heated on a steam-bath for 30 minutes. The resulting hot solution was neutralized to *p*H 5.5 with dilute aqueous sodium hydroxide, cooled and extracted continuously with ether for three days. During this time considerable material crystallized from the ether solution.

The ether was cooled and filtered, and the resulting crystalline material was recrystallized from benzene–hexane to give 1.0 g. (22% yield based on crude starting material) of deschloroanhydroaureomycin, m.p. 215–220° dec., $[\alpha]^{25}D$ +25°[23] (cellosolve). This compound shows ultraviolet absorption properties substantially identical with those of anhydroterramycin (*cf.* Fig. 4).

Anal. Calcd. for $C_{22}H_{22}N_2O_7$: C, 61.96; H, 5.20; N, 6.57. Found: C, 62.01; H, 5.18; N, 6.45.

Desoxydesdimethylaminoanhydroaureomycin (XV or a Tautomer).—A solution of 3.6 g. of amorphous desoxydesdimethylaminoaureomycin in 100 ml. of methanol was treated with 100 ml. of saturated methanolic hydrogen chloride solution. A rapid reaction occurred during which an orange-red powder separated. After 30 minutes at room temperature, the solid was collected and washed with methanol. There was obtained 2.9 g. of amorphous product. This was boiled in 40 ml. of dioxane, filtered hot and washed with dioxane. Two grams (57% yield) of crystalline product of good quality was obtained. Recrystallization from boiling nitrobenzene gave orange-red needles which decomposed without liquefying at about 240–250° and showed a specific rotation at 25° (0.1% in dimethylformamide) of +955°. This compound shows ultraviolet absorption peaks at 275, 338, 391 and 450 mμ in glacial acetic acid solution.

Anal. Calcd. for $C_{20}H_{16}NO_6Cl$: C, 59.78; H, 4.02; N, 3.49; Cl, 8.83. Found: C, 60.13; H, 4.14; N, 3.57; Cl, 8.90.

Naphthacene from Desoxydesdimethylaminoanhydroaureomycin.—Seventy-five milligrams of desoxydesdimethylaminoanhydroaureomycin was mixed in a mortar with 5 g. of purified[24] zinc dust, packed between asbestos plugs in an 8 × 300 mm. Pyrex tube, flushed with hydrogen, and heated to a dull red heat in a slow stream of hydrogen. The small amount of orange-red distillate which collected in the cooler parts of the tube was sublimed twice at 180° (0.05 mm.) to yield 0.5 mg. of pure naphthacene—identified by its very characteristic ultraviolet absorption peaks at 280.5, 295, 355, 375, 395, 418, 444 and 475 mμ in benzene solution. An additional 0.5–1.0 mg. of less pure naphthacene was also recovered from the reaction.

Acknowledgments.—We should like to express our gratitude to Messrs. G. B. Hess, T. J. Toolan and Dr. J. R. Means for the analytical data and for physical measurements recorded in this paper. We are also indebted to Mr. E. J. Bianco for certain preparative work, and to Dr. D. B. Seeley and associates for the microbiological assays.

(23) This product was also prepared, in a different manner, by C. W. Waller and co-workers who report m.p. 225–226° dec. and $[\alpha]^{25}D$ +24° (cellosolve); *cf.* reference 5e.

(24) The purification procedure is described in the Experimental section of reference 4b.

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The Stability of Coenzyme A[1]

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RECEIVED FEBRUARY 1, 1954

The stability of a high purity preparation of coenzyme A has been followed by use of the transacetylase assay system. It was found that the transacetylase method measured only intact CoA, while the sulfanilamide method was active for fragments of the CoA molecule. Acetone powders and high purity CoA preparations in the free acid form were found to be stable for several years when stored under dry conditions, at room temperature. Drying of high purity powders *in vacuo* produced marked decreases in activity. Aqueous solutions subjected to autoclave temperatures showed considerable destruction. Alkaline solutions of CoA were found to be unstable, while acidic solutions were much more stable and even showed an increase in activity under certain conditions of time, temperature and *p*H.

The great interest in coenzyme A (CoA) and its wide current research use has prompted the present study of its stability under a variety of commonly encountered conditions. Most of the work was carried out on a single highly purified preparation, although a few observations were made on a crude concentrate.

Experimental

Materials.—Crude concentrates containing 2–4% of CoA (acetone powder stage) were prepared from brewers' yeast as previously described.[3] To obtain CoA of the highest purity, the previous procedure[3] was modified by including a reduction step after the second charcoal adsorption and before the copper-glutathione precipitation. The pyridine-free eluate from the second charcoal column was made 0.5 *N* in sulfuric acid and forced by air pressure through a Jones reductor consisting of a 1 × 15 cm. column of freshly amalgamated, granulated zinc (20 mesh). The effluent was kept at 0–5° until all the solution had passed through (about 30 minutes). The solution was passed through a Dowex-50 column, then warmed to 40°, immediately precipitated in the usual manner and carried through to the lyophilized powder as previously described.[3]

The freshly purified material (approximately 95% CoA) was dissolved in water at a concentration of 2.5 mg. per ml. and 0.2-ml. aliquots were dispersed into a series of 10-ml. ampules. The solutions were lyophilized, and the ampules filled with nitrogen, sealed, and stored at −5° in the dark until needed for stability trials. No detectable change in activity occurred under these conditions during the two months required to complete the stability studies.

Analytical Methods.—CoA activity was determined by the transacetylase method,[4] using transacetylase from *E. coli*. Occasional samples were also checked by the sulfanilamide method.[5] Pantothenic acid (PA) was determined microbiologically after enzymatic liberation,[6] and ribose by Drury's method.[7] Adenine was estimated spectrophotometrically after hydrolysis in *N* HCl at 100° for one hour. The molar extinction value of 13,300 for free adenine in acid solution at the maximum wave length of 264 mμ was used for the calculations. The Beckman model DU spectrophotometer was used for all measurements in the ultraviolet.

(1) Presented in part at the 124th Meeting of the American Chemical Society, Chicago, Ill., September, 1953. Supported in part by grants from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

(2) National Science Foundation Predoctoral Fellow, 1952–1953.

(3) H. Beinert, R. W. Von Korff, D. E. Green, D. A. Buyske, R. E. Handschumacher, H. Higgins and F. M. Strong, *J. Biol. Chem.*, **200**, 385 (1953), and preceding papers.

(4) E. R. Stadtman, G. D. Novelli and F. Lipmann, *ibid.*, **191**, 365 (1951).

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

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

(7) H. F. Drury, *ibid.*, **19**, 455 (1948).

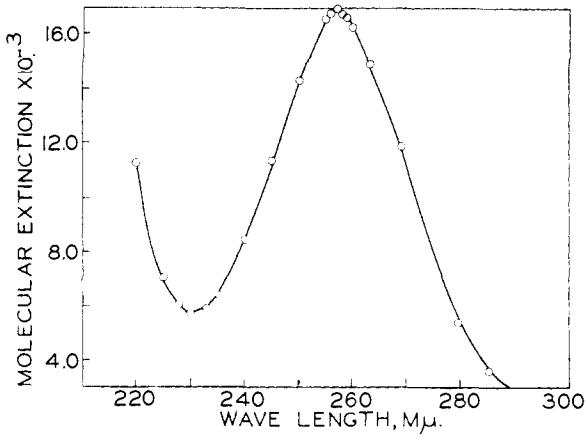

Fig. 1.—Absorption spectrum of CoA in aqueous solution at *p*H 2.5.

Total phosphorus was determined after sulfuric acid–hydrogen peroxide digestion by the Fiske–SubbaRow procedure or its modification described by Griswold, *et al.*[8] Microanalytical determinations of total carbon, hydrogen, nitrogen and sulfur were carried out by C. W. Beazley.[9]

Stability Trials.—The contents of one of the ampules described above containing 0.5 mg. of highly purified CoA were dissolved in a few ml. of water. An aliquot was immediately removed, frozen, and stored in Dry Ice to be later assayed together with other aliquots which had been subjected to the desired experimental conditions. Changes in activity resulting from the experimental treatment are reported as a percentage of the activity found on the corresponding untreated aliquot. For experiments on the effect of drying, the solid contents of an ampule were weighed out directly. For the experiments reported in Table III the *p*H of the test solutions was adjusted to the desired level by addition of 0.1 *N* potassium hydroxide.

Results and Discussion

The composition of the high-purity CoA sample used in these studies is given in Table I. The analytical values agree with theory for the currently accepted structure of CoA[10] within about ±4%, except for total nitrogen and pantothenic acid. The latter may well be low because of incomplete enzymatic hydrolysis prior to the *L. arabinosus* assay. No explanation for the high nitrogen value is available. In view of the large number of components determined and the generally close agreement with theory, it appears that the purity of this preparation was of the order of 95%.

TABLE I

COMPOSITION OF A HIGHLY PURIFIED CoA PREPARATION

Component	Found,[a] %	Theory,[b] %	% of theory
Carbon	32.7	32.86	99.5
Hydrogen	4.56	4.73	96.4
Nitrogen	13.6	12.78	106.3
Sulfur	4.01	4.18	95.9
Phosphorus	11.6	12.11	96.0
Adenine[c]	18.1	17.61	102.6
Pantothenic acid[c]	26.5	28.56	92.9
Ribose[c]	20.4	19.56	104.3
Loss on drying[d]	1.3	...	...

[a] Dry basis. [b] Calcd. for $C_{21}H_{36}O_{16}N_7SP_3$: mol. wt., 767.56. [c] Based on the free component after hydrolysis. [d] Four hours over P_2O_5 at 1 mm. and 100°.

The ultraviolet absorption spectrum of aqueous solutions of the same preparation is shown in Fig. 1. The molecular extinction at the maximum, 257 mμ, was 16,838. The ratio of the extinction at 280 mμ to that at 260 mμ was 0.33. The absorption curve was the same at *p*H 2.5, 7.0 and 11.0, except that the extinction at the minimum, 230 mμ, was 7,400 at *p*H 11.0 and 5,695 at 2.5 or 7.0.

For the stability studies it was necessary to use an assay system which would measure only intact CoA. The transacetylase system was selected since it gives no response to degradation fragments of the coenzyme. In contrast, the commonly used sulfanilamide assay[5] shows a high response to such fragments. For example, highly purified diphosphoadenosine from barium hydroxide hydrolysates of CoA[11] was mixed with a solution containing the other main hydrolysis product, presumably pantetheine phosphate, and assayed. The sulfanilamide method showed a response equal to 70% of an equivalent amount of intact CoA while the transacetylase system showed no activity.

Assay of Acetone Powders.—The preparation of dried acetone powders is a step common to the published methods for obtaining high purity CoA.[3,12,13] Since the acetone powder is a convenient stopping point in the preparation, it was of interest to investigate the stability of these powders. The original CoA content ranged from 1.5 to 4.0%. It was found that such powders were stable for at least three years when stored at room temperature. Aqueous solutions of these powders did not decrease in activity after several days at 4°, or even at room temperature if the *p*H was below 7.

Storage of CoA.—Lyophilized CoA powders of high purity are highly hygroscopic, and if exposed to the atmosphere rapidly absorb moisture and lose activity. However, it was found that dry, high purity powders stored as the free acid in a desiccator at room temperature for almost two years showed no loss in activity.

Drying *in vacuo*.—The effect of drying high purity powders *in vacuo* over P_2O_5 is shown in Table II. Destruction increases rapidly with temperature, but there is little additional loss after four hours. This loss may have been due to the formation of an inner anhydride of some sort; however, the activity was not restored by dissolving the dried sample in water, adjusting to *p*H 7.8, and warming at 40° for two hours.

TABLE II

LOSS OF COENZYME A ACTIVITY DURING DRYING *in vacuo*[a]

Time, hours	Temperature 40°	76.8°	100°
	% of original activity lost		
4	14.3	21.3	70.5
24	15.0	21.6	89.0

[a] 1 mm.

Autoclaving Aqueous Solutions.—The destruction of aqueous solutions of CoA submitted to auto-

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

(9) Micro Tech Laboratories, Skokie, Illinois.

(10) J. Baddiley, E. M. Thain, G. D. Novelli and F. Lipmann, *Nature*, **171**, 76 (1953).

(11) R. E. Handschumacher, Ph.D. Thesis, University of Wisconsin, 1953.

(12) F. Lipmann, N. O. Kaplan, G. D. Novelli and L. C. Tuttle, *J. Biol. Chem.*, **186**, 235 (1950).

(13) E. R. Stadtman and A. Kornberg, *ibid.*, **203**, 47 (1953).

clave temperatures of 120° (15 p.s.i.) is shown in Fig. 2. Only 23% loss occurred in 30 minutes at *p*H 7. This result indicates the feasibility of using CoA in studies involving the necessity of sterilization.

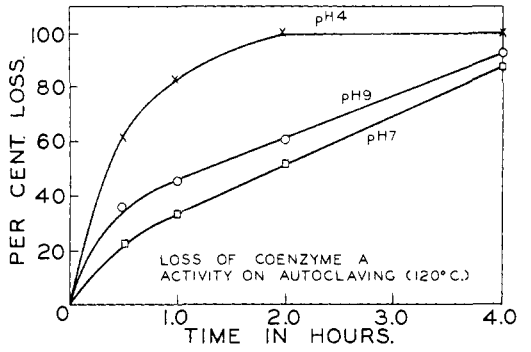

Fig. 2.—Stability of CoA to autoclaving (120°) in aqueous solutions of various *p*H values.

Acid and Alkali Treatments.—Table III shows the loss of CoA activity at alkaline *p*H values. The results show the inadvisability of keeping CoA solutions at room temperature for long periods of time, particularly if alkaline. Pyridine appeared to have a specific destructive effect, since solutions of CoA in aqueous pyridine lost up to 90% of their activity after 24 hours even though the *p*H was only slightly alkaline.

TABLE III

LOSS OF COENZYME A ACTIVITY AT ALKALINE *p*H VALUES

*p*H	Temp., °C.	Time in hours: 1.5	3.0	24
		% of original activity lost		
7	0	0	0	6.1
	25	0	5.0	..
	40	0	11.5	..
8	0	0	0	6.0
	25	0	10.4	31.0
	40	0	11.3	42.0
9	0	0	0	7.7
	25	0	17.4	..
	40	0	24.4	..
10	0	0	0	21.7
	25	4.3	23.4	..
	40	13.0	28.6	..

One of the steps in the preparation of CoA by the method of Beinert, *et al.*,[3] involves precipitation from 0.5 *N* sulfuric acid solution at 40°. This precipitation requires from 10 to 20 minutes. It was found in the present study that this exposure to acid causes a 20 to 30% loss in activity.

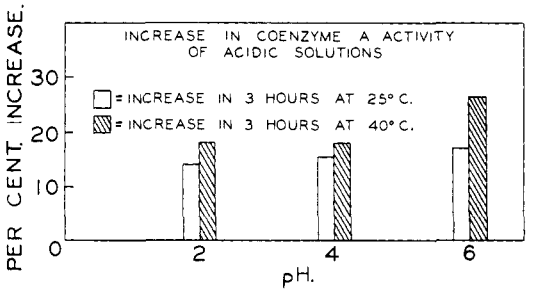

Fig. 3.—Increase in CoA activity of acidic solutions.

Figure 3 shows an interesting phenomenon that was observed when studying the stability of coenzyme A in aqueous solution at acid *p*H values. At *p*H 2 to 6 under the conditions shown in Fig. 3 there was no destruction, but actually an increase in activity. This increase was time and temperature dependent and reached a maximum of 20–25% in three hours at 40°. After four hours at 40° the activity began to decrease and had returned to its original level after 24 hours. Previously published work has suggested the possible existence of two active forms of CoA.[3] The main evidence for a second active component was a minor transacetylase-active peak beside the major peak in the electrophoretic pattern of CoA. Whether or not this minor peak and the transitory existence of a more active form are related has not yet been determined.

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The Metalation of Dialkyl Sulfones

BY WILLIAM E. TRUCE AND KENNETH R. BUSER

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Dimethyl sulfone (I) was monometalated by ethylmagnesium bromide, methylmagnesium iodide and *n*-butyllithium; the resulting organometallic compounds were added to benzaldehyde and benzophenone. With the intermediate methanesulfonylmethylmagnesium bromide (II), superior yields were obtained using anisole rather than benzene as the solvent. Tetrahydrothiophene-1,1-dioxide was metalated in poor yield by ethylmagnesium bromide.

The recent report of mono- and dimetalation of dimethyl sulfone (I) by ethylmagnesium bromide[1] has prompted the presentation of the following related work. The only earlier reference to the action of organometallic compounds on dialkyl sulfones is the report[2] that Grignard reagents have no effect on diisoamyl sulfone; however, this work was not properly designed to detect metalation. Several workers[1,3] have investigated the action of organometallic reagents on alkyl aryl sulfones.

The present investigation concerns the metalation of I by methylmagnesium iodide, ethylmagnesium bromide and *n*-butyllithium. The metalated derivatives were added successfully to benzophenone

(1) L. Field and J. W. McFarland, THIS JOURNAL, **75**, 5583 (1953).
(2) H. Hepworth and H. W. Clapham, *J. Chem. Soc.*, 1192 (1921).
(3) E. P. Kohler and H. Potter, THIS JOURNAL, **57**, 1316 (1935); W. M. Ziegler and R. Conner, *ibid.*, **62**, 2596 (1940); F. J. Webb, *Iowa State Coll. J. Sci.*, **17**, 152 (1942); H. A. Potter, paper presented to the Spring Meeting of the Midland Section of the A.C.S. in 1951; L. Field, THIS JOURNAL, **74**, 3920 (1952).