gave 11.5, 12.1% (calcd. for three methyls, 10.7%), thus four terminal methyls (three branches) are indicated. Infrared spectra[5] first indicated a carbon–carbon double bond, and quantitative hydrogenation resulted in absorption of one equivalent of hydrogen for a molecular weight of 423. Ultraviolet absorption spectra showed λ maximum at 217 mμ, log ε 4.03, whereas pure synthetic 3-methyl-2-nonenoic acid showed λ maximum at 219 mμ, log ε 3.92. Thus, it seems definitely established that this substance, for which we propose the name C_{28}-phthienoic acid, is an α,β-unsaturated acid.

For the hydrogenated acid, $[\alpha]^{25}D + 2.8°$, eq. wt., 421.6, $n^{25}D$ 1.4565. Thus, the high molecular rotation of phthioic acid fractions, which has previously been inexplicable,[4] may now be attributed to the presence of unsaturation near an asymmetric center. Thus, there must be a branching alkyl in the γ- or δ-position, probably in the γ-position. This type of structure also explains the large change in optical rotation in going from acid to ester, and failure of the acid to betray its unsaturation by addition of halogen.[2]

The ultraviolet absorption spectrum of the hydrogenated acid (C_{28}-phthianoic acid) has been compared with that of numerous synthetic branched-chain acids, but the absorption band due to carboxyl is weak (log ε about 2) and broad, and traces of impurities may have a large effect. It is felt that these data cannot yet be interpreted with certainty, but the presence of alkyls at the α- and β-positions seems possible. Both the natural product and model synthetic acids are being studied further.

(5) Determined by Dr. K. N. Freeman, Department of Medical Physics, University of California, Berkeley.

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PURIFICATION OF COENZYME A FROM FERMENTATION SOURCES AND ITS FURTHER PARTIAL IDENTIFICATION

Sir:

The pantothenic acid (p.a.) derivative, coenzyme A (Co A)[1], a co-factor in enzymatic acetyl transfer reactions, had been isolated from animal sources. Many microörganisms contain large amounts of the coenzyme. A particularly rich source is *Streptomyces fradiae*, originally isolated by Dr. S. A. Waksman of Rutgers University. A fermentation carried out at 32° for 88 hours produced up to 5 units[2] of Co A per ml., from which a preparation of approximately 64 μ per mg. was obtained by repeated acid adsorption on charcoal and elution with alkaline acetone–water. Further purification was carried out on charcoal columns, giving products of up to 240 u. p. mg. activity, corresponding probably to around 60% purity. From a larger batch of 210 μ per mg. the following data were obtained:

	μM/mg.	molar ratio	%
Pantothenic acid[3]	0.71	1	15.4
Adenosine[4]	0.91	1.28	24.4
Adenine, from U. V. absorption at 260 mμ	0.99	1.45	
Ribose	1.14	1.6	
Reducing sugar, after acid hydrolysis	1.03	1.44	
Phosphorus	2.15	3.0	6.7
Sulfur	1.31	1.86	4.2
Cystine equivalent (= half cystine, after acid hydrolysis)	0.89	1.26	10.8
Glutamic acid		<0.1	

Besides adenosine and phosphate, a sulfur-compound resembling cystine had been found earlier. In chromatograms of acid hydrolysates, the sulfur fragment, revealed by cyanide–nitroprusside test, also reacts with ninhydrin, but its chemical identity remains to be decided. Strong evidence appears now that it is part of the coenzyme molecule. (a) The sulfur content increases parallel with activity. (b) On paper chromatography of phosphatase-treated Co A,[3] three major bands developed with butanol–water, all showing p. a. associated with the sulfur-containing moiety. The fastest moving component contained p. a. and sulfur compound only, but no adenine. The liberation of p. a. from this fragment by liver extract[5] should be due to a split of the link between p. a. and sulfur compound. These findings assume increased interest through recent work by McRorie, *et al.*,[6] and by Brown, *et al.*[7] They find their growth factor for *Lactobacillus bulgaricus* (LBF) to be a p. a. derivative,[6] namely, the residue of Co A after intestinal phosphatase treatment.[7] Activity of LBF is destroyed by liver extract. Comparison of our data with those on LBF suggest that LBF is, or at least contains, the p. a.–sulfur compound. A sample of LBF, containing 12.5% pantothenate, kindly supplied to us by Dr. W. L. Williams of the Lederle Laboratories, gave the cyanide–nitroprusside reaction and behaved chromatographically analogously to our fast moving p. a.–sulfur compound.

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(1) Lipmann, Kaplan, Novelli, Tuttle, and Guirard, *J. Biol. Chem.*, **167**, 869 (1947); **186**, 235 (1950).

(2) Kaplan and Lipmann, *J. Biol. Chem.*, **174**, 37 (1948).

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(4) Kalckar, *ibid.*, **167**, 445 (1947).

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(7) Brown, Craig and Snell, *Arch. Biochem.*, **27**, 473 (1950).

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