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
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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 0.7γ 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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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).

leads in one purification step from a crude concentrate to a product of high purity. This method is based on the observation[2] that CoA, owing to its character as a sulfhydryl compound,[3] can be precipitated in strong acid solution by Cu_2O in the presence of reduced glutathione (GSH). This step supplemented by available column procedures[4] for initial concentration and for elimination of carrier GSH permits preparation of CoA containing about 20% pantothenic acid from yeast in 10–15% yield.

CoA is adsorbed on a charcoal column from an aqueous extract (100°) of dried brewers' yeast and eluted with 5% pyridine (yield 80%). The eluate is shaken with chloroform, concentrated, and the coenzyme precipitated with 5 volumes of acetone (yield 60–70%). A solution of the acetone powder is again passed through a charcoal column and eluted as before after washing with dilute alkali (yield 80%). The eluate is freed from pyridine and concentrated to contain 5% solids.[5] GSH is added (10–20 mg./ml.) and the *p*H adjusted to 7. After a few minutes 0.05 volume of 10 *N* H_2SO_4 is added and Cu_2O is stirred in slowly as outlined by Hopkins.[6] The precipitate is washed with 0.5 *N* H_2SO_4, then with water until sulfate-free and finally decomposed with H_2S (yield 30–35%). After removal of CuS and H_2S the solution is passed through Dowex 50 (H^+) to remove GSH. The effluent is concentrated and lyophilized (yield 85%, over-all yield 10–15%). In a typical run from 8 kg. of dried yeast, 620 mg. of powder was obtained which analyzed as follows:

	%	Molar ratio
Pantothenic acid		
Microbiological[7]	22	1.0
Spectrophotometric, DPN reduction[8]	21	1.0
Transacetylase[9]	24	1.1
Sulfanilamide acetylation[10]	22	1.0
Adenine, from ultraviolet absorption at 260 mμ	13.7	1.0
Ribose	18.9	1.3
Glutathione, glyoxalase[11]		
GSH	3	0.1
GSSG	4	0.065
L-Glutamic acid, after acid hydrolysis[12]		
Microbiological, *L. arabinosus*	14	0.95
Decarboxylase, *E. Coli*[13]	11	0.75
Phosphorus, Fiske-SubbaRow[14]		
Inorganic	<0.1	
Total	10.3	3.3
Nitrogen, Kjeldahl	13.3	9.5
Sulfur[15]	4.68	1.45
Carbon[15]	32.3	27
Hydrogen[15]	4.83	48
Ash[15]	0	—

Electrophoresis and paper chromatography of such preparations revealed that the bulk of the material and CoA activity moved as a single component, although two to four minor components were detected.

(2) H. Beinert, R. W. Von Korff and D. E. Green, unpublished.

(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); G. M. Brown, J. A. Craig and E. E. Snell, *Arch. Biochem.*, **27**, 473 (1950); F. Lynen and E. Reichert, *Angew. Chem.*, **63**, 47 (1951).

(4) D. A. Buyske, R. E. Handschumacher, Harvey Higgins, Tsoo E. King, F. M. Strong, V. H. Cheldelin, L. J. Teply and G. C. Mueller, *J. Biol. Chem.*, **193**, 307 (1951); D. A. Buyske, R. E. Handschumacher, Harvey Higgins, and F. M. Strong, unpublished.

(5) The second charcoal treatment may be omitted if a final pantothenic acid content of about 12–15% is satisfactory.

(6) F. G. Hopkins, *J. Biol. Chem.*, **84**, 269 (1929).

(7) J. B. Neilands and F. M. Strong, *Arch. Biochem.*, **19**, 287 (1948).

(8) Unpublished method of R. W. Von Korff.

(9) E. R. Stadman, G. D. Novelli and F. Lipmann, *J. Biol. Chem.*, **191**, 365 (1951).

(10) N. O. Kaplan and F. Lipmann, *ibid.*, **174**, 37 (1948).

(11) G. E. Woodward, *ibid.*, **109**, 1 (1935).

(12) This amount of glutamic acid obviously cannot be contained in the glutathione present, but may still have been derived in some manner from the glutathione used in the preparation. The data available do not permit the conclusion that glutamic acid is a component of the CoA molecule. In fact a similar subsequent preparation contained 24% P.A. but less than 2% glutamic acid.

(13) W. W. Umbriet and I. C. Gunsalus, *J. Biol. Chem.*, **159**, 333 (1945).

(14) B. L. Griswold, F. L. Humoller and A. R. McIntyre, *Anal. Chem.*, **23**, 192 (1951).

(15) Microanalyses by C. W. Beazley, Micro-Tech Laboratories, Skokie, Illinois.

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RECEIVED NOVEMBER 26, 1951

A SERIES OF NEW, BIOLOGICALLY SIGNIFICANT DIHYDROTRIAZINES

Sir:

We wish to report the synthesis (by E. J. M.) of a hitherto unreported class of compounds (I) with anti-vitamin and anti-malarial activity. In the course of a program of synthesis of arylbiguanides,[1] a new compound (m.p. 189–191°; $\lambda_{max.}^{H_2O}$ 236 mμ, log ε 4.24; *Anal.* Calcd. for $C_{14}H_{19}N_5O_2 \cdot HCl$: C, 51.61; H, 6.19; N, 21.50. Found: C, 51.97; H, 6.12; N, 21.22) was produced by the condensation of ethyl *p*-aminobenzoate, dicyandiamide and concentrated hydrochloric acid in acetone. The structure Ia·HCl, 4,6-diamino-1-(*p*-carbethoxyphenyl)-1,2-dihydro-2,2-dimethyl-*s*-triazine hydrochloride, is proposed for this substance. This reaction is general for a ring-substituted aniline hydrochloride, dicyandiamide or N^1-monosubstituted dicyandiamide and a number of ketones or aldehydes. Another synthesis of compounds with structure I has been developed through condensation of arylbiguanides with ketones or aldehydes under acid conditions.

(1) Synthesis of a group of arylbiguanides was undertaken originally in these laboratories at the suggestion of M. M. Pechet as one part of a broad program in the chemotherapy of cancer initiated by Sidney Farber.