

hexane solution: 277.7 (2370) and 270.5 (2440);  $\lambda_{\min.}$  (and  $\epsilon$ ): 275.0 (1590) and 246.5 (470).

**228° Isomer.**—This isomer has been prepared from ethyl silicate, tri-*o*-tolylchlorosilane, and tri-*o*-tolylmethoxysilane. Tri-*o*-tolylchlorosilane (0.175 mole), for example, was refluxed under nitrogen for 48 hours with *o*-tolyllithium (0.25 mole) in ether. The ether was distilled and the residue heated at 140–180° for 5 hours. After hydrolysis,<sup>2a</sup> the ether soluble material was separated and distilled. The fraction boiling 215–220° (1 mm.) and the adjoining fractions gave 11 g. (16%) tetra-*o*-tolylsilane melting 215–225° after treatment with ligroin. Recrystallizations from ethanol–benzene (9:1) and from ligroin raised the melting point to 227.5–228.0°. Additional recrystallizations from these solvents and from acetic acid did not change the melting point. *Anal.* Calcd. for C<sub>28</sub>H<sub>28</sub>Si: C, 85.66; H, 7.19; Si, 7.15. Found: C, 85.63; H, 7.03; Si, 7.11. *Ultraviolet data.*<sup>6</sup>  $\lambda_{\max.}$  in m $\mu$  (and  $\epsilon$ ) for cyclohexane solution: 277.8 (2520) and 270.5 (2470);  $\lambda_{\min.}$  (and  $\epsilon$ ): 275.0 (1640) and 246.5 (440).

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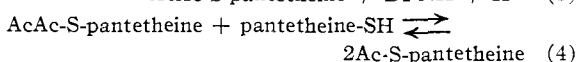
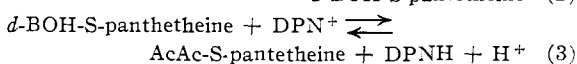
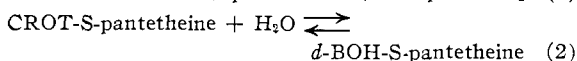
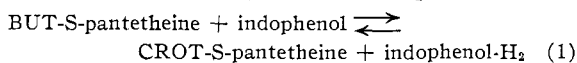
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#### AN ALTERNATE FATTY ACID CYCLE INVOLVING THIOESTERS OF PANTETHEINE<sup>1</sup>

Sir:

It is now well established<sup>2–4</sup> that fatty acid oxidation and synthesis can proceed via a reversible cycle of enzymes utilizing thioesters of CoA.<sup>5</sup> Recent experiments demonstrate that the same or similar enzymes catalyze an analogous sequence of reactions involving thioesters of pantetheine.<sup>6</sup>



Enzymes catalyzing reaction 1 have been found in soluble extracts of pigeon and ox liver (Table I). Using a modified indophenol assay,<sup>7</sup> the enzymatic

(1) Supported by grants from the U. S. Public Health Service and the Williams–Waterman Fund of Research Corporation.

(2) F. Lynen and S. Ochoa, *Biochim. Biophys. Acta*, **12**, 299 (1953).

(3) H. R. Mahler, *Federation Proc.*, **12**, 694 (1953).

(4) G. D. Greville and H. B. Stewart, *Chem. Soc. Ann. Reports*, **50**, 301 (1954).

(5) Abbreviations: Coenzyme A (reduced), CoA-SH; thioesters, acyl-S-R; acids: acetic, Ac; butyric, BUT; crotonic, CROT;  $\beta$ -hydroxybutyric, BOH; acetoacetic, AcAc; *d* refers to direction of rotation; DPN<sup>+</sup> and DPNH, oxidized and reduced diphosphopyridine nucleotide; TRIS, tris-(hydroxymethyl)-aminomethane;  $\beta$ -MEA,  $\beta$ -mercaptoethylamine; ATP, adenosine triphosphate; 5-AMP, adenosine-5'-phosphate; PP, pyrophosphate.

(6) Pantetheine was kindly supplied by Dr. O. D. Bird, Parke, Davis and Co.

(7) D. E. Green, S. Mii, H. R. Mahler and R. M. Bock, *J. Biol. Chem.*, **206**, 1 (1954).

oxidation of BUT-S-pantetheine was measured (a) as the decrease in light absorption at  $\lambda$  600  $\mu\mu$  due to dye reduction and (b) by demonstrating a concomitant increase in absorption at  $\lambda$  240  $\mu\mu$  due to formation of CROT-S-pantetheine. This reaction proceeds to an equilibrium, at which point addition of *trans*-CROT-S-pantetheine causes re-oxidation of dye and attainment of a new equilibrium. The *cis*-isomer also is reduced, but less rapidly. Crystalline liver crotonase<sup>8</sup> was found to hydrate both *trans*- and *cis*-isomers of CROT-S-pantetheine. Although the rates were only 0.013% and 0.0023% of those for the respective CoA esters, they are significant in view of the very high turnover number (730,000) of crystalline crotonase. A partially purified preparation of crotonase-free heart *d*-BOH-S-CoA dehydrogenase<sup>9</sup> reacts almost equally well with the pantetheine as with the CoA thioesters of AcAc and *d*-BOH.

TABLE I

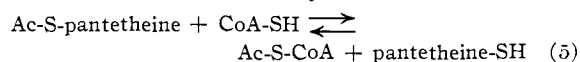
#### REACTIVITY OF PANTETHEINE AND CoA ESTERS

Reactants as indicated: 0.01 *M* TRIS–HCl buffer, 1–2  $\times 10^{-4}$  *M* thioester, 8.2  $\times 10^{-5}$  *M* dye, 3  $\times 10^{-4}$  *M* DPN<sup>+</sup>, 1.7  $\times 10^{-4}$  DPNH. Specific activity =  $\mu\text{M}$ . thioester reacting per minute per mg. protein at 25°.

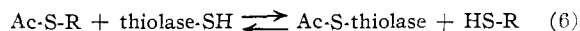
Reaction	pH	Specific activity	
		R = Pantetheine	R = CoA
1 BUT-S-R + dye	7.5	0.0039 <sup>a</sup>	0.0055
BUT-S-R + dye	7.5	.0018 <sup>b</sup>	.0025
2 <i>trans</i> -CROT-S-R + H <sub>2</sub> O	7.5	.220 <sup>c</sup>	1700
<i>cis</i> -CROT-S-R + H <sub>2</sub> O	7.5	.012	510
3 <i>d</i> -BOH-S-R + DPN <sup>+</sup>	9.1	.52 <sup>d</sup>	0.68
AcAc-S-R + DPNH	7.0	3.1	3.1

<sup>a</sup> Dialyzed pigeon liver extract. <sup>b</sup> Ox liver mitochondrial extract after salt fractionation. <sup>c</sup> Crystalline liver crotonase<sup>10</sup> (representing 800-fold purification). <sup>d</sup> Partially purified pig heart *d*-BOH-S-CoA dehydrogenase.

A highly purified preparation of heart thiolase<sup>9,10</sup> (Table II) was found to catalyze (a) the thiolysis of AcAc-S-pantetheine<sup>11</sup> by pantetheine, (b) the condensation of two molecules of Ac-S-pantetheine to AcAc-S-pantetheine, and (c) the transfer of the acetyl group from Ac-S-pantetheine (but not Ac-S-Ac-N- $\beta$ -MEA) to CoA-SH (reaction 5). The rate of this thioltransacetylation reaction is rather



more rapid than the rate of the condensation reaction and is further evidence for the hypothesis of Lynen that reaction (6) (R = CoA or pantetheine) is a partial reaction of the overall thiolase reaction (4).



Ac-S-pantetheine was inactive as substrate for the crystalline citrate condensing enzyme.<sup>12</sup> Ac-Ac- and succinyl-S-pantetheine compounds did not react with highly purified CoA transferase.<sup>9,10</sup>

(8) J. R. Stern and A. del Campillo, *THIS JOURNAL*, **75**, 2277 (1953).

(9) J. R. Stern, M. J. Coon and A. del Campillo, *ibid.*, **75**, 1517 (1953).

(10) J. R. Stern, in S. P. Colowick and N. O. Kaplan, "Methods in Enzymology," New York **1**, 559 and 573 (1955).

(11) See reference 2 for preparation of thioesters and optical methods of assay.

(12) S. Ochoa, J. R. Stern and M. C. Schneider, *J. Biol. Chem.*, **193**, 691 (1951).

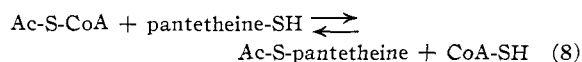
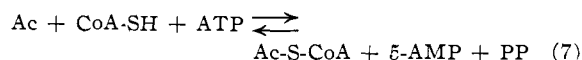
TABLE II  
REACTIONS CATALYZED BY THIOLASE

110-fold purified heart thiolase (specific activity 37 when prepared). Final concentrations: 0.01 M TRIS-HCl buffer pH 8.1,  $1.0 \times 10^{-4}$  M AcAc-SR,  $2.0 \times 10^{-4}$  M R-SH,  $5 \times 10^{-8}$  M MgCl<sub>2</sub>,  $1.5 \times 10^{-3}$  M Ac-S-pantetheine.

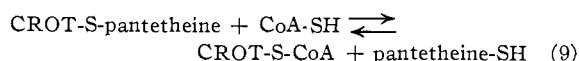
Reaction	Specific activity
AcAc-S-CoA + CoA-SH	4.05
AcAc-S-Pantetheine + CoASH	3.02
AcAc-S-Pantetheine + pantetheine	0.80
AcAc-S-CoA + pantetheine	.79
2-Ac-S-pantetheine	.09 <sup>c</sup>
Ac-S-Pantetheine <sup>a</sup> + CoA-SH	.15
AcAc-S-Ac-N-βMEA <sup>b</sup> + HS-AcN-βMEA <sup>b</sup>	0

<sup>a</sup>  $1.0 \times 10^{-3}$  M; pH 7.5; spontaneous rate was 0.2 times the enzymatic one. <sup>b</sup>  $3.3 \times 10^{-3}$  M. <sup>c</sup> Indirect assay<sup>11</sup> pH 7.1. By direct assay (pH 9.2) 0.035.

In liver extracts pantetheine did not substitute for CoA in the aceto-CoA-kinase reaction<sup>13</sup> nor in the butyro-CoA-kinase reaction.<sup>14</sup> However, the synthesis of Ac-S-pantetheine<sup>15</sup> from Ac, ATP and pantetheine occurs in pigeon liver extract provided catalytic amounts of CoA-SH are added. This indicated the coupling of the aceto-CoA-kinase (reaction 7) with reaction 8 (the reverse of reaction 5):



The reversibility of reaction 8 was shown by coupling it with the crystalline citrate condensing enzyme and purified malic dehydrogenase<sup>16</sup> and observing a CoA-SH dependent reduction of DPN in the presence of Ac-S-pantetheine, L-malate, and pigeon liver extract. The specific activity was 0.014. The enzyme catalyzing reaction 8 belongs to the class of thioltransacylases recently described in pigeon liver by Brady and Stadtman<sup>17</sup> who did not test pantetheine. Pigeon liver extracts were also found to catalyze a CoA-SH dependent hydration of CROT-S-pantetheine to *d*-BOH-S-CoA (specific activity 0.0056) indicating the presence of a thioltranscrotonylase catalyzing reaction 9



Since liver also catalyzes a stepwise conversion of sorbyl-S-CoA through β-ketohexenoyl-S-CoA to CROT-S-CoA and Ac-S-CoA,<sup>18</sup> the exact relation of the thioltransacylases present in liver extract to thiolase-type enzymes remains to be determined.

It would seem that, as with crystalline crotonase, the pantetheine thioesters serve as substrates at physiological concentrations for the enzymes of the

(13) M. E. Jones and F. Lipmann, in S. P. Colowick and N. O. Kaplan, *Methods in Enzymology*, New York, 1, 585 (1955).

(14) H. R. Mahler, S. J. Wakil and R. M. Bock, *J. Biol. Chem.*, **204**, 453 (1953).

(15) Optical assay (λ240) according to E. R. Stadtman, *ibid.*, **203**, 501 (1953).

(16) J. R. Stern, S. Ochoa and F. Lynen, *ibid.*, **198**, 313 (1952).

(17) R. O. Brady and E. R. Stadtman, *ibid.*, **211**, 621 (1954).

(18) J. R. Stern, unpublished observations.

CoA fatty acid cycle. The demonstration of enzymes which synthesize pantetheine thioesters is further evidence for the occurrence of a pantetheine fatty acid cycle. Unlike the CoA cycle, the pantetheine cycle is not directly linked to the citric acid cycle; and, since crotonase appears to be the rate-limiting step in this cycle, it may play a role in isoprenoid synthesis. The relative amount of CoA and pantetheine in bacteria and tissues,<sup>19</sup> and hence the quantitative significance of the respective cycles, has still to be assessed.

(19) E. E. Snell and G. M. Brown, *Advances in Enzymology*, **14**, 49 (1953).

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SIMILARITIES BETWEEN SO-CALLED CHLOROPHYLL b'' AND OXIDIZED CHLOROPHYLL b, AND BETWEEN SO-CALLED CHLOROPHYLL a'' AND OXIDIZED CHLOROPHYLL a

Sir:

Heat, alkalis and oxygen convert chlorophylls a and b into several chromatographically unique pigments that are spectrally similar to the parent chlorophylls. Some of these pigments, chlorophylls a' and b', are isomers or other reconvertible alteration products of the common chlorophylls.<sup>1</sup> Some are oxidation or allomerization products that have not been reconverted to the chlorophylls.<sup>2</sup> Others, chlorophylls a'' and b'', are regarded as chlorophyll isomers although they also resemble the non-reconvertible allomerized chlorophylls.<sup>3</sup> Additional indications that a'' and b'' are oxidation products are presented herein.

In repetition of the experiments of Strain and Manning<sup>1</sup> and of Strain,<sup>2</sup> heat or traces of alkali converted chlorophyll a, in *n*-propyl alcohol solution, into a' plus a. The less sorbed a', isolated in a column of powdered sugar and redissolved in propyl alcohol, was reconverted into a plus a' by heat or alkalis. These preparations of a and a' were readily interconverted again by heat or alkalis. Consequently, a and a' are isomers or other readily interconvertible products. In similar experiments, chlorophyll b yielded the reconvertible, less sorbed b'. Neither a nor b yielded more sorbed green pigments.

Freed, *et al.*,<sup>3</sup> found the strongly sorbed b'' (yield ca. 14%), in addition to weakly sorbed b', when very dilute propyl alcohol solutions of b were heated in vacuum. This pigment was regarded as a chlorophyll, because it was prepared in vacuum, its spectral properties resembled those of b, and it was more sorbed than b. Freed's allomerized b (method of preparation not described) was less sorbed than b. Unlike b and b', b'' did not give the phase test, and it was not reconvertible to b plus b'.

Strain<sup>2</sup> has found, however, that b yields a par-

(1) H. H. Strain and W. M. Manning, *J. Biol. Chem.*, **146**, 275 (1942).

(2) H. H. Strain, *Agricultural and Food Chem.*, **2**, 1222 (1954).

(3) S. Freed, K. M. Sancier and A. H. Sporer, *THIS JOURNAL*, **76**, 6006 (1954).