loss during the addition there was an excess of hydrogen fluoride in the final mixture. Little heat was evolved. To make more certain that reaction did occur the loosely stoppered bottle with contents was warmed to 50–55° for about an hour.

Distillation at 11.5 to 12.0 mm. gave between 1 and 2 g. of distillate at 54.5 to 55.5° and 12 to 13 g. at 55.5 to 55.8°. After redistillation of the larger fraction the distillate was analyzed.

*Anal.* Calcd. for $(C_2H_5O)_2PSF$: F, 11.0; P, 18.0; S, 18.6. Found: F, 10.4; P, 18.2; S, 18.3.

Fluorine was determined by refluxing for two hours with alcoholic sodium hydroxide solution followed by distillation from perchloric acid and titration of the distillate with thorium nitrate in the presence of sodium alizarin sulfonate. The phosphorus and sulfur contents were determined by conventional methods following decomposition in a Parr peroxide bomb. Properties: $d^{25}_4$ 1.1387, $n^{25}D$ 1.4188, b.p. 58.0–58.7° at 12.9 mm., 164.0–164.7° at 740 mm.; soluble in alcohol, acetone and ether; only slightly soluble in water; hydrolyzes only slowly, no effect on glass noticeable after two years storage. The compound has a sharp, nauseating odor but the toxicity is not particularly high; $LD_{50}$ for rats is about 350 mg./kg. by intramuscular injection.[3] The chymotrypsin inhibitory potency is about one-tenth that shown by diisopropylmonofluorophosphate.[4]

(3) Private communication from Dr. Willy Lange, January 6, 1949.

(4) Private communication from Dr. Arnold Kent Balls, January 9, 1950.

OZARK-MAHONING COMPANY WAYNE E. WHITE
TULSA, OKLAHOMA ARCHIE HOOD

RECEIVED SEPTEMBER 17, 1951

# COMMUNICATIONS TO THE EDITOR

## THE COMPOSITION OF COENZYME A[1]

*Sir:*

After the presence of a sulfhydryl group in coenzyme A (CoA) had been established,[2,3,4] the contamination of CoA preparations by disulfide formation with other mercaptans was recognized. Therewith, the high sulfur content in CoA, amounting in some preparations to nearly 2 atoms per mole of pantothenic acid,[3] was explained. It was found that the contaminating mercapto derivative could be removed through inclusion of a reduction step.[5] In this manner, preparations were obtained with close to 1 atom of sulfur per mole of pantothenic acid. We wish to report here on a compound assaying 384 units per mg. and approaching ultimate purity (413 units per mg., calculated for a pantothenic acid content of 0.7$\gamma$ per unit, and a molecular weight of 767 for CoA). CoA was concentrated by adsorption on charcoal from a large-scale fermentation of *Streptomyces fradiae*. Elution with alkaline acetone, followed by a second acid adsorption and alkaline elution from charcoal, gave a preparation of 64 units per mg. in about 40% yield.[3]

This compound is reduced in 1% solution with zinc and 0.5 *N* hydrochloric acid for 30 minutes, then precipitated with excess mercuric acetate solution. The washed product is suspended, decomposed with hydrogen sulfide, and the supernatant passed through a column of Duolite CS-100 resin (100–200 mesh, acid form). Most of the impurities are removed by washing with 0.2 *N* hydrochloric acid, and the coenzyme is eluted with water and freeze-dried. This gives a compound of an average of 384 units per mg. in 20% yield, having the following analyses:

| | Calcd. %[a] | % Found | Ratio |
|---|---|---|---|
| Pantothenic acid | 28.6 | 26.8 (enzymatic assay)<br>25.6 (microbiological) | 1 |
| Adenine | 17.6 | 17.0 (spectrophotometric) | 1.05 |
| Phosphorus (total) | 12.12 | 10.6 | 2.83 |
| Mono-ester phosphorus[b] | ... | 3.6 | 0.96 |
| Sulfur | 4.18 | 4.13 | 1.07 |

[a] Pantothenic acid, 2-mercaptoethylamine, 3 phosphoric acid, adenosine, $-5H_2O$; molecular weight 767. [b] Liberated by prostate phosphomonoesterase.

On paper chromatography of the acid hydrolysate, such a substance shows the presence of $\beta$-alanine and 2-mercaptoethylamine disulfide, but no other ninhydrin-reacting compound. By comparison with earlier data,[3,5] this indicates the removal by the reduction step of all cross-linked sulfur-containing amino acid.

Due to the danger of decomposition, the preparation was dried *in vacuo* over phosphorus pentoxide for one hour at 34°. Assuming this to be sufficient to remove all water, this preparation is at least 90 to 93% pure CoA.

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RECEIVED DECEMBER 15, 1951

(1) This investigation was supported by a research grant from the National Cancer Institute of the National Institutes of Health, Public Health Service, and from the Commonwealth Fund.

(2) F. Lipmann, N. O. Kaplan, G. D. Novelli and B. Guirard, *J. Biol. Chem.*, **167**, 869 (1947); **186**, 235 (1950).

(3) W. H. DeVries, W. M. Govier, J. S. Evans, J. D. Gregory, G. D. Novelli, M. Soodak and F. Lipmann, THIS JOURNAL, **72**, 4838 (1950).

(4) E. E. Snell, G. M. Brown, V. J. Peters, J. A. Craig, E. L. Wittle, J. A. Moore, V. M. McGlohon and O. D. Bird, *ibid.*, **72**, 5349 (1950).

(5) J.D. Gregory and F. Lipmann, *Abstracts*, 12th Interntl. Cong. of Pure and Applied Chem., p. 74 (1951).

## A METHOD FOR PURIFICATION OF COENZYME A

*Sir:*

The following method for purification of coenzyme A (CoA), Lipmann's[1] acetylation coenzyme,

(1) F. Lipmann, N. O. Kaplan, G. D. Novelli, L. C. Tuttle and R. M. Guirard, *J. Biol. Chem.*, **167**, 869 (1947).