80% of which was removed by filtration. The balance did not interfere since <0.001% SiO_2 was found in the product. All filtrations were carried out in fritted-glass funnels, since any filter paper shreds in the final product caused reduction of AgCl during single crystal growth. Scrap silver salts, washed free of oil and grease with petroleum ether, reagent grade silver salts and silver metal were used as starting materials.

Experimental

The silver salts[2] were reduced with granular zinc (20 mesh, low in As, Fe and Pb) in (1:10) HCl, and the resulting metal was washed thoroughly, first by decantation, and then by filtration. The metallic silver was dissolved in a minimum amount of dilute (1:1) HNO_3. The resulting solution was diluted and tin, antimony, and the insoluble chlorides allowed to settle out. After filtration, the solution was heated, made ammoniacal, and filtered, removing Fe, Al, most of the Tl and some of the SiO_2. The filtrate was made just acid with HNO_3 and evaporated to a small volume, cooled, filtered and the resulting $AgNO_3$ was dissolved in water and filtered. AgCl was precipitated from the filtrate with concentrated HCl in slight excess.[3] After filtration, AgCl was dissolved in a minimum amount of NH_4OH and any residue filtered off. The solution was gently heated with continuous stirring until crystallization began. Removed from the heat, the solution was placed in the dark. Vigorous stirring was continued to prevent formation of a crust and to allow NH_3 to escape. After a sufficient crop of crystals had formed, they were washed, first with water, then with HCl and finally with water. The solution was reheated and a second and third crop of crystals gathered in the same manner. Proper care was taken throughout to recover silver from filtrates and residues.

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(2) Silver metal was dissolved in a minimum amount of HNO_3; AgCl was precipitated and washed free of nitrates, then treated as above.

(3) When thallium was present in excess of 0.50%, repetition of the preceding steps was necessary; and when copper was present in large quantities, repetition was deemed advisable.

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RECEIVED OCTOBER 1, 1951

NEW COMPOUNDS

Preparation of N-Acetylphenyl-2-thienylamine[1,2]

Ten grams (0.075 mole) of acetanilide, 20 g. (0.123 mole) of 2-bromothiophene, 5 g. (0.037 mole) of anhydrous potassium carbonate, about 0.1 g. of a mixture of powdered potassium iodide and powdered copper, a crystal of iodine and 50 ml. of nitrobenzene[3] were stirred in a three-necked 250-ml. flask in a nitrogen atmosphere for 25 hours at 160–170°. The dark mixture was neutralized, steam distilled and the residue cooled. The oil layer was extracted with ether, the ether solution dried with calcium chloride, and the ether removed by vacuum distillation at 100°. The solid, which weighed 14 g., was washed with 50 ml. of Skellysolve A, then dissolved in 25 ml. of hot absolute ethanol, treated with Norite A and filtered. The crystals which separated on cooling were collected on a filter and dissolved in 400 ml. of boiling water by addition of the minimum amount of ethyl alcohol. The precipitate which separated on cooling was collected on a filter and dried in vacuum over sulfuric acid, yielding 2.2 g. (14%) of white crystals melting at 100–101°.

Anal. Calcd. for $C_{12}H_{11}ONS$: N, 6.44; S, 14.74. Found: N, 6.33; S, 14.76.

Experimental conditions sufficiently vigorous to cause hydrolysis of N-acetylphenyl-2-thienylamine invariably resulted in formation of tars.

When 2-iodothiophene was substituted for 2-bromothiophene in the above procedure, a yield of 2.5 g. (31%) of crude N-acetyl phenyl 2-thienylamine was obtained. When 2-chlorothiophene was used, no product was obtained. Use of the method with N-acetyl-2-aminothiophene and iodobenzene gave less than a gram of crude N-acetyl phenyl 2-thienylamine. From N-acetyl-2-aminothiophene and 2-bromothiophene no acetyl-di-2-thienylamine could be obtained.

DEPARTMENT OF CHEMISTRY
UNIVERSITY OF KENTUCKY REEDUS RAY ESTES
LEXINGTON, KENTUCKY PETER PANZERA
RECEIVED OCTOBER 15, 1951

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(1) From the M.S. thesis of Peter Panzera, June, 1949.

(2) This work was supported in part by a Research Corporation Grant-in-aid.

(3) I. Goldberg, *Ber.*, **40**, 4541 (1907).

Preparation of Ethyl Pyrazinoylacetate

A mixture of 13.8 g. of methyl pyrazinoate and 14.8 g. of ethyl acetate was added slowly with stirring to 10.7 g. of alcohol-free sodium ethoxide. After standing at room temperature for one hour, the mixture was refluxed for five hours. The reaction mixture was then cooled, dissolved in 125 ml. of water and extracted with ether to remove the unreacted esters. The solution was neutralized to a *p*H of 7 with hydrochloric acid and exhaustively extracted with ether. The ether extract was dried over sodium sulfate and evaporated to a small volume to give 13 g. (67%) of ethyl pyrazinoylacetate (yellow crystals), which melted at 66–67° when recrystallized from petroleum ether.

Anal. Calcd. for $C_9H_{10}O_3N_2$: C, 55.6; H, 5.15; N, 14.4. Found: C, 55.7; H, 5.28; N, 14.4.

The following derivatives of ethyl pyrazinoylacetate were prepared: 2,4-dinitrophenylhydrazone, yellow crystals which melted at 187–189° when recrystallized from ethanol.

Anal. Calcd. for $C_{15}H_{16}O_6N_6$: N, 22.5. Found: N (Dumas), 22.8.

Phenylhydrazone, yellow crystals which melted at 131–132° when recrystallized from ethanol.

Anal. Calcd. for $C_{15}H_{16}N_4O_2$: N, 19.7. Found: N (Dumas), 19.5.

3-(2-Pyrazyl)-pyrazolone-5 light tan crystals which melted with decomposition at 245–246° when recrystallized from methanol.

Anal. Calcd. for $C_7H_6N_4O$: N, 34.6. Found: N (Dumas), 34.6.

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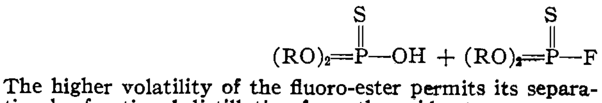
RECEIVED OCTOBER 5, 1951

Diethylthionomonofluorophosphate

The preparation of dialkylmonofluorophosphoric esters, $(RO)_2POF$, by the interaction of anhydrous hydrogen fluoride with symmetrical pyrophosphoric acid diesters has been previously described.[1] A similar procedure has now been found satisfactory for obtaining analogous thioesters. The general reaction is



$$(RO)_2{=}\overset{\overset{S}{\|}}{P}{-}O{-}\overset{\overset{S}{\|}}{P}{=}(OR)_2 + HF \longrightarrow (RO)_2{=}\overset{\overset{S}{\|}}{P}{-}OH + (RO)_2{=}\overset{\overset{S}{\|}}{P}{-}F$$

The higher volatility of the fluoro-ester permits its separation by fractional distillation from the acid ester.

To 7.8 g. of anhydrous hydrogen fluoride in a platinum bottle cooled in ice 101.2 g. of tetraethyldithionopyrophosphate[2] was slowly added. In spite of some vaporization

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(1) A. Hood and W. Lange, THIS JOURNAL, **72**, 4956 (1950).

(2) The tetraethyldithionopyrophosphate was kindly supplied by the Victor Chemical Works. For description of this and related compounds, see A. D. F. Toy, *ibid.*, **73**, 4670 (1951).

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: $d^{23}{}_4$ 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]

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RECEIVED SEPTEMBER 17, 1951

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[3] Private communication from Dr. Willy Lange, January 6, 1949.

[4] Private communication from Dr. Arnold Kent Balls, January 9, 1950.

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 0.7γ 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) 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 β-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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FRITZ LIPMANN

RECEIVED DECEMBER 15, 1951

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[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,

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