of I was also attempted by the coupling of S-benzyl-N-carbobenzoxy-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine[3] with S-benzyl-L-cysteinyl-L-prolyl-ε-tosyl-L-lysylglycinamide (II) according to the pyrophosphite procedure,[4] followed by removal of the protecting groups from the resulting nonapeptide derivative and subsequent oxidation to the disulfide form. This approach yielded a synthetic product assaying approximately 100 pressor units/mg. after purification by electrophoresis at *p*H 5.6 in pyridine acetate and countercurrent distribution between *sec*-butyl alcohol and 0.08 *M* *p*-toluenesulfonic acid.

In addition to these approaches, another method was utilized which has led to material which is as biologically active as the most potent preparation of lysine-vasopressin obtained from natural sources. The key intermediate in this synthesis is S-benzyl-N-tosyl-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinyl-L-prolyl-ε-tosyl-L-lysylglycinamide (III), which has solubility properties favorable for its purification.

III was synthesized by the coupling of S-benzyl-N-tosyl-L-cysteinyl-L-tyrosyl-L-phenylalanyl-L-glutaminyl-L-asparagine (IV) with the tetrapeptide amide II. For the synthesis of II, ethyl L-prolyl-ε-tosyl-L-lysylglycinate,[5] m.p. 81–84°, was coupled with S-benzyl-N-carbobenzoxy-L-cysteine[6] by the N,N′-dicyclohexylcarbodiimide method[7] and the resulting protected tetrapeptide ester was converted to the corresponding crystalline amide with ammonia in ethanol; m.p. 101–104°, $[\alpha]^{21}D$ −29.3° (*c* 1, $CHCl_3$) (calcd. for $C_{38}H_{48}O_8N_6S_2$: C, 58.5; H, 6.19; N, 10.8; S, 8.21. Found: C, 58.3; H, 6.34; N, 10.6; S, 8.10); the carbobenzoxy group was then removed with HBr–HOAc.[4] IV was prepared by the coupling of L-phenylalanyl-L-glutaminyl-L-asparagine[8] with S-benzyl-N-tosyl-L-cysteinyl-L-tyrosine[9] by the isobutyl chlorocarbonate mixed anhydride procedure[10] in a yield of 55% after recrystallization; m.p. 203–204°, $[\alpha]^{21}D$ +4.4° (*c* 2.08, dimethylformamide) (calcd. for $C_{44}H_{51}N_7O_{11}S_2 \cdot 1/2H_2O$: C, 57.0; H, 5.65; N, 10.6. Found: C, 57.1; H, 5.66; N, 10.6).

Compounds IV and II were coupled by the N,N′-dicyclohexylcarbodiimide method[7] to give a 39% yield of analytically pure III, m.p. 226–230°, $[\alpha]^{18}D$ −23.0° (*c* 2.11, dimethylformamide) (calcd. for $C_{74}H_{91}O_{16}N_{13}S_4 \cdot H_2O$: C, 56.8; H, 5.99; N, 11.6; H_2O, 1.15. Found: C, 56.7; H, 6.02; N, 11.6; H_2O, 1.02).

(3) V. du Vigneaud, D. T. Gish and P. G. Katsoyannis, *ibid.*, **76**, 4751 (1954).

(4) G. W. Anderson, J. Blodinger and A. D. Welcher, *ibid.*, **74**, 5309 (1952).

(5) This tripeptide ester was prepared from α-carbobenzoxy-ε-tosyl-L-lysine, m.p. 85–88°, and ethyl glycinate, removal of the carbobenzoxy group, and coupling of the product with carbobenzoxy-L-proline by the *o*-phenylene chlorophosphite procedure [G. W. Anderson and R. W. Young, THIS JOURNAL, **74**, 5307 (1952)] followed by removal of the carbobenzoxy group with HBr–HOAc.

(6) C. R. Harington and T. H. Mead, *Biochem. J.*, **30**, 1598 (1936).

(7) J. C. Sheehan and G. P. Hess, THIS JOURNAL, **77**, 1067 (1955).

(8) E. A. Popenoe and V. du Vigneaud, *ibid.*, **76**, 6202 (1954).

(9) This compound was prepared independently by a method similar to that recently published by J. Honzl and J. Rudinger [*Collection Czechoslov. Chem. Communs.*, **20**, 1190 (1955)]. They also reported the synthesis of a product of high oxytocic activity from a nonapeptide derivative containing a tosylcysteine residue.

(10) J. R. Vaughan, Jr., and J. A. Eichler, THIS JOURNAL, **75**, 5556 (1953).

The N-tosyl and S-benzyl groups were removed from III (300 mg.) by reduction with sodium in liquid ammonia and the resulting product was oxidized to the disulfide form by aeration in aqueous solution at *p*H 6.4. The yield of pressor activity[11] ranged in several experiments from 50,000 to 70,000 units (per 300 mg. of III). After concentration and lyophilization, the product was purified by countercurrent distribution between *sec*-butyl alcohol and 0.08 *M* *p*-toluenesulfonic acid followed by electrophoresis in pyridine acetate buffer (*p*H 5.6) on a cellulose-supporting medium.[12,13] The purified material had a pressor activity of 250–290 units/mg.[14] when assayed as usual against the U.S.P. Standard Powder.[15] The ratios between pressor, antidiuretic, milk-ejecting and avian depressor activities for the synthetic material are the same as those for natural lysine-vasopressin.[12,14,16] Starch column chromatography[17] of a hydrolysate of this material showed the eight amino acids to be present in molar ratios to each other of approximately 1:1 and ammonia in a ratio to any one amino acid of approximately 3:1. The natural hormone and the synthetic product showed the same infrared spectrum.[18] They showed the same behavior on countercurrent distribution between *sec*-butyl alcohol and 0.08 *M* *p*-toluenesulfonic acid and also had the same electrophoretic mobility on Whatman No. 1 paper in pyridine acetate buffer at *p*H 5.6 and 4.0.

(11) J. Dekanski, *Brit. J. Pharmacol.*, **7**, 567 (1952).

(12) D. N. Ward and V. du Vigneaud, *J. Biol. Chem.*, in press.

(13) H. G. Kunkel in "Methods of Biochemical Analysis," Vol. 1, D. Glick, Ed., Interscience Publishers, Inc., New York, p. 141.

(14) A sample of natural lysine-vasopressin with approximately the same pressor activity as the synthetic material was kindly supplied by Dr. Albert Light of this laboratory. This material was obtained by purification by countercurrent distribution followed by chromatography on Amberlite IRC-50.

(15) "The Pharmacopeia of the United States of America," fifteenth revision, Mack Printing Co., Easton, Pa., 1955, p. 776. These assay values are based on an activity of 0.4 U.S.P. Posterior Pituitary Unit/mg. for the Standard Powder.

(16) For the ratios between these four activities in natural lysine-vasopressin, see H. B. van Dyke, S. L. Engel and K. Adamsons, Jr., *Proc. Soc. Exptl. Biol. Med.*, **91**, 484 (1956). The ratios of antidiuretic and milk-ejecting activities to pressor activity for the synthetic material were determined by Professor H. B. van Dyke and Mr. S. L. Engel of the College of Physicians and Surgeons.

(17) S. Moore and W. H. Stein, *J. Biol. Chem.*, **178**, 53 (1949).

(18) The authors wish to thank Dr. Julian R. Rachele of this laboratory for determination of the infrared spectra.

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THE PREPARATION OF ACID-STABILIZED SUBHALIDES FROM MOLTEN METAL-METAL HALIDE SOLUTIONS[1]

Sir:

The formation of slightly stable subhalides by the solution of certain metals in their fused halides

(1) Work was performed in the Ames Laboratory of the Atomic Energy Commission.

has been suggested several times.[2,3,4] Among the post-transition metal–metal halide systems, the variations in metal solubility both within the group and with change in halide ion are consistent with such an interpretation, and, moreover, there is a direct correspondence between appreciable solubility in the molten halide and the existence of a known gaseous subhalide.[5] However, with the possible exception of CaCl,[6] the isolation of lower oxidation states from these melts apparently has not been achieved; the systems characteristically revert to the original components on solidification.

The effect of certain foreign salts on the metal solubility has been reported for the cadmium[7] and bismuth[8] systems. An interpretation of the results that appears more satisfactory than those previously presented[3,7] can be obtained from a consideration of the possible acid-base or "complexing" interactions between the added salt and the two oxidation states present. Added base, *i.e.*, halide ion, would be expected to reduce the amount of subhalide formed through stabilization of the more acidic, higher oxidation state. Conversely, addition of an acid capable of complexing halide ion would increase the amount of subhalide formed. Use of the strongly acidic $AlCl_3$ in such systems has resulted in the preparation of stable Bi(I), Cd(I) and Ga(I) compounds.

The solubility of Bi in $BiCl_3$ at 260° corresponds to 46% conversion to BiCl; a black, asphalt-like mixture is obtained on solidification. At the same temperature, addition of $AlCl_3$ results in the reaction $BiCl_3 + 2Bi + 3AlCl_3 = 3BiAlCl_4$ taking place quantitatively [56.3, 55.7% Bi, 55.3% theor.]. The product, m.p. *ca.* 253°, is maroon in bulk and reddish-brown as a powder. It disproportionates to metal and trihalide essentially quantitatively in water, dioxaneor alcohol, and darkens rapidly in air.

Similarly, the solubility of Cd in $CdCl_2$ at 740° corresponds to 17.6% conversion of Cd_2Cl_2; a black mixture is obtained on quenching. With $2AlCl_3/CdCl_2$, the solubility of cadmium at 330° indicates 71.5% conversion to the Cd(I) oxidation state. The solid, very light gray in bulk, white as a powder, also readily disproportionates in contact with the above solvents. Similar results have been obtained in the iodide system.

The compound Ga_2Cl_4, which has been found to be $Ga(I)[Ga(III)Cl_4]$,[5] is an example of such an acid-stabilized lower oxidation state. Further reduction of the Ga(III) therein by metal gives a solution containing 7.4% GaCl at 180°.[4] In the presence of sufficient $AlCl_3$ the amount of metal dissolved at 180° is within 0.1% of that for the reaction $Ga(GaCl_4) + 2Ga + 4AlCl_3 = 4GaAlCl_4$. The white product, m.p. 175°, is, as expected, physically very similar to $Ga(GaCl_4)$.

(2) G. von Hevesy and E. Löwenstein, *Z. anorg. allgem. Chem.*, **187**, 266 (1930).

(3) K. Grjotheim, F. Grönvold and J. Krogh-Moe, THIS JOURNAL, **77**, 5824 (1955).

(4) J. D. Corbett and S. von Winbush, *ibid.*, **77**, 3964 (1955).

(5) S. von Winbush, R. K. McMullan and J. D. Corbett, to be published.

(6) (a) P. Ehrlich and L. Gentsch, *Naturwiss.*, **40**, 460 (1953); (b) The compound has been found to be CaHCl, P. Ehrlich, B. Ait, and L. Gentsch, *Z. anorg. allgem. Chem.*, **283**, 58 (1956).

(7) D. Cubicciotti, THIS JOURNAL, **74**, 1198 (1952).

(8) G. Cleary and D. Cubicciotti, *ibid.*, **74**, 557 (1952).

All the solid products described are diamagnetic, and give powder pattern lines different from those obtained with any binary mixture of the components.

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THE STRUCTURE OF THE ANTIBIOTIC METHYMYCIN

Sir:

Methymycin[1] belongs chemically to a class of antibiotics[2] which contains such therapeutically important representatives as erythromycin[3] and carbomycin (magnamycin)[4] and partial structures have been advanced for two of them (erythromycin[3] and pikromycin[5]). We should now like to report certain degradation experiments which, coupled with earlier results,[6] lead us to propose structure I as a complete expression for methymycin.

Mild hydrolysis of methymycin with aqueous sulfuric acid led to the desosamine free fragment II (m.p. 163–165°, $[\alpha] + 79°$ (all rotations in chloroform), λ_{max}^{EtOH} 225 mμ, log ε 4.03, $\lambda_{max}^{CHCl_3}$ 2.80, 2.95, 5.76, 5.91 and 6.08 μ; Found: C, 65.17; H, 9.11; C—CH_3, 19.34) which was further characterized as the acetate III (m.p. 198–200°, $[\alpha]_D + 93°$; Found: C, 64.39; H, 8.29; acetyl, 12.11) and as the ketone IV (m.p. 173–179°, $[\alpha]_D +177°$, λ_{max}^{EtOH} 224 mμ, log ε 3.96; Found: C, 65.87; H, 8.47; C—CH_3, 19.95). The ketone IV gave a negative Schiff test and yielded 58% of carbon dioxide upon treatment with alkali followed by acidification (a parallel experiment with II furnished no carbon dioxide).

When methymycin or II was treated with methanolic sulfuric acid, there was produced the spiroketal V (m.p. 79–81°, $[\alpha]_D - 68°$, no high selective ultraviolet absorption, $\lambda_{max}^{CHCl_3}$ 5.77 μ; Found: C, 66.51; H, 9.25; OCH_3, 9.81; active hydrogen, 0.00), which upon lithium aluminum hydride reduction led to the corresponding diol (m.p. 159–161°, $[\alpha]_D + 118°$; Found: C, 65.77: H, 10.17; OCH_3, 9.40).

Permanganate oxidation of II in acetone solution furnished three products: VI (m.p. 164–172°, $[\alpha]_D +52°$ (acetone), λ_{max}^{KBr} 2.90, 5.66 and 5.78 μ; Found: C, 58.35; H, 7.79; C—CH_3, 21.01; neut. equiv., 318), VII (m.p. 55–56°, $[\alpha]_D + 69°$, $\lambda_{max}^{CHCl_3}$ 5.68–5.76 μ (broad); Found: C, 63.40; H, 8.39; C—CH_3, 22.51) and VIIIa (m.p. 126–128°,

(1) M. N. Donin, J. Pagano, J. D. Dutcher and C. M. McKee, "Antibiotics Annual 1953–1954," Medical Encyclopedia, Inc., New York, p. 179.

(2) For leading references see R. Corbaz, L. Ettlinger, E. Gäumann, W. Keller-Schierlein, F. Kradolfer, E. Kyburz, L. Neipp, V. Prelog, A. Wettstein and H. Zähner, *Helv. Chim. Acta*, **39**, 304 (1956).

(3) *Cf.* P. F. Wiley, K. Gerzon, E. H. Flynn, M. V. Sigal and U. C. Quarck, THIS JOURNAL, **77**, 3677 (1955).

(4) R. L. Wagner, F. A. Hochstein, K. Murai, N. Messina and P. P. Regna, *ibid.*, **75**, 4684 (1953).

(5) H. Brockmann and R. Oster, *Naturwiss.*, **42**, 155 (1955).

(6) (a) C. Djerassi, A. Bowers and H. N. Khastgir, THIS JOURNAL, **78**, 1729 (1956); (b) C. Djerassi, A. Bowers, R. Hodges and B. Riniker, *ibid.*, **78**, 1733 (1956).