melt. Due to the ease with which dihydroperlolidine underwent air oxidation upon standing in solution, we were never able to obtain this compound completely free of perlolidine.

Anal. Calcd for $C_{12}H_{10}N_2O$: mol wt, 198.07931. Found: mol wt, 198.07923.

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The Structure of Acetone-Oxytocin with Studies on the Reaction of Acetone with Various Peptides[1]

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Abstract: The structure of acetone-oxytocin has been shown to possess a 2,2-dimethyl-4-imidazolidinone ring structure in which the isopropylidene group from acetone forms a bridge between the nitrogen of the free amino group of one of the half-cystine residues of oxytocin and the nitrogen of the peptide bond between this half-cystine residue and the succeeding tyrosine residue. These results were obtained by extensive comparison of the chemical and spectral properties of the acetone derivative of S-benzyl-L-cysteinyl-L-tyrosine, from which acetone-oxytocin can be synthesized, with those of 2,2-dimethyl-4-imidazolidinone and 5-imino-2,2-dimethyloxazolidine. When L-prolyl-L-leucylglycinamide was treated with acetone under anhydrous conditions, a product was isolated which similarly was shown to be a substituted 2,2-dimethyl-4-imidazolidinone. On the other hand, treatment of S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide which can form neither an imidazolidinone nor an oxazolidine with acetone afforded no isolable product. However, the rapid formation of a Schiff base could be demonstrated by a trapping experiment using sodium borohydride which led to the formation of N-isopropyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide.

In a recent communication,[3] the inactivation of oxytocin (Figure 1) by acetone was reported, and the new compound which formed, referred to as acetone-oxytocin, was isolated. Analytical and chemical evidence showed acetone-oxytocin to be a monoisopropylidene derivative of oxytocin. Further, the N-terminal amino group of oxytocin was shown to be involved since deamino-oxytocin, a highly potent analog of oxytocin in which the free amino group is replaced by hydrogen,[4,5] is not inactivated by acetone. Parallel experiments with lysine-vasopressin[6] led to similar inactivation, and a monoisopropylidene derivative of lysine-vasopressin, referred to as acetone-lysine-vasopressin, was isolated. As in the case of deamino-oxytocin, 1-deamino-8-lysine-vasopressin[7] was not inactivated by treatment with acetone, no isolable derivative of the deamino analog was formed, and only 1-deamino-lysine-vasopressin was recovered. This indicated that the amino group at position 1 of lysine-vasopressin, as in oxytocin, is necessary for formation of acetone-lysine-vasopressin.

The full biological activities of oxytocin and lysine-vasopressin could be recovered from acetone-oxytocin and acetone-lysine-vasopressin, respectively, by treatment of the derivatives with 0.25% acetic acid at 90° for 30 min. Acetone is liberated from both compounds mole for mole when either is heated at 100° in 0.1 *N* acetic acid for a short period of time.

More recently it was found[8] that S-benzyl-L-cysteinyl-L-tyrosine (I), the N-terminal dipeptide segment of S,S′-dibenzyl-oxytoceine, gave a crystalline monoisopropylidene derivative with acetone from which total synthesis of acetone-oxytocin was accomplished. The synthesis was achieved by first coupling the isopropylidene dipeptide with the heptapeptide L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide. The resulting isopropylidene derivative of S,S′-dibenzyl-oxytoceine was treated with sodium in liquid ammonia for removal of the S-benzyl groups, the disulfhydryl intermediate was oxidized with potassium ferricyanide, and the product was isolated. Since the synthetic product was found to be identical with acetone-oxytocin obtained by inactivation of oxytocin with acetone, the isopropylidene groups in both acetone-oxytocin and the isopropylidene derivative of S-benzyl-L-cysteinyl-L-tyrosine

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(3) D. Yamashiro, H. L. Aanning, and V. du Vigneaud, *Proc. Nat. Acad. Sci. U. S.*, **54**, 166 (1965).

(4) V. du Vigneaud, G. Winestock, V. V. S. Murti, D. B. Hope, and R. D. Kimbrough, Jr., *J. Biol. Chem.*, **235**, PC 64 (1960); D. B. Hope, V. V. S. Murti, and V. du Vigneaud, *ibid.*, **237**, 1563 (1962).

(5) D. Jarvis and V. du Vigneaud, *Science*, **143**, 545 (1964).

(6) D. Yamashiro, R. T. Havran, H. L. Aanning, and V. du Vigneaud, *Proc. Nat. Acad. Sci. U.S.*, **57**, 1058 (1967).

(7) R. D. Kimbrough, Jr., W. D. Cash, L. A. Branda, W. Y. Chan, and V. du Vigneaud, *J. Biol. Chem.*, **238**, 1411 (1963).

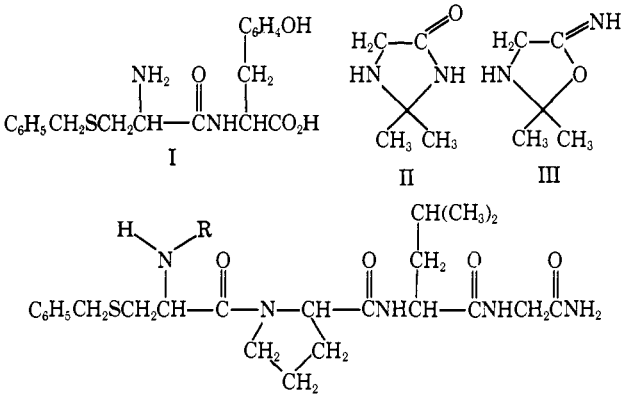
(8) D. Yamashiro and V. du Vigneaud, *J. Amer. Chem. Soc.*, **90**, 487 (1968).

are part of the same structural moiety in the two compounds. Thus if the structure of the dipeptide derivative could be elucidated, the structure of acetone-oxytocin would be established.

In the initial study,[3] a Schiff base and a substituted derivative of 2,2-dimethyl-4-imidazolidinone (II) were among the types of isopropylidene derivatives considered as possible structures for acetone-oxytocin. A substituted derivative of 5-imino-2,2-dimethyloxazolidine (III) also has been considered.[6] In the communication on the total synthesis of acetone-oxytocin,[8] the Schiff base structure for the isopropylidene-S-benzyl-L-cysteinyl-L-tyrosine, and therefore for acetone-oxytocin, was excluded by infrared data, since the absence of the amide II band in the spectrum of the isopropylidene dipeptide, indicating the absence of a hydrogen on the nitrogen of the peptide bond, excluded the possibility that the isopropylidene group could be attached only to the amino nitrogen.

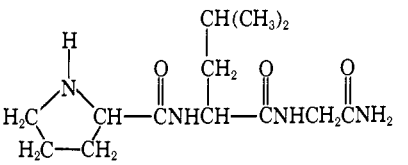
We have therefore further investigated chemical and spectral properties of the isopropylidene derivative of S-benzyl-L-cysteinyl-L-tyrosine to distinguish between the imidazolidinone and oxazolidine structures and report the results of these studies. During the course of these studies we also investigated the reaction of S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (IVa) and L-prolyl-L-leucylglycinamide (V) with acetone (see Chart I).

Chart I. Various Compounds Studied



I II III

IVa, R = H
b, R = CH(CH$_3$)$_2$



V

It is known that glycinamide reacts with acetone to form the isopropylidene derivative 5-imino-2,2-dimethyloxazolidine which in turn rearranges in hot pyridine to the isomeric 2,2-dimethyl-4-imidazolidinone.[9] On the other hand, carbobenzoxy amino acid amides react with aldehydes and ketones to give directly the corresponding substituted 4-imidazolidinones.[10] Recently the very interesting preparation of hetacillin, an isopropylidene derivative of the peptide antibiotic 6-[D-(−)-α-aminophenylacetamido]penicillanic acid, by reaction of the peptide with acetone has been reported.[11] The structure of hetacillin was demonstrated by X-ray diffraction analysis to be a substituted 2,2-dimethyl-4-imidazolidinone.

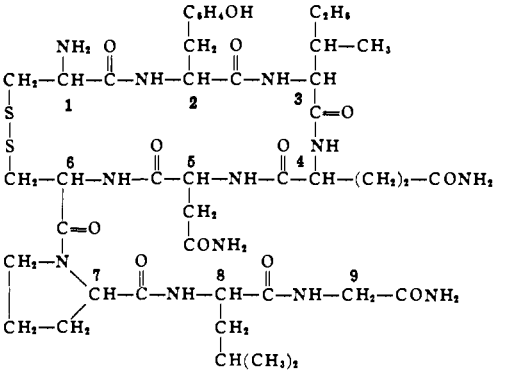


Figure 1. Structure of oxytocin with numbers indicating the position of individual amino acid residues.

The stability of 2,2-dimethyl-4-imidazolidinone in aqueous solution is greater than that of 5-imino-2,2-dimethyloxazolidine since the latter liberates acetone in cold water whereas the former requires heating in hot water to liberate acetone.[9] The imidazolidinones described by Zehavi and Ben-Ishai[10] and the hetacillin reported by Hardcastle, *et al.*,[11] possessed hydrolytic stabilities resembling 2,2-dimethyl-4-imidazolidinone. In comparison, acetone-oxytocin[3] and the isopropylidene-S-benzyl-L-cysteinyl-L-tyrosine[8] on contact with water at room temperature do not liberate acetone, but upon heating aqueous solutions of these acetone derivatives at 90°, acetone is detected in the solution by the Legal test. Thus, the hydrolytic behavior of acetone-oxytocin and the isopropylidene-S-benzyl-L-cysteinyl-L-tryosine resembles that of known imidazolidinones. It may be noted that after hydrolysis of the isopropylidene-S-benzyl-L-cysteinyl-L-tyrosine, it was possible to isolate the S-benzyl-L-cysteinyl-L-tyrosine in its original optical purity.

The structural moiety containing the isopropylidene group in acetone-oxytocin survives treatment with sodium in liquid ammonia.[3] When 2,2-dimethyl-4-imidazolidinone (II) was treated with sodium in liquid ammonia, practically no consumption of sodium was observed, as indicated by the persistent blue color in the solution, and the imidazolidinone II was recovered. Treatment of 5-imino-2,2-dimethyloxazolidine (III) in the same manner resulted in a large uptake of sodium, and the oxazolidine III could not be recovered. Hence acetone-oxytocin resembles more closely the behavior of the imidazolidinone than the oxazolidine under these conditions.

In efforts to study the possibility of forming the substituted derivative of 2,2-dimethyl-4-imidazolidinone (II) or 5-imino-2,2-dimethyloxazolidine (III) with a compound for which Schiff base formation is excluded, experiments on the reaction of the tripeptide, L-prolyl-L-leucylglycinamide (V), with acetone were carried out since this peptide contains an N-terminal proline residue. It was found that an acetone derivative could be obtained provided the reaction was carried out under

(9) A. C. Davis and A. L. Levy, *J. Chem. Soc.*, 3479 (1951).
(10) U. Zehavi and D. Ben-Ishai, *J. Org. Chem.*, **26**, 1097 (1961).
(11) G. A. Hardcastle, Jr., D. A. Johnson, C. A. Panetta, A. I. Scott, and S. A. Sutherland, *ibid.*, **31**, 897 (1966).

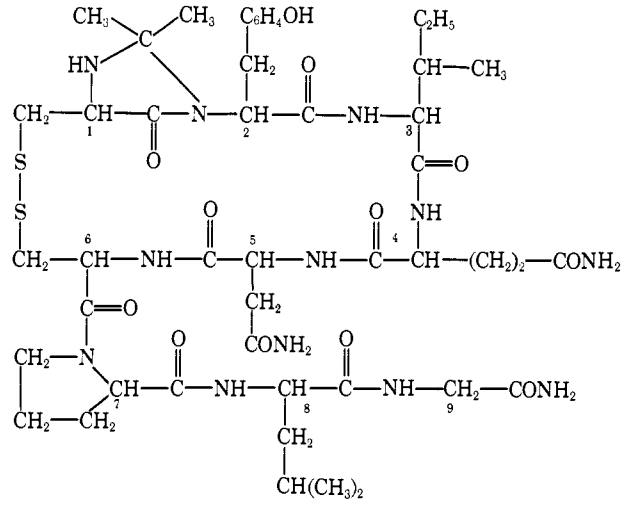


Figure 2. Structure of acetone-oxytocin with numbers indicating the position of the individual amino acid residues.

anhydrous conditions. The acetone derivative also proved to be a monoisopropylidene derivative of L-prolyl-L-leucylglycinamide which could be isolated in two crystalline forms, a low-melting form (83–85°) and a high melting form (143–145°), both of which gave identical infrared spectra in chloroform solution. It is interesting that this derivative liberates acetone on contact with cold water.

The chemical and analytical evidence which indicated the presence of the isopropylidene group in the acetone derivative of S-benzyl-L-cysteinyl-L-tyrosine is supported by the infrared spectrum of the derivative in which peaks due to the *gem*-dimethyl groups at 1385 and 1370 cm^{-1} are present. Similar bands at 1385 and 1375 cm^{-1} were also observed in the spectrum of the acetone derivative of L-prolyl-L-leucylglycinamide.

The evidence so far obtained suggests that the isopropylidene group might be part of a 4-imidazolidinone ring, but further evidence for this and for exclusion of the oxazolidine structure was desired. For this purpose the nuclear magnetic resonance spectrum[12] of 2,2-dimethyl-4-imidazolidinone (II), 5-imino-2,2-dimethyloxazolidine (III), and the isopropylidene derivative of L-prolyl-L-leucylglycinamide (VI) and of S-benzyl-L-cysteinyl-L-tyrosine (VII) were determined. Of particular interest for our purposes are the absorptions of the *gem*-dimethyl groups. It was observed (see Table I) that the absorptions of the methyl hydrogens for compounds VI (1.50 and 1.40 ppm) and VII (1.45 and 1.20 ppm) agree well with those of 2,2-dimethyl-4-imidazolidinone (II) at 1.44 ppm, but not with those of 5-imino-2,2-dimethyloxazolidine (III) at 1.89 ppm. The different absorptions of the hydrogens of the two methyl groups (H_b) in compound VI undoubtedly result from their slightly different steric and chemical environments. A similar result obtains in compound VII, and in this case examination of space-filling molecular models shows that the hydrogens of one of the methyl groups may be slightly influenced by the aromatic ring current of the tyrosine phenyl ring. This would account for the much larger shift upfield for the hydrogens of one of the methyl groups at 1.20 ppm. Thus, the nmr data afford direct evidence that the isopropylidene group in the acetone derivatives of both L-prolyl-L-leucylglycinamide and S-benzyl-L-cysteinyl-L-tyrosine is part of a 4-imidazolidinone structure.

Since the Schiff base structure was excluded for the acetone derivatives of the peptides thus far mentioned, it was of interest to investigate under what conditions a Schiff base would be formed. In the tetrapeptide S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (IVa), a proline residue is present next to the N-terminal residue, and the possibility of forming either the imidazolidinone or the oxazolidine derivative with acetone is excluded since there is no hydrogen on the proline nitrogen of the tetrapeptide. Repeated attempts to isolate an acetone derivative with this tetrapeptide under conditions used for the preparation of acetone-oxytocin and the isopropylidene-S-benzyl-L-cysteinyl-L-tyrosine (VII) led to the recovery of the tetrapeptide. However, it was observed that a solution of the tetrapeptide in 94% aqueous acetone exhibited a rapid change in optical rotation which occurred within 30 min. This led us to suspect that the tetrapeptide in the presence of acetone forms a Schiff base which is unstable under the conditions we had employed for its isolation. Since further attempts to isolate the Schiff base in pure form under anhydrous conditions were unsuccessful, we decided to try to detect the presence of such a derivative, if it were formed, by the technique used for demonstrating the formation of a Schiff base acetone derivative of oxytocin as an intermediate in the formation of acetone-oxytocin.[13] Thus a 60% aqueous acetone solution of S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (IVa) was treated with sodium borohydride at pH 5 to trap the Schiff base as the isopropyl derivative IVb. After the reaction mixture was neutralized, the solvents were removed in a vacuum, and the mixture was purified by partition chromatography on Sephadex.[14] N-Isopropyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (IVb) was isolated in good yield. This indicates that the Schiff base of IVa had been formed, but was not isolable under conditions used for the isolation of acetone-oxytocin. Thus, although IVa cannot form an imidazolidinone derivative, the evidence indicates that a Schiff base can be formed and undoubtedly accounts for the change in optical rotation when IVa is exposed to acetone.

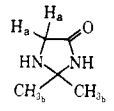
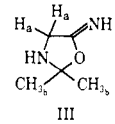
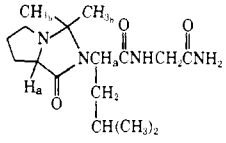
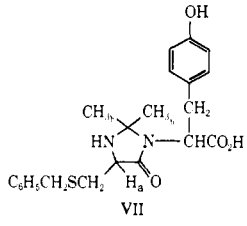
The combination of chemical and physical data presented has shown that the structure of the acetone derivative of S-benzyl-L-cysteinyl-L-tyrosine (I) is that of the 2,2-dimethyl-4-imidazolidinone VII (Table I). Since acetone-oxytocin has been synthesized from this acetone dipeptide, it may be concluded that acetone-oxytocin likewise possesses a 2,2-dimethyl-4-imidazolidinone ring structure in which the isopropylidene group from acetone forms a bridge between the nitrogen atom 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 as shown in Figure 2.

(12) The nmr spectra were determined using a Varian A-60 spectrometer. Absorptions are given in parts per million (ppm) downfield from internal tetramethylsilane (TMS).

(13) Unpublished results of V. J. Hruby and V. du Vigneaud.

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

Table I. Pertinent Nmr Data

Compound	Solvent	H_a	H_b	Area found H_b
 II	$CDCl_3$	3.50 (d)	1.44 (s)	6.0
	Acetone-d_6	3.33 (s)	1.38 (s)	6.0
 III	$CDCl_3$	3.76 (s)	1.89 (d)	6.0
	Acetone-d_6	3.75 (s)	1.88 (t)	6.0
 VI	$CDCl_3$	4.0–4.1 (m)[a]	1.50 (s)	3.1
			1.40 (s)	3.1
	Acetone-d_6	~3.9 (bm)[a]	1.50 (s), 1.41 (s)	5.4
 VII	Acetone-d_6	~4.18 (m)	1.45 (s)	2.8
			1.20 (d)	2.9

[a] The hydrogen α to a peptide bond is usually found between 3.9 and 4.3 ppm in $CDCl_3$ or acetone-d_6 solution and shifts very little in these instances.

Experimental Section

Methods. Sephadex G-25 partition chromatography columns were prepared and operated as previously described.[14] Acetone was detected qualitatively by a modified Legal test.[3] For quantitative determination of acetone liberated by a compound, a sample (~1 mg) was boiled in water (5 ml) with simultaneous distillation, and the distillate was analyzed for acetone as previously described.[3] Nmr spectra were determined on solutions of the compounds containing tetramethylsilane as an internal standard using a Varian A-60 spectrometer at ambient temperatures. Infrared spectra were taken on a Perkin-Elmer Model 237 spectrophotometer and the absorptions are given in cm^{-1}. Assignments are given in parentheses.

Materials. The 2,2-dimethyl-4-imidazolidinone (II) and 5-imino-2,2-dimethyloxazolidine (III) used in these studies were prepared by the method of Davis and Levy.[9] The isopropylidene-S-benzyl-L-cysteinyl-L-tyrosine (VII) was prepared as previously described.[8] The isopropylidene-L-propyl-L-leucylglycinamide (VI) was prepared as described herein.

Infrared Results. The infrared spectra for the following compounds were determined.

5-Imino-2,2-dimethyloxazolidine (III) in $CHCl_3$: 1680 (C=N), 1560 (-NH), and 1390 and 1370 (*gem*-dimethyls); in KBr: 1670 (C=N), 1600 (-NH), and 1380 and 1360 (*gem*-dimethyls), no absorption at 1175.

2,2-Dimethyl-4-imidazolidinone (II) in $CHCl_3$: 1710 (C=O) and 1390 and 1375 (*gem*-dimethyls); in KBr: 1680 (C=O) 1385 and 1370 (*gem*-dimethyls), and 1175 (isopropylidene).

Isopropylidene-S-benzyl-L-cysteinyl-L-tyrosine (VII) in $CHCl_3$: 1755 ($-CO_2H$) and 1710 (amide I); in KBr: 1720 ($-CO_2H$), 1690 (amide I), 1610 and 1515 (aromatic), 1385 and 1370 (*gem*-dimethyls), and 1175 (isopropylidene).

Isopropylidene-L-prolyl-L-leucylglycinamide (VI) in $CHCl_3$: 1590 ($CONH_2$ amide II), 1550–1520 (CONH amide II), and 1390 and 1370 (*gem*-dimethyls); in KBr: 1650 ($CONH_2$ amide II), 1520 (CONH amide II), and 1385 and 1375 (*gem*-dimethyls).

Isopropylidene-L-prolyl-L-leucylglycinamide (VI). A sample (1.00 g) of L-prolyl-L-leucylglycinamide hemihydrate[15] was stirred in anhydrous acetone (100 ml) for 1 hr at room temperature. The resulting solution was allowed to stand over Drierite (20 g) for 21 hr. After separation from the drying agent, the solution was evaporated *in vacuo* to a volume of about 50 ml, diluted with dry benzene (50 ml), and evaporated to dryness. The powder was dissolved in dry acetone (20 ml). One-half of the solution was removed and to the other half hexane (30 ml) was added. The latter solution was stored at 4° overnight. The thick mass of crystalline material formed was collected on a filter at 4° and washed with 10 ml of anhydrous acetone–hexane (2:5) and hexane (20 ml) to give 455 mg, mp 93–95° dec. The product was recrystallized by dissolving it in anhydrous acetone (6 ml) and diluting the solution with anhydrous ether (15 ml). After 2 hr at 4°, the heavy crystalline product was collected on a filter and washed with 10 ml of anhydrous acetone–anhydrous ether (1:4) and anhydrous ether (10 ml) to give 262 mg, mp 143–145°, $[\alpha]^{22}D$ +42° (*c* 1, anhydrous acetone).[16] For analysis, a sample was dried over P_2O_5 at 25° (0.2 mm) for 10 hr.

Anal. Calcd for $C_{16}H_{28}N_4O_3$: C, 59.2; H, 8.70; N, 17.3. Found: C, 59.3; H, 8.77; N, 17.3.

A sample of the compound dissolved in water (5 mg/ml) at room temperature liberated acetone as indicated by the qualitative Legal test. Under conditions described in the Methods section, a sample (1.182 mg) of the derivative liberated 95% of the theoretical amount of acetone.

A sample (19 mg) was dissolved in water (0.12 ml) and allowed to stand at room temperature for 1 hr and then at 4° for 2 days. The solution was concentrated *in vacuo* to about one-third its original volume and stored at 4° until a crystalline product separated. The product (4 mg) was identified as L-prolyl-L-leucylglycinamide hemihydrate by the melting point (121–125°) (lit.[15] mp 122–123°).

N-Isopropyl-S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide (IVB). A stirred solution of 0.956 g (2 mmol) of S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycinamide[17] in 10 ml of water and 15 ml of acetone (112.5 mmol) was cooled to 0°. The pH was adjusted to 5.0 with glacial acetic acid under nitrogen and 4.0 g (107 mmol) of sodium borohydride (Sigma Chemical Co., St. Louis) was

(15) M. Zaoral and J. Rudinger, *Collect. Czech. Chem. Commun.*, **20**, 1183 (1955).

(16) In an earlier preparation this material crystallized in the form of light, feathery needles, mp 83–85° dec and $[\alpha]^{20}D$ +41° (*c* 1, anhydrous acetone). This material gave satisfactory analytical values for the isopropylidene derivative (Found: C, 59.1; H, 8.88; N, 16.8) and its infrared spectrum in chloroform solution was identical with that of the higher melting form.

(17) C. Ressler and V. du Vigneaud, *J. Amer. Chem. Soc.*, **76**, 3107 (1954).

added over a 45-min period. During this addition, the mixture was maintained at pH 5.0–5.5 with glacial acetic acid. The mixture was stirred for 1 hr at 0° to destroy any $NaBH_4$ that might have been present, the pH was adjusted to 6.8 with ammonium hydroxide, and the solvents were removed *in vacuo*. The resulting oil was dissolved in 30 ml of 1-butanol and 6 ml of benzene. A 2-ml aliquot was placed on a 64 × 2.8 cm column of Sephadex G-25 (100–200 mesh) that had been equilibrated with the lower and upper phases of the solvent system 1-butanol–benzene–3.5% acetic acid in 1.5% aqueous pyridine (5:1:6) according to the method of Yamashiro.[14] The product was eluted with the upper phase, and 60 7.2-ml fractions were collected. Folin-Lowry color values[18] were determined and the contents from the major peak (R_f 0.42) were pooled. Then 200 ml of distilled water was added and the mixture was evaporated to dryness *in vacuo* to give a viscous oil (~45 mg). The entire procedure was repeated on a large scale with the remaining 1-butanol–benzene solution to give 0.820 g of the product as a semi-solid foam. All attempts to crystalline the material failed. A sample was prepared for elemental and amino acid analysis by dissolving a 100-mg portion of the product in 3 ml of methylene chloride, filtering the solution, removing the solvent *in vacuo*, and drying the residue at 25° for 24 hr *in vacuo* to give the product as a foam, mp 55–58°, $[\alpha]^{22}D$ −59.3° (*c* 1.0, ethanol).

Anal. Calcd for $C_{26}H_{41}N_3O_4S$: C, 60.1; H, 7.95; N, 13.5. Found: C, 59.5; H, 7.95; N, 13.2.

A sample was hydrolyzed for 24 hr in 6 *N* HCl at 110° and analyzed[19] on a Beckman-Spinco amino acid analyzer. The following molar ratios were obtained with the value of glycine taken as 1.0: proline, 1.0; glycine, 1.0; leucine, 1.0; and ammonia, 1.0. Authentic N-isopropyl-S-benzyl-L-cysteine has no ninhydrin color value.[13]

Acknowledgments. We wish to thank Mr. Joseph Albert for the elemental analyses and Mr. Roger Sebbane for the amino acid analysis.

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

(19) D. H. Spackman, W. H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

Metal Ion Binding to Adenosine Triphosphate. III. A Kinetic Analysis[1]

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Abstract: Previous attempts to elucidate the mechanism of metal–adenosine triphosphate (ATP) binding have resulted in apparent disagreement between the results of two fast reaction techniques, namely, nmr and temperature jump. We have extended the nmr measurements on manganese(II) to low ATP concentrations (*ca.* 5×10^{-4} *M*). Competition studies in mixtures of adenosine monophosphate (AMP), ATP, and Mn^{2+} have also been carried out. The results of these measurements confirm the existence of a 1:2 metal–ligand complex at high total ATP. The low concentration studies support the assignment of temperature-jump spectra to the formation of a 1:1 complex. The kinetic scheme at room temperature reconciling the results of the different experimental techniques is shown in Scheme I. By using the rate constants for step $1 \rightleftharpoons 2$ obtained by temperature jump, and an estimate of the equilibrium quotient for the metal-independent step $1 \rightleftharpoons 4$, we have been able either to determine or to estimate the remaining rate constants and equilibrium quotients for the metal-dependent steps. These constants are shown for Mn^{2+}. This scheme predicts that the phosphorus and proton magnetic resonance line broadening studies should reach a low concentration asymptote consistent with pathway $1 \rightleftharpoons 2$. This limit was experimentally observed in the proton case, where ATP concentrations as low as 5×10^{-4} *M* could be studied using computer enhancement. In the MA_2 complex the metal ion simultaneously binds to the phosphate moiety of one nucleotide and to the adenine ring nitrogen of the second nucleotide. The MA_2 complexes in which the metal ion binds to the N-7 position predominate. At low nucleotide concentration where the MA complex becomes accessible to the nmr, we show that the metal ion is *ca.* 3.8 Å from the H_8 proton. This distance could arise either (1) from the metal ion binding predominantly to the N-7, or (2) from the metal ion being near the H_8 but separated from the adenine ring by a coordination shell water molecule. An earlier uv difference study strongly suggests the second alternative.

In recent years there have been a number of studies of transition metal binding to adenosine triphosphate (ATP). Nuclear magnetic resonance,[3] temperature jump,[4,5] ultraviolet spectroscopy,[6] and electron spin resonance[7] have all been used to determine the nature of the metal–ATP interaction. The conclusions of these studies have apparently been in disagreement with respect to the detailed kinetics, and to whether the metal does[3,5] or does not[6,7] simultaneously bind to the phosphates and the adenine ring of the ATP molecule as first proposed by Szent-Györgyi.[8] We have reexamined the problem of metal binding to ATP

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