least as high. Hydriodic acid[2] and hypophosphorous acid[3] have been used previously to reduce the perrhenate in the preparation of potassium hexachlororhenate(IV).

Potassium hexabromorhenate(IV) is formed in the same manner when chromium(II) bromide and hydrobromic acid are substituted for the chloride reagents. This rhenium salt may be prepared in higher yields, in better purity, and in considerably less time than with the existing method of preparation.[3]

Procedure.—Add approximately 3 g. of potassium perrhenate, a stoichiometric amount of potassium chloride (0.77 g.), 100 ml. of concentrated hydrochloric acid (12.4 *N*), and 20 ml. of water to a 250-ml. Erlenmeyer flask. Heat the contents of the flask for several minutes until the potassium perrhenate goes into solution. While the solution is still warm, add about 25 ml. of 1.7 *M* chromium(II) chloride, preferably under an atmosphere of nitrogen. Prepare the chromium(II) chloride by running a 1.7 *M* chromium(III) chloride solution, which is about 0.2 *M* in hydrochloric acid, through a Jones reductor. After cooling the flask in an ice-bath, suction filter the green precipitate into a sintered glass filter crucible. Wash the salt with two 5-ml. volumes of ice-cold 10% hydrochloric acid and then, twice each, with ethyl alcohol and diethyl ether. Air dry the salt by drawing air through the filter by means of suction, and finally dry in an oven at 110° for one hour.

The yield of the potassium hexachlororhenate is about 55%, with a purity of about 98.5%. The yield can be increased to about 85%, with no appreciable sacrifice in purity, by boiling the solution down to a volume of about 30 ml. before filtering.

The same procedure is used for the preparation of potassium hexabromorhenate except that the chloride reagents are replaced with bromide. The 3 g. of potassium perrhenate are dissolved in 120 ml. of 48% hydrobromic acid. The chromium(III) bromide solution may be prepared conveniently by addition of hydrobromic acid to chromium(III) carbonate.

The yield of potassium hexabromorhenate is about 60%, with a purity of 99%. Here also the yield can be increased to about 85% with no sacrifice in purity by boiling the solution down to a volume of about 30 ml. before filtering.

Either of the preparations can be completed in less than an hour, not including the final drying.

The extension of this procedure for the preparation of potassium hexaiodorhenate(IV) and potassium hexafluororhenate(IV) was not successful.

Analyses.—The rhenium in the hexahalorhenates was oxidized to perrhenate with hydrogen peroxide after hydrolysis of the tetravalent salt. After boiling to destroy the excess hydrogen peroxide, aliquots of the solutions were analyzed for rhenium spectrophotometrically with alpha furil dioxime.[4] Other aliquots, after acidification with nitric acid, were assayed for halide by conventional gravimetric techniques.

(4) V. W. Meloche, R. L. Martin and W. H. Webb, submitted for publication.

Acknowledgment.—The authors wish to thank the Wisconsin Alumni Research Foundation for financial assistance in this project.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF WISCONSIN
MADISON, WISCONSIN
VILLIERS W. MELOCHE
RONALD MARTIN

RECEIVED OCTOBER 8, 1956

FORMATION OF α,γ-DIAMINOBUTYRIC ACID FROM ASPARAGINE-CONTAINING PEPTIDES[1]

Sir:

During the recent synthesis of the cyclic disulfide of L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-L-cysteinamide (I),[2] of interest because of its relationship to the pentapeptide ring moiety of oxytocin,[3] an unusual side reaction of some interest was observed. The route to I utilized several standard methods of peptide synthesis currently in wide use: namely, (1) couplings using the tetraethyl pyrophosphite reagent,[4] for the preparation of tosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinamide (II) from tosyl-L-isoleucyl-L-glutaminyl-L-asparagine and S-benzyl-L-cysteinamide, and of carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinamide (III) from L-isoleucyl-L-glutaminyl-L-asparaginyl-S-benzyl-L-cysteinamide and carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosine; and (2) removal of tosyl, benzyl, and carbobenzoxy protecting groups with sodium in liquid NH_3,[5,6] from II and III. Another product (IV) (K = 0.14), found to be present in 23% yield during the final isolation of I (K = 0.19) by countercurrent distribution,[7] has now been further purified by distribution in *sec*-butyl alcohol–0.01 *M* NH_3 (K = 0.77). Unlike I, it possessed no significant oxytocic activity.

Analysis of an acid hydrolysate of IV by the starch column procedure[8] in the solvent system (A) 1:2:1 *n*-BuOH–*n*-PrOH–0.1 *N* HCl followed by 2:1 *n*-PrOH–0.5 *N* HCl indicated cystine, tyrosine, isoleucine, and glutamic acid in molar amounts, 2 moles of ammonia, and an unidentified ninhydrin-positive substance (V), which was eluted at fraction 352 soon after cystine. These results were confirmed qualitatively by paper chromatography. V showed chromatographic behavior characteristic of the basic amino acids. Treatment of IV with 2,4-dinitrofluorobenzene (DNFB) followed by hydrolysis, resulted in the replacement of V by a yellow, acid-soluble, ninhydrin-sensitine DNP derivative (VI), R_f 0.52, 5:1:5 *n*-BuOH-HAc–H_2O; R_f 0.79, phenol, as well as affecting the cystine and tyrosine.

(1) This work was supported in part by a grant (H-1675) from the National Heart Institute, Public Health Service.

(2) C. Ressler, *Proc. Soc. Exp. Biol. and Med.*, **92**, 725 (1956).

(3) V. du Vigneaud, C. Ressler and S. Trippett, *J. Biol. Chem.*, **205**, 949 (1953); H. Tuppy, *Biochim. et Biophys. Acta*, **11**, 449 (1953).

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

(5) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).

(6) V. du Vigneaud and O. K. Behrens, *ibid.*, **117**, 27 (1937).

(7) L. C. Craig, *Anal. Chem.*, **22**, 1346 (1950).

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

On the basis of results of extensive comparisons with an authentic sample[9] using paper and starch chromatography, V has been identified as α,γ-diaminobutyric acid (VII), and it has apparently been derived from asparagine. The unknown and VII showed the same mobilities on paper in: 10:1:10 *n*-BuOH–HAc–H_2O, R_f 0.02; phenol, 0.28; 3:1 *n*-PrOH–0.2 *N* NH_3, 0.28; phenol–NH_3, 0.69; 4:1 pyridine–H_2O, 0.07, and on starch in 2:1 *n*-PrOH–0.5 *N* trichloroacetic acid[8] as well as in A. Chromatography of V in the presence of VII in several of these systems resulted in no separation. In addition, comparison showed the DNP derivative VI to be chromatographically identical with γ-DNP-α,γ-diaminobutyric acid.[10]

Preliminary experiments have traced this side reaction at least partly to initial changes in asparagine occurring during the preparation of the tetrapeptide II. Hydrolysis of the crude product yielded only aspartic acid and the other expected amino acids, while after reduction and hydrolysis VII also was detected. In contrast, reduction of purified II, as well as asparagine, carbobenzoxy-L-asparaginyl-S-benzyl-L-cysteinamide, and other asparagine peptides led only to expected products. The asparagine residue appears to have been converted partially to one with reactivities reminiscent of, among other possibilities, a nitrile, being hydrolyzable to aspartic acid and reducible, at least in part, to a basic grouping, which yields after hydrolysis α,γ-diaminobutyric acid. This effect on asparagine has been noticed during the preparation of other similar asparagine peptides and may perhaps suggest a basis for a synthetic route to peptides of α,γ-diaminobutyric acid from the more readily available asparagine, if as seems possible, the α,γ-diaminobutyric acid residue is formed prior to hydrolysis.

Although the mechanism of this interesting reaction remains to be determined, it is stimulating to envision a comparable biosynthetic pathway which might account for the origin in nature from asparagine of α,γ-diaminobutyric acid, which occurs as an important constituent in a group of polypeptide antibiotic substances, the polymyxins.

(9) α,γ-L-Diaminobutyric acid dihydrochloride was obtained from Mann Chemical Company; m.p. 223–227°, $[\alpha]^{28}D$ +23.9° (2.5% in 5 *N* HCl); reported m.p. 195–196° (D. W. Adamson, *J. Chem. Soc.*, 1564 (1939)), m.p. 218–220° (A. C. Kurtz, *J. Biol. Chem.*, **180**, 1253 (1949)), $[\alpha]^{25}D$ +24.2° (2% monohydrochloride in 5 *N* HCl) (S. J. Fu, K. R. Rao, S. M. Birnbaum, and J. P. Greenstein, *J. Biol. Chem.*, **199**, 207 (1952)).

(10) Appreciation is expressed to Drs. L. C. Craig and W. Hausmann of the Rockefeller Institute for Medical Research for a sample of γ-DNP-α,γ-diaminobutyric acid which was derived from a hydrolysate of DNP-polymyxin B_1.

(11) An anhydro product has recently been isolated from a similar reaction (D. T. Gish, P. G. Katsoyannis, G. P. Hess, and R. J. Stedman, THIS JOURNAL, **78**, 5954 (1956)). Its possible relationship to the formation of α,γ-diaminobutyric acid from asparagine-containing peptides remains to be determined.

(12) The author wishes to thank Dr. Paula Zimmering and Mrs. Lorraine S. Abrash for assistance with the starch chromatography and Mrs. Sylvia Kirsimagi White for the bioassay. Appreciation is expressed to Dr. Vincent du Vigneaud for his encouragement and interest in the problem.

DEPARTMENT OF BIOCHEMISTRY
CORNELL UNIVERSITY MEDICAL COLLEGE
NEW YORK, N. Y. CHARLOTTE RESSLER[12]

RECEIVED SEPTEMBER 7, 1956

ENTROPY CHANGE ASSOCIATED WITH HYDROTHERMAL SHRINKAGE OF COLLAGEN

Sir:

Native tendon fibers, when heated above 60° in water, undergo a dramatic, essentially irreversible shrinkage. Although the native fiber is nearly inextensible, the shrunken fiber (about one-quarter the original length) is quite rubbery, and can be stretched to nearly the original length. It is known that the shrink temperature, T_s, may be raised by external stress.[1] If as has been suggested,[2,3] shrinkage is a first-order phase transition, it should be possible, as suggested by Gee,[4] to apply an appropriately modified form of the Clausius–Clapeyron equation, *viz.*

$$df/dT = \Delta S/\Delta l \qquad (1)$$

In preliminary studies of this phenomenon, we first determined the increase in stress necessary to *prevent* hydrothermal shrinkage of strips of bovine foreleg tendon.[3,5] The resulting stress–temperature curves were entirely consistent with the force–length and length–temperature curves predicted by Flory.[6] Over the temperature range from 75–90°, df/dT = 630 g.cm.$^{-2}$ per °C. The small value of df/dT (*i.e.*, marked effect of stress on T_s) suggested strongly that ΔS must be quite small also. This is in contrast to the well-known small effect of pressure in lowering the m.p. of ice, where ΔS is large.

Several strips of tendon were then heated in water under various fixed loads, so that the rise of T_s with stress could be directly measured. From these results, df/dT was calculated to be about 330 g.cm.$^{-2}$ per °C. The calculated value of ΔS is given in Table I, where it is compared with ΔS for several more orthodox phase transitions.

TABLE I

ENTROPY OF MELTING OF SEVERAL SUBSTANCES

Substance	M.p., °C.	ΔS, cal.-g.$^{-1}$ – °C.$^{-1}$
Tendon	65 (T_s)	0.005
Ice	0	0.292
Polyethylene[7]	*ca.* 140	0.136
Poly-(N,N′-sebacoylpiperazine)[7]	180	0.054
Cellulose tributyrate[7]	207	0.017

If we assume[7,8] that each mole of chain bonds freed for rotation contributes 1.5–2.0 cal.-°C.$^{-1}$ to the entropy, we must conclude that the molecular weight of a freely-rotating unit of shrunken collagen is 300–400. Since the average weight per residue of the amino acids in collagen is 93,[9]

(1) E. Wohlisch, *Z. Biol.*, **85**, 406 (1927); *Biochim. Z.*, **247**, 329 (1932); F. G. Lennox, *Biochem. et Biophys. Acta* **3**, 170 (1949); C. E. Weir, *J. Research. Natl. Bur. of Standards*, **42**, 17 (1949).

(2) R. R. Garrett and P. J. Flory, *Nature*, **177**, 176 (1956).

(3) A. V. Tobolsky, *J. Applied Phys.*, **27**, 673 (1956).

(4) Geoffrey Gee, *Quarterly Rev.*, **1**, 265 (1947).

(5) R. Haselkorn, B.A. Thesis, Princeton University, Princeton, N. J., 1956.

(6) P. J. Flory, *Science*, **124**, 53 (1956).

(7) P. J. Flory, "Principles of Polymer Chemistry," Cornell University Press, Ithaca, N. Y., 1953, p. 573.

(8) H. Mark and A. V. Tobolsky, "The Physical Chemistry of High Polymers," Interscience Publication, N. Y., 1950, pp. 105–106.

(9) J. C. Kendrew, in "The Proteins," H. Neurath and K. Bailey (eds.), Academic Press, Inc., New York, N. Y., 1954, pp. 909–936.