heated at 90° for 20 min, a positive test was obtained. The synthetic material gave a positive 2,4-dinitrophenylhydrazine test. A sample of the synthetic material (1.082 mg) in 5.0 ml of 0.1 *N* acetic acid was heated near and at the boiling point for 10 min and a quantitative determination of acetone in the distillate showed that 99% of the theoretical amount of acetone had been liberated from the compound. The results in these three tests were the same as those obtained with an authentic sample of acetone-oxytocin.[3]

The synthetic material (2.3 mg) was subjected to partition chromatography on Sephadex G-25 in a second solvent system under the following conditions: solvent, 1-butanol-benzene-pyridine-water (7:9:2:18) at 25°; column size, 1.05 × 52.9 cm; hold-up volume, 11.4 ml; flow rate, 3.3 cc/hr; fraction size, 1.0 ml; aliquots for Folin-Lowry determination, 0.20 ml; solvent for regeneration of column, pyridine-0.2 *N* aqueous acetic acid (5:4). One sharp, symmetrical peak was detected with R_f 0.29 which agrees, within experimental error, with the value of 0.33 obtained with an authentic sample of acetone-oxytocin. Partition chromatography of a mixture of the synthetic material (1.25 mg) and authentic acetone-oxytocin (1.43 mg) under the same conditions gave one sharp, symmetrical peak with R_f 0.31 which agrees, within experimental error, with the R_f values obtained when the preparations were chromatographed separately.

The synthetic preparation (1.96 mg) was subjected to gel filtration on Sephadex G-25 as follows: solvent, 0.2 *N* acetic acid at 25°; column size, 1.38 × 83.5 cm; flow rate, 7 cc/hr; fraction size, 0.86 ml; aliquots for Folin-Lowry determination, 0.6 ml. Only one peak was detected with a maximum at effluent volume 103 ml, the same effluent volume at which the maximum occurred on gel filtration of authentic acetone-oxytocin. Gel filtration of a mixture of synthetic material (0.60 mg) and authentic acetone-oxytocin (0.65 mg) under exactly the same conditions gave one peak with a maximum at the same effluent volume.

The synthetic material exhibited a specific rotation of $[\alpha]^{19}D$ −13.6° (*c* 1, 95% ethanol) as compared to $[\alpha]^{20}D$ −13° (*c* 1, 95% ethanol) reported for acetone-oxytocin.[3]

(15) A. I. Schepartz, *J. Chromatogr.*, **6**, 185 (1961).

(16) J. A. Behre and S R. Benedict, *J. Biol. Chem.*, **70**, 487 (1926); R. N. Harger and R. B. Forney, "Standard Methods of Chemical Analysis," Vol. 2, F. J. Welcher, Ed., D. Van Nostrand Co., Inc., Princeton, N. J., 1963, p 2139.

(17) G. L. Ellman, *Arch. Biochem. Biophys.*, **82**, 70 (1959).

(18) R. A. Munsick, W. H. Sawyer, and H. B. van Dyke, *Endocrinology*, **66**, 860 (1960).

(19) (a) H. S. Bachelard and V. M. Trikojus, *J. Chem. Soc.*, 4541 (1958); (b) K. Jošt, and J. Rudinger, *Collection Czech. Chem. Commun.*, **26**, 2345 (1961).

(20) Initial crystallization was effected by storage of the oil at 4° for several days. In some runs crystallization from isopropyl alcohol-hexane required more hexane.

The synthetic material had an avian vasodepressor potency of approximately 0.5 unit/mg, the same as that previously reported for acetone-oxytocin.[8] To test for the liberation of oxytocin from the synthetic material, 9.05 mg of the compound was dissolved in 9.0 ml of 0.25% acetic acid and heated at 90° for 30 min. The solution was lyophilized, and the product was subjected to partition chromatography on Sephadex in the manner previously described for the purification of oxytocin.[13] The conditions were as follows: solvent, 1-butanol-benzene-pyridine-0.1% aqueous acetic acid (6:2:1:9) at 25°; column size, 1.05 × 52.9 cm; hold-up volume, 12.2 ml; flow rate, 3.5 cc/hr; fraction size, 1.0 ml; aliquots for Folin-Lowry determination, 0.05 ml; solvent for regeneration of column, pyridine-0.2 *N* aqueous acetic acid (3:5). One peak was detected with R_f 0.28, the same value obtained under these conditions with an authentic sample of oxytocin. The R_f value for acetone-oxytocin under these conditions is about 0.7, well ahead of the position of oxytocin.[8] The material isolated from the eluates represented by the peak with R_f 0.28 weighed 6.4 mg and had an avian vasodepressor potency of approximately 500 units/mg. These results are practically identical with those previously reported for the regeneration of oxytocin from acetone-oxytocin.[8]

Acknowledgments. The authors wish to thank the following members of this laboratory for their cooperation: Mr. Joseph Albert for the elemental analyses, and Miss Margitta Wahrenburg, Mrs. Maxine Goldberg, and Mrs. Jessie Lawrence for the avian vasodepressor assays under the direction of Dr. W. Y. Chan.

Communications to the Editor

The Effect of Alcohol on Proton Exchange Reactions in Dimethyl Sulfoxide. I. Low Alcohol Concentrations

Sir:

Chemical reactions in solutions prepared by dissolving alkoxides in dimethyl sulfoxide (DMSO) have been widely studied.[1] Measurements of rates of proton transfer from carbon acids have been made,[2,3] and equilibrium constants have been measured.[4] Although the "active" base in these solutions has generally been assumed to be alkoxide, recent work by Ritchie and Uschold has indicated that an appreciable amount of the "kinetic" and equilibrium basicity may be due to the presence of methylsulfinylcarbanion, $CH_3SOCH_2^-$, the conjugate base of DMSO.[3c]

We have previously measured the rate of proton exchange between DMSO and $CH_3SOCH_2^-$ by ^{13}C satellite proton nmr line broadening.[5] Our method of measurement allows a unique insight into proton-transfer processes in these solutions because rates can be measured under equilibrium conditions while reactions are proceeding continuously. In this communication we wish to report the effect of added *t*-butyl alcohol (ROH) on the rate of proton removal from carbon acids in the presence of $CH_3SOCH_2^-K^+$. For total base strengths in the range ~0.01-~0.25 *M*, *addition of ROH causes a significant increase in the rate of proton removal from DMSO.* This effect reaches a maximum at ~2 equiv of ROH/equiv of base. The rates then decrease rapidly, but even at ROH:base of 5:1 the rates are faster than those in the absence of ROH. At its largest, the acceleration amounts to a factor of about 20. Typical results are summarized in Table I.

The rates of proton abstraction were determined by measuring the width of the ^{13}C satellite of DMSO.[5] The constant half-width of an internal standard showed that neither paramagnetic impurities nor viscosity effects were responsible for the line broadening. The increased rate of exchange is clearly demonstrated by the observation that the ^{13}C satellite of DMSO is substantially broadened when ROH is added to a solution of $CH_3SOCH_2^-K^+$ in DMSO. This additional broadening implies that the lifetime of the protons of DMSO is shortened. Results from solutions made from RO^-K^+ + ROH were identical with those from solutions made from $NH_2^-K^+$ + ROH or KH + ROH.[6]

Table I. Width at Half-Height of ^{13}C Satellite (0.0153 *M* Base)[a]

Expt	*t*-BuOH, *M*[b]	Width, Hz
317	0	1.4
319	0.0134	4.0
320	0.0209	6.1
321	0.0271	6.8
322	0.0362	2.9
323	0.0576	2.8
324	0.0842	1.6

[a] Solutions made from KNH_2 + DMSO. [b] *t*-BuOH added to original solution.

We are unable to specify the kinetically active base under these conditions. However, the absence of an OH peak in the nmr spectrum implies that RO^-K^+ is involved to some extent in the exchange process. A rather sharp maximum in rate at ROH:base of 2:1 suggests possible formation of a kinetically reactive 2:1 complex which becomes less reactive, or is destroyed, as ROH is further increased.

We have obtained additional evidence that *proton removal from other carbon acids in DMSO is also ac-*

(1) See D. J. Cram, "Fundamentals of Carbanion Chemistry," Academic Press Inc., New York, N. Y., 1965, for a good general discussion and references; A. J. Parker, *Quart. Rev.* (London), **16**, 163 (1962).

(2) J. E. Hofmann, R. J. Muller, and A. Schriesheim, *J. Am. Chem. Soc.*, **85**, 3002 (1963).

(3) C. D. Ritchie and R. E. Uschold: (a) *ibid.*, **86**, 4488 (1964); (b) *ibid.*, **89**, 1730 (1967); (c) *ibid.* **89**, 2960 (1967).

(4) E. C. Steiner and J. M. Gilbert, *ibid.*, **85**, 3054 (1963); **87**, 382 (1965); E. C. Steiner and J. D. Starkey, *ibid.*, **89**, 2751 (1967).

(5) J. I. Brauman and N. J. Nelson, *ibid.*, **88**, 2332 (1966).

(6) Solutions were made up and handled entirely with vacuum line techniques. Solutions were filtered prior to placement in nmr tubes; base concentrations were determined by titration. Spectra were obtained with a Varian A-60 spectrometer.