

TABLE II

YNA	Flattened ellipsoid					Elongated ellipsoid				
	a/b	f/f_0	M	$a(\text{\AA.})$	$b(\text{\AA.})$	b/a	f/f_0	M	$a(\text{\AA.})$	$b(\text{\AA.})$
I	43.0	2.26	19,000	115	2.7	17.2	1.88	33,000	15	262
II	26.2	1.94	17,000	93	3.6	12.7	1.68	25,000	15	196
II'	23.2	1.87	23,000	99	4.3	11.8	1.64	35,000	17	207

YNA-I is the sample prepared from the fresh bakers' yeast ("Oriental" Yeast Co., Tokyo) by the method of Clarke and Schryver.³ A trace of protein impurity can be further removed by shaking with chloroform-amyl alcohol mixture according to the method of Sevag.⁴ These protein-free samples, YNA-II and -II', contain 8.90 and 9.01% phosphorus, respectively. The diffusion constant obtained by us is far smaller than that found by previous investigators⁵ and corresponds to a molecular weight of 120,000–220,000 when YNA is assumed to be an unhydrated sphere.

We have tried to explain the large value of intrinsic viscosity by assuming the molecule of YNA is either a hydrated sphere or an ellipsoid of revolution. In the former case, however, the hydration becomes unreasonably large, *i.e.*, 6.6, 3.9 and 3.4 g. of water per one gram of nucleic acid for YNA-I, -II and -II', respectively. Therefore, the molecule of YNA must be ellipsoidal.

In the latter case, the axial ratio (a/b or b/a) of an ellipsoid of revolution considered to be a model of YNA molecules can be evaluated from the volume fraction intrinsic viscosity by the Simha equation.⁶ From this axial ratio, Svedberg's fric-

tional ratio (f/f_0) can be calculated by using the equation derived by Perrin⁷ and by Herzog, Illig and Kudar.⁸ With this frictional ratio, diffusion constant and partial specific volume, the molecular weight (M), the length of axis of revolution (b) and the length of equatorial axis (a) of ellipsoid of revolution can easily be evaluated. In Table II are shown these values.

From these results, it seems impossible to consider YNA as a flattened molecule, for its thickness (b) is found to be far too small (not greater than about 4 \AA.). The data appear to be more consistent with the concept of an elongated molecule. It is very interesting in this case to find that the diameter of YNA molecule (a) is about 15–17 \AA. and is quite identical with that of thymonucleic acid⁹ and of the pentosenucleic acid of tobacco mosaic virus.¹⁰

It might be, therefore, reasonable at the present time to conclude that the shape of a YNA molecule is essentially rod-like, *i.e.*, 15–17 \AA. in diameter and 200–260 \AA. long, and its molecular weight is at least about 30,000.

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RECEIVED MARCH 15, 1950

COMMUNICATIONS TO THE EDITOR

STRUCTURAL FEATURES OF AN ACID OF THE PHTHIOIC TYPE¹

Sir:

Phthioic acid, the physiologically active fraction isolated from tubercle bacillus,² has been described as 3,13,19-trimethyl-tricosanoic acid,³ but this structure has been shown untenable.⁴ A continuation of the study of this interesting material has become possible through the generosity of Prof. R. J. Anderson, who has supplied us with 24 g. of a crude methyl phthioate fraction.

(1) This investigation was supported in part by a research grant from the National Institute of Health, Public Health Service.

(2) M. A. Spielman and R. J. Anderson, *J. Biol. Chem.*, **112**, 759 (1936).

(3) N. Polgar and R. Robinson, *J. Chem. Soc.*, **399** (1945).

(4) J. Cason and F. S. Prout, *THIS JOURNAL*, **70**, 879 (1948).

Fractionation and systematic refractionation of this material through a four-foot column of the Podbielniak type has yielded 39 fractions, whose rotations and indices of refraction indicate the presence of at least eleven components. About one-fourth of the total appeared to be one component, and four successive fractions showed the same boiling point, optical rotation and index of refraction; b. p. 232.0° (2.0 mm), $[\alpha]^{25}_D + 14.7^\circ$, $n^{25}_D 1.4600$. Saponification yielded a polymorphic acid whose rotation was not significantly changed by recrystallization from acetone; m.p. 26–27° and 38–41°, $[\alpha]^{25}_D + 17.8^\circ$, $n^{25}_D 1.4666$. *Anal.* Calcd. for $C_{28}H_{54}O_2$: C, 79.55; H, 12.88; eq. wt., 422.7. Found: C, 79.18; H, 12.56; eq. wt., 423.7, 426.8. Analysis for terminal methyl

gave 11.5, 12.1% (calcd. for three methyls, 10.7%), thus four terminal methyls (three branches) are indicated. Infrared spectra⁵ 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 m μ , log ϵ 4.03, whereas pure synthetic 3-methyl-2-nonenic acid showed λ maximum at 219 m μ , log ϵ 3.92. Thus, it seems definitely established that this substance, for which we propose the name C₂₈-phthienoic acid, is an α,β -unsaturated acid.

For the hydrogenated acid, $[\alpha]^{25}_D + 2.8^\circ$, 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 (C₂₈-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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RECEIVED AUGUST 18, 1950

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 microorganisms 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 units² 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,

(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).

corresponding probably to around 60% purity. From a larger batch of 210 μ per mg. the following data were obtained:

	μ M/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 m μ	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 extract⁵ 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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RECEIVED AUGUST 15, 1950

(3) Novelli, Kaplan and Lipmann, *ibid.*, **177**, 97 (1949).

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

(5) Novelli, Kaplan and Lipmann, *Fed. Proc.*, **9**, 209 (1950).

(6) McRorie, Masley and Williams, *Arch. Biochem.*, **27**, 471 (1950).

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(8) This group was supported by research grants from the National Cancer Institute, the U. S. Public Health Service, and from the Nutrition Foundation.