

most elements exhibit a higher tendency to form negatively charged complexes in hydrochloric acid media than in nitric acid media, these results may be useful for separations and purification of thorium with anion-exchange resins.

On the basis of ionic radius considerations alone, one would conclude that the tendency of the chloride ion to form complexes should be higher than that of the nitrate ion. It has been shown that reversal in this order can be expected as a consequence of the structure of the nitrate ion and resulting high polarizability.<sup>7</sup> However, it is interesting to observe that among the elements previously studied only Nd(III), Pr(III), Sm(III), Eu(III)<sup>7</sup> and the transuranic elements in the +3 and +4 state such as Pu(III), Pu(IV),<sup>7</sup> Am(III)<sup>8</sup> and possibly Np(IV)<sup>7</sup> have less tendency to complex formation with the chloride ion than with the nitrate ion.

(7) J. C. Hindman, "Ionic and Molecular Species of Plutonium in Solution," National Nuclear Energy Series, Vol. 14-A, pp. 333, 346, 463.

(8) G. N. Yakolev and V. N. Kosyakov, "Spectrophotometric Studies of the Behaviour of Americium Ions in Solution," Proceedings of the International Conference on Peaceful Uses of Atomic Energy, Vol. 7, p. 363, United Nations, 1956.

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#### UNEXPECTED FORMATION OF ANHYDRO COMPOUNDS IN THE SYNTHESIS OF ASPARAGINYL AND GLUTAMINYL PEPTIDES<sup>1</sup>

Sir:

It was of considerable interest to find that some peptide-forming reagents lead to what appear to be intramolecular dehydrations during the formation of asparaginyl peptide bonds and, to a minor degree, during the formation of glutaminyl peptide bonds. Carbobenzoxy-L-asparagine previously has been coupled with the benzyl and methyl esters of S-benzyl-L-cysteine by the *o*-phenylene chlorophosphite,<sup>2</sup> diethyl chlorophosphite,<sup>3</sup> tetraethyl pyrophosphite,<sup>3</sup> phosphorazo<sup>4</sup> and *sec*-butyl chlorocarbonate<sup>4</sup> methods of peptide synthesis. We wish to report that the use of the tetraethyl pyrophosphite or N,N'-dicyclohexylcarbodiimide reagents for the preparation of asparaginyl-S-benzyl-L-cysteine peptides leads to the formation of compounds having the composition of anhydrides of the expected products, in addition to the expected protected peptides. The coupling of carbobenzoxy-L-asparagine with S-benzyl-L-cysteine methyl ester by the pyrophosphite procedure in diethyl phosphite solution gave the expected product (C<sub>23</sub>H<sub>27</sub>O<sub>6</sub>N<sub>3</sub>S, m.p. 199–200°) in 35% yield, and 27% of a com-

(1) Alteration of asparagine during the course of peptide synthesis using tetraethyl pyrophosphite has been independently demonstrated in this laboratory by C. Ressler (THIS JOURNAL, **78**, 5956 (1956)). In the synthesis of the cyclic disulfide of L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminy-L-asparaginyl-L-cysteinamide, a side product was isolated in which it was found that the asparagine had undergone change.

(2) S. J. Leach and H. Lindley, *Aust. J. Chem.*, **7**, 173 (1954).

(3) R. A. Boissonnas, St. Guttman, P.-A. Jaquenoud and J.-P. Waller, *Helv. Chim. Acta*, **38**, 1491 (1955).

(4) J. Rudinger, J. Honzl and M. Zaoral, *Collection Czechoslov. Chem. Commun.*, **21**, 202 (1956).

pound of m.p. 128–129°, [ $\alpha$ ]<sub>D</sub><sup>24</sup> –42.1° (*c* 1, acetic acid) (*Anal.* Calcd. for C<sub>23</sub>H<sub>27</sub>O<sub>6</sub>N<sub>3</sub>S: C, 60.6; H, 5.53; N, 9.22; S, 7.04. Found: C, 60.6; H, 5.73; N, 9.11; S, 7.08). The use of dicyclohexylcarbodiimide in tetrahydrofuran solution for this coupling gave 39% of the protected dipeptide ester and 26% of the anhydro compound. Treatment of tosyl-L-glutaminy-L-asparagine and S-benzyl-L-cysteine benzyl ester with dicyclohexylcarbodiimide in dimethylformamide solution yielded 40% of the tosyl tripeptide ester, m.p. 228–229°, [ $\alpha$ ]<sub>D</sub><sup>23</sup> –30.7° (*c* 1, dimethylformamide) (*Anal.* Calcd. for C<sub>33</sub>H<sub>39</sub>O<sub>8</sub>N<sub>5</sub>S<sub>2</sub>: C, 56.8; H, 5.63; N, 10.0; S, 9.17. Found: C, 56.6; H, 5.75; N, 10.0; S, 9.04), and 15% of its anhydro derivative, m.p. 210–211°, [ $\alpha$ ]<sub>D</sub><sup>23</sup> –33.5° (*c* 1, dimethylformamide) (*Anal.* Calcd. for C<sub>33</sub>H<sub>37</sub>O<sub>7</sub>N<sub>5</sub>S<sub>2</sub>: C, 58.0; H, 5.40; N, 10.3; S, 9.43. Found: C, 58.0; H, 5.56; N, 10.3; S, 9.14). No by-product of the type described could be detected when carbobenzoxy-L-asparaginyl-S-benzyl-L-cysteine methyl ester was prepared in tetrahydrofuran solution through the mixed anhydrides of carbobenzoxy-L-asparagine with carboxylic or alkyl carbonic acids. The best preparative procedure employed isovaleryl chloride to form the mixed anhydride, and gave a 58% yield of the protected dipeptide ester.

Dehydration took place to a lesser extent during the preparation of carbobenzoxy-L-glutaminy-L-S-benzyl-L-cysteine methyl ester. Tetraethyl pyrophosphite in diethyl phosphite gave a 70% yield of the expected product, m.p. 201°, [ $\alpha$ ]<sub>D</sub><sup>23</sup> –28.0° (*c* 1, dimethylformamide) (*Anal.* Calcd. for C<sub>24</sub>H<sub>29</sub>O<sub>6</sub>N<sub>3</sub>S: C, 59.1; H, 5.99; N, 8.62. Found: C, 59.2; H, 6.07; N, 8.53), and only 5% of the anhydro compound, m.p. 103–104°, [ $\alpha$ ]<sub>D</sub><sup>23</sup> –35.0° (*c* 1, dimethylformamide) (*Anal.* Calcd. for C<sub>24</sub>H<sub>27</sub>O<sub>5</sub>N<sub>3</sub>S: C, 61.4; H, 5.75; N, 8.94. Found: C, 61.7; H, 5.90; N, 8.75). The dicyclohexylcarbodiimide coupling, in tetrahydrofuran solution, gave 76% of the protected dipeptide ester, and no detectable anhydro compound.

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#### ON THE MOLECULAR WEIGHT, SIZE AND SHAPE OF THE MYOSIN MOLECULE

Sir:

Despite progress in several laboratories on the macromolecular characterization of myosin, no conclusive results have hitherto appeared. Part of the difficulty has recently been ascribed to spontaneous, strongly temperature dependent, side-to-side molecular aggregation.<sup>1</sup> Consequently, the molecular unit can be studied only by extremely rapid preparation and experimental analysis of the protein, working entirely in the cold up to the moment of actual measurement. We present here a summary of results obtained observing these precautions.

(1) A. Holtzer, *Arch. Biochem. & Biophysics*, in press.