scribed in our investigations of copper and silver porphyrin and phthalocyanine.[6] Gold was detected in the divalent state.

The paramagnetic resonance spectrum obtained shows hyperfine splitting for both gold ($I = {}^3/_2$) and the four isoindole nitrogens of the phthalocyanine. The perpendicular side to the spectrum is shown in Figure 1 and from it we calculate $g_\perp = 1.996$ (± 0.001); the gold hyperfine splitting parameter B is 6.1×10^{-3} cm.$^{-1}$, and the nitrogen hyperfine splitting D, 1.58×10^{-3} cm.$^{-1}$. g_0 is approximately 2.065 (± 0.005), in comparison with 2.042 for copper and 2.093 for silver phthalocyanine,[7] while Vånngård and Åkerström calculate 2.040, 2.046, and 2.019 as the g_0 values for gold, copper, and silver dialkyl dithiocarbamates, respectively. The perpendicular nitrogen hyperfine constant is 1.78×10^{-3} cm.$^{-1}$ for copper phthalocyanine and 2.32×10^{-3} cm.$^{-1}$ for silver. Solution spectra could not be obtained at room temperature.

The σ-covalency of the I-B group phthalocyanines, therefore, exhibits a maximum at silver, which shows the minimum stability in the series toward demetalation in concentrated sulfuric acid; copper phthalocyanine is by far the most stable of the three. Gold(II) phthalocyanine is the most soluble in 1-chloronaphthalene, and the samples for e.s.r. could be readily prepared without recourse to supersaturation, which is necessary to achieve proper resolution of the hyperfine spectrum of silver, and particularly of copper phthalocyanine, in this solvent.[7b,8]

The reaction between gold monobromide (or finely divided metallic gold) and 1,3-diiminoisoindoline was accomplished by mixing the two components intimately, in a ratio of 1:2, and heating for 5 min. at a temperature between 240 and 250°. The reaction mixture was quickly cooled in an ice bath. The product was purified (but not completely separated from other organic components) by repeated extraction with benzene and acetone. Synthesis at temperatures above 280° often leads to the formation of some metal-free phthalocyanine, and gold phthalocyanine is not obtained beyond 300°.

The visible spectrum of gold(II) phthalocyanine shows absorption peaks at 662, 633, 601, and 348 mμ, on an instrument which reproduces Whalley's values for copper phthalocyanine.[9]

It is of interest to note that a porphyrin of gold, the magnetic properties of which are different from those of gold phthalocyanine, has also been synthesized. Gold mesoporphyrin was prepared by the room temperature rearrangement of the mesoporphyrin-IX dimethyl ester–gold acid trichloride association complex[10] (prepared in ethyl acetate–ethanol) in benzene. The Soret absorption band of this compound in methanol is at 384 mμ while the visible bands are at 508 and 541 mμ; an ultraviolet shift with respect to silver mesoporphyrin comparable to that observed for platinum mesoporphyrin with regard to the palladium derivative.[11] These shifts have been interpreted in the past[12] as an indication of stability, and, indeed, gold mesoporphyrin is undecomposed in concentrated sulfuric acid at room temperature. Its proton magnetic resonance spectrum in pyridine and chloroform is similar to that of copper mesoporphyrin but appears diamagnetic by the n.m.r. method of Fritz and Schwarzhaus.[13] A more complete report on this compound will appear in a forthcoming paper.

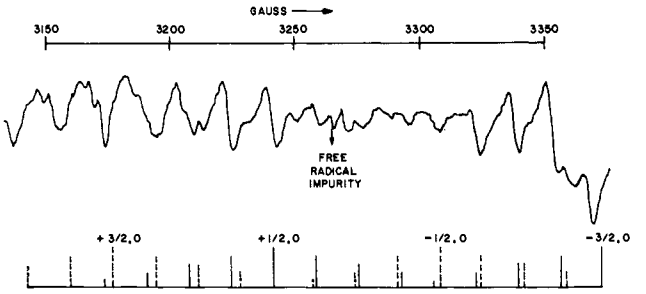

Figure 1. The perpendicular side of the electron spin resonance spectrum of gold phthalocyanine.

The stability of gold(II) phthalocyanine with respect to the silver(II) compound, apart from possible mechanistic differences, is in agreement with Linstead's correlation[14] between the metallic radii and the resistance of the phthalocyanines to demetalation. Because of the lesser degree of covalency (as shown by spin resonance) and the lanthanide contraction, it may be supposed that the effective radius of the metal atom in the gold complex is closer than in silver phthalocyanine to the estimated "ideal" fit of 1.35 Å. The greater ionicity of the gold derivative in this series also agrees with the greater electronegativity of gold.[15] This would help to explain why within the same subgroup of the periodic table the stabilities of porphyrin derivatives are in general less sensitive to variations in the metallic radii than those of the phthalocyanines.

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On the Configuration of a Lysine-vasopressin Dimer

Sir:

Wade, Winitz, and Greenstein[1] reported the formation of "parallel" dimer during the oxidation of cysteinyl-l-cysteine. Heaton, Rydon, and Schofield[2] assigned "antiparallel" cyclic structures to the polymers formed from the oxidation of L-cysteinyl-polyglycyl-l-cysteines. In view of recent reports on the preparation and

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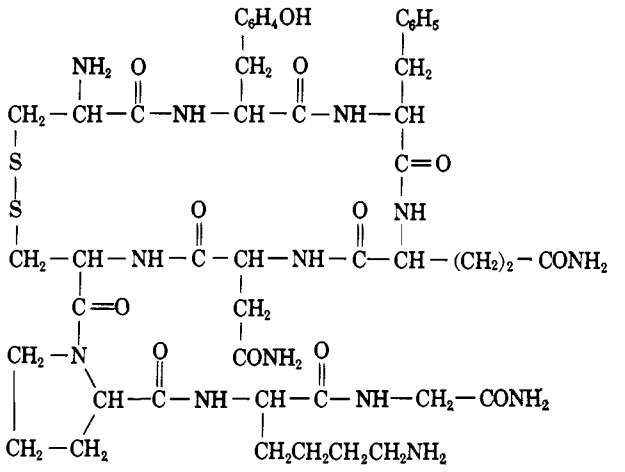

Figure 1. Structure of lysine-vasopressin.

pyridine–acetic acid–water (100:4:900, v./v.) at 1000 v. and 30 ma. for 3 hr. and by paper chromatography. The fragments were eluted from paper with 2 *N* acetic acid, evaporated to dryness, and hydrolyzed in 6 *N* hydrochloric acid for 20 hr. The amino acids were identified after separation by paper chromatography in butanol–acetic acid–water (4:1:5, v./v.) on Whatman No. 52[7] filter paper or on an automatic Beckman-Spinco Model 120 B analyzer. The determination of NH_2-terminal amino acids of the fragments was carried out by the phenylthiohydantoin (PTH) method of Edman[8] as described by Fraenkel-Conrat, *et al.*[9] The PTH amino acids were separated by paper chromatography.[10,11]

The dimer after chymotryptic digestion and electrophoretic separation gave five major and two minor fragments. Four of the fragments (1–4) contained all the amino acid residues of vasopressin. All these

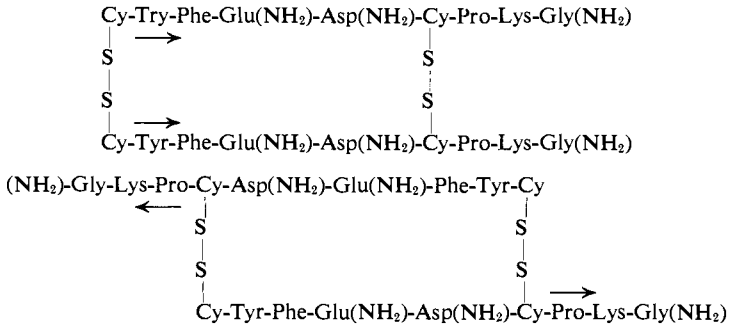

Figure 2. Upper, cyclic parallel dimer of lysine-vasopressin. Lower, cyclic antiparallel dimer of lysine-vasopressin. The arrows indicate the direction of peptide linkages.

properties of a lysine-vasopressin dimer[3] and studies of its physiological properties[4] it seemed important to carry out a preliminary examination of its structure.

The possibility that the dimer might be linear was rendered unlikely by the absence of SH groups as ascertained by a qualitative nitroprusside test for thiols. Other possibilities were that the dimer might have a cyclic parallel or cyclic antiparallel structure (Figure 2). In theory, these possibilities can be studied (or distinguished from one another) by hydrolysis with chymotrypsin. This enzyme has a preference for bonds on the carboxyl side of aromatic acid residues. On such treatment the parallel and the antiparallel dimer should yield different digestion products.

Eight hydrolyses with chymotrypsin were performed. The digestions were carried out with 0.3–1.4 mg. of dimer in 0.1 *M* ammonium acetate buffer, pH 7.4–8.7, for 15–24 hr. at 38°. In four digestions 2 mg. of N-ethylmaleimide/ml. of solution was added to prevent disulfide interchanges.[5] The enzyme:substrate ratio was about 1:40 to 1:30.

After enzymic hydrolyses, the dimer was acidified with glacial acetic acid and taken to dryness twice over phosphorus pentoxide *in vacuo* (<0.1 mm.) to remove ammonium acetate. The fragments were separated by high voltage electrophoresis in a system[6] consisting of amino acids except phenylalanine were present in fragment 5. Fragment 6 was similar to fragments 1–4 but had more than an equimolar proportion of phenylalanine. A slow liberation of free phenylalanine was also noted. The Edman degradation carried out after chymotryptic digestion showed that the peptide chain of the dimer was disrupted with the liberation of the NH_2-terminal groups of phenylalanine and glutamine. Though one of the amino groups of cystine in vasopressin and two in the dimer are free, the cystine residue is anchored to the peptide chain and does not form a PTH derivative.[9] Consequently in the Edman degradation of the digested dimer, as well as in model studies with chymotrypsin-digested lysine vasopressin monomer, only PTH-phenylalanine and PTH-glutamine were formed.

Chymotryptic digestion of the cyclic parallel dimer should have yielded tyrosylcysteinyltyrosine (I) and/or phenylalanyltyrosylcystyltyrosine (II) or tyrosylcystyltyrosylphenylalanine (III) among the fragments and a larger fragment containing no tyrosine (VI). The linear dimer should have also yielded cysteinyltyrosine (IV) or cysteinyltyrosylphenylalanine (V) among the products. Acid hydrolysis of fragments I and IV would have yielded only cystine and tyrosine. Fragments II, III, or V on hydrolysis would

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have produced cystine, tyrosine, and phenylalanine. No such fragments were found, though the cleavage of peptide chains undoubtedly occurred. The fragments separated from the digestion products and containing all the amino acids of lysine-vasopressin and the one fragment that had no phenylalanine corresponded in principle to the expected (theoretical) cleavage products of the antiparallel dimer.

The results of chymotryptic hydrolyses are thus compatible with a cylic antiparallel structure. Structural studies (J. Meienhofer, Deutsches Wollforschungsinstitut, Aachen, Germany) using a C^{14}-labeled cross-linking reagent, 1,5-difluoro-2,4-dinitrobenzene (FFDNB),[12] gave inconclusive results. A negligible content of 2,4-dinitrophenylene-1,5-bis-Nϵ-lysine[13] among the products of the reaction followed by total hydrolyses pointed to antiparallel structure. Other reaction products[13] of cross linking with FFDNB were identified but offered no arguments in favor of parallel or antiparallel structure due to the difficulty in estimating distances between reactive centers and possibilities of "folding-back" of the tripeptide side chain at the proline residue.

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Carbonium Ion Salts. X. Hydroxytropenium Iodide Monohydrate[1]

Sir:

Many carbonium ion salts react rapidly with moisture. Some deliquesce with decomposition as do the tropenium halides,[2-4] and most of the less stable ones react irreversibly to form carbinols or olefins. The three carbonium ion hydrates reported to date[5-7] are all of salts of hydroxy cations; this suggests that hydrogen bonding from the substituent hydroxyl to hydrate water may play an important role in the formation of such hydrates, but no spectral nor thermodynamic evidence for such hydrogen bonding has been reported.

Hydroxytropenium iodide is prepared in 73.5% yield by treatment of tropone with anhydrous hydrogen iodide in methylene chloride as brilliant red microneedles, m.p. 151–152°. *Anal.* Calcd. for C_7H_7IO: $C_7H_6OH^+$, 45.77; I^-, 54.23. Found: $C_7H_6OH^+$, 45.9; I^-, 54.24. The red iodide quickly turns yellow in moist air and with excess moisture deliquesces to form a red solution; this solution on standing dries to red or yellow crystals depending on the moisture content of the atmosphere. These observations strongly suggested the formation of a stable yellow hydrate.

A portion of the red iodide was sealed in a vacuum system at 30.00° and water vapor admitted. Uptake of water commenced at a pressure of 6.15 mm., and a pressure plateau was maintained until 0.6 mole of water had been consumed; after this point water uptake slowed to an impractical rate. This was attributed to conversion of the surface of the crystals to the hydrate with concurrent shielding of the crystal center from water vapor.

To obviate this difficulty, an oxygen-free glove box was converted to a humidistat by inclusion of a saturated solution of calcium chloride hexahydrate, and a solution of the iodide in water was allowed to evaporate under 8 mm. of water vapor. This gave a quantitative yield of hydroxytropenium iodide monohydrate as bright yellow needles. *Anal.* Calcd. for $C_7H_9IO_2$: $C_7H_6OH^+$, 42.50; I^-, 50.75. Found: $C_7H_6OH^+$, 42.4; I^-, 50.71. At 30° the vapor pressure of water over this compound was 6.15 mm., identical with the pressure plateau found above. The hydrate seems stable indefinitely as long as the pressure of water is slightly above this value.

The variation of water vapor pressure over the hydrate with temperature was investigated in the range 26–47°. These data fit an excellent straight line of the form $\ln P_{atm} = -a/T + b$, where $a = 7288$ and $b = 19.24$. From these constants the thermodynamic values at 25° are calculated as $\Delta F° = 3.09$ kcal./mole, $\Delta H° = 14.48$ kcal./mole, and $\Delta S° = 38.22$ e.u. for the reaction

$$(C_7H_6OH^+I^- \cdot H_2O)(s) = (C_7H_6OH^+I^-)(s) + H_2O(g)$$

Consideration of the infrared spectra of hydroxytropenium iodide and its hydrate casts considerable doubt on the previous assignment[8] of the O–H stretching frequency of the hydroxytropenium ion. In the region 1700–3700 cm.$^{-1}$, the anhydrous iodide shows a single broad peak centered at 2600 cm.$^{-1}$ (Figure 1); in the hydrate this peak shifts to 1830 cm.$^{-1}$ and a new intense absorption appears at 3250 cm.$^{-1}$. We have assigned the 2600-cm.$^{-1}$ band in the iodide to the O–H stretch of the hydroxytropenium ion for the following reasons: (1) hydroxytropenium bromide and fluoroborate also show only a single, very similarly shaped

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