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

228° Isomer.—This isomer has been prepared from ethyl silicate, tri-*o*-tolylcholorosilane, 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,[2a] 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 $C_{28}H_{28}Si$: C, 85.66; H, 7.19; Si, 7.15. Found: C, 85.63; H, 7.03; Si, 7.11. *Ultraviolet data.*[6] $\lambda_{max.}$ in mμ (and ϵ) for cyclohexane solution: 277.8 (2520) and 270.5 (2470); $\lambda_{min.}$ (and ϵ): 275.0 (1640) and 246.5 (440).

DEPARTMENT OF CHEMISTRY
MUHLENBERG COLLEGE
ALLENTOWN, PENNSYLVANIA G. N. RUSSELL SMART
DEPARTMENT OF CHEMISTRY HENRY GILMAN
IOWA STATE COLLEGE HARRY W. OTTO
AMES, IOWA

RECEIVED AUGUST 22, 1955

AN ALTERNATE FATTY ACID CYCLE INVOLVING THIOESTERS OF PANTETHEINE[1]

Sir:

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

BUT-S-pantetheine + indophenol ⇌ CROT-S-pantetheine + indophenol-H_2 (1)

CROT-S-pantetheine + H_2O ⇌ *d*-BOH-S-pantetheine (2)

d-BOH-S-panthetheine + DPN^+ ⇌ AcAc-S-pantetheine + DPNH + H^+ (3)

AcAc-S-pantetheine + pantetheine-SH ⇌ 2Ac-S-pantetheine (4)

Enzymes catalyzing reaction 1 have been found in soluble extracts of pigeon and ox liver (Table I). Using a modified indophenol assay,[7] the enzymatic oxidation of BUT-S-pantetheine was measured (a) as the decrease in light absorption at λ 600 mμ due to dye reduction and (b) by demonstrating a concomitant increase in absorption at λ 240 mμ due to formation of CROT-S-pantetheine. This reaction proceeds to an equilibrium, at which point addition of *trans*-CROT-S-pantetheine causes reoxidation of dye and attainment of a new equilibrium. The *cis*-isomer also is reduced, but less rapidly. Crystalline liver crotonase[8] 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[9] 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 × 10^{-4} *M* thioester, 8.2 × 10^{-5} *M* dye, 3 × 10^{-4} *M* DPN^+, 1.7 × 10^{-4} DPNH. Specific activity = μM. thioester reacting per minute per mg. protein at 25°.

	Reaction	*p*H	Specific activity R = Pantetheine	R = CoA
1	BUT–S–R + dye	7.5	0.0039[a]	0.0055
	BUT–S–R + dye	7.5	.0018[b]	.0025
2	*trans*-CROT–S–R + H_2O	7.5	.220[c]	1700
	cis-CROT–S–R + H_2O	7.5	.012	510
3	*d*-BOH–S–R + DPN^+	9.1	.52[d]	0.68
	AcAc–S–R + DPNH	7.0	3.1	3.1

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

A highly purified preparation of heart thiolase[9,10] (Table II) was found to catalyze (a) the thiolysis of AcAc-S-pantetheine[11] 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-βMEA) to CoA-SH (reaction 5). The rate of this thioltransacetylation reaction is rather

Ac-S-pantetheine + CoA-SH ⇌ Ac-S-CoA + pantetheine-SH (5)

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-R + thiolase-SH ⇌ Ac-S-thiolase + HS-R (6)

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

(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; β-hydroxybutyric, BOH; acetoacetic, AcAc; *d* refers to direction of rotation; DPN^+ and DPNH, oxidized and reduced diphosphopyridine nucleotide; TRIS, tris-(hydroxymethyl)-aminomethane; β-MEA, β-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).

(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 *p*H 8.1, 1.0×10^{-4} *M* AcAc–SR, 2.0×10^{-4} *M* R–SH, 5×10^{-3} *M* $MgCl_2$, 1.5×10^{-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[c]
Ac–S–Pantetheine[a] + CoA–SH	.15
AcAc–S–Ac–N–βMEA[b] + HS–AcN–βMEA[b]	0

[a] 1.0×10^{-3} *M*; *p*H 7.5; spontaneous rate was 0.2 times the enzymatic one. [b] 3.3×10^{-3} *M*. [c] Indirect assay[11] *p*H 7.1. By direct assay (*p*H 9.2) 0.035.

In liver extracts pantetheine did not substitute for CoA in the aceto-CoA-kinase reaction[13] nor in the butyro-CoA-kinase reaction.[14] However, the synthesis of Ac-S-pantetheine[15] 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):

$$\text{Ac} + \text{CoA-SH} + \text{ATP} \rightleftharpoons \text{Ac-S-CoA} + \delta\text{-AMP} + \text{PP} \quad (7)$$

$$\text{Ac-S-CoA} + \text{pantetheine-SH} \rightleftharpoons \text{Ac-S-pantetheine} + \text{CoA-SH} \quad (8)$$

The reversibility of reaction 8 was shown by coupling it with the crystalline citrate condensing enzyme and purified malic dehydrogenase[16] 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 thioltransacetylases recently described in pigeon liver by Brady and Stadtman[17] 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

$$\text{CROT-S-pantetheine} + \text{CoA-SH} \rightleftharpoons \text{CROT-S-CoA} + \text{pantetheine-SH} \quad (9)$$

Since liver also catalyzes a stepwise conversion of sorbyl-S-CoA through β-ketohexenoyl-S-CoA to CROT-S-CoA and Ac-S-CoA,[18] 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 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,[19] and hence the quantitative significance of the respective cycles, has still to be assessed.

(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.

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

DEPARTMENT OF PHARMACOLOGY
SCHOOL OF MEDICINE
WESTERN RESERVE UNIVERSITY
CLEVELAND 6, OHIO
JOSEPH R. STERN

RECEIVED JULY 12, 1955

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

Sir:

Heat, alkalies 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.[1] Some are oxidation or allomerization products that have not been reconverted to the chlorophylls.[2] Others, chlorophylls a″ and b″, are regarded as chlorophyll isomers although they also resemble the non-reconvertible allomerized chlorophylls.[3] Additional indications that a″ and b″ are oxidation products are presented herein.

In repetition of the experiments of Strain and Manning[1] and of Strain,[2] 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 alkalies. These preparations of a and a′ were readily interconverted again by heat or alkalies. 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.*,[3] 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[2] 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. Sttain, *Agricultural and Food Chem.*, **2**, 1222 (1954).

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