

TABLE II  
SUMMARY OF THERMODYNAMIC VALUES FOR THE FIVE HEXANES IN THE GASEOUS STATE<sup>a,b</sup>

Isomer	$H^\circ$ (isomer) - $H^\circ$ ( <i>n</i> -hexane), cal./mole				$F^\circ/T$ (isomer) - $F^\circ/T$ ( <i>n</i> -hexane), cal./deg. mole			Relative amounts present at equilibrium		
	0°K.	298°K.	600°K.	1000°K.	298°K.	600°K.	1000°K.	298°K.	600°K.	1000°K.
<i>n</i> -Hexane (g)	0	0	0	0	0	0	0	1.0	1.0	1.0
2-Methylpentane (g)	-1108 ± 220	-1748 ± 200	-1468	-1248	-3.56 ± 0.77	-0.85	+0.17	6.0 ± 2.3	1.5	0.9
3-Methylpentane (g)	-399 ± 220	-1069 ± 200	-759	-339	-1.18 ± 0.77	+0.43	+0.88	1.8 ± 0.7	0.8	0.7
2,3-Dimethylbutane (g)	-1856 ± 220	-2546 ± 200	-2016	-1596	-2.67 ± 0.77	+1.20	+2.52	3.8 ± 1.5	0.6	0.3
2,2-Dimethylbutane (g)	-3535 ± 220	-4375 ± 200	-4095	-3375	-8.00 ± 0.77	-0.80	+1.75	56 ± 22	1.5	0.4

<sup>a</sup> The values of energy are given in terms of the artificial calorie defined as equal to 4.1833 international joules. <sup>b</sup> The uncertainty in the difference in heat content or free energy between any two isomers is substantially the same as that given here for the difference between normal hexane and each of the other isomers.

$T$  become values for the standard free energy change for the reaction,  $n\text{-C}_6\text{H}_{14} = i\text{-C}_6\text{H}_{14}$ ; columns 9, 10, and 11 give values for the relative amounts of each of the five isomers present at equilibrium with one another at 298, 600, and 1000°K., with the amount of normal hexane taken as unity. Estimated uncertainties are assigned to the values for 0 and 298°K., the uncertainties for 600 and 1000°K. will be greater.

NATIONAL BUREAU OF STANDARDS    FREDERICK D. ROSSINI  
WASHINGTON, D. C.                    EDWARD J. R. PROSEN

RECEIVED JULY 5, 1940

#### UNSATURATED FAT OXIDASE

Sir:

In 1928 R. Bohn and L. W. Haas found that the soy bean, navy bean and other beans contain an enzyme which oxidizes carotene and unsaturated fats. They obtained and assigned patents to the J. R. Short Milling Company of Chicago for bleaching of wheat flour by this method. Recently J. B. Sumner and A. L. Dounce [*Enzymologia*, 7, 190 (1939)] verified the claims of Bohn and Haas and stated that the carotene oxidase present in soy meal also oxidizes xanthophyll of egg yolk.

The following experiments show that carotene oxidase as described by these authors does not exist and that the oxidation of carotene is caused indirectly by an "unsaturated fat oxidase." To 30 cc. of water, 2 g. of soy meal and 0.15 cc. of commercial carotene in vegetable oil (a mixture of  $\alpha$ - and  $\beta$ -carotene) was added. A second enzyme sample was prepared containing 0.03 mg. of crystalline  $\beta$ -carotene in 0.15 cc. of mineral oil and to a third sample containing 0.03 mg. of crystalline  $\beta$ -carotene in 0.15 cc. of mineral oil, 0.15 cc. of olive oil was added. All samples of course

contained 2 g. of soy meal. To all samples 10 cc. of 0.1 *M* phosphate of pH 6.5 was added and they were mechanically shaken in open containers at 23°. The sample with the commercial carotene in vegetable oil was completely oxidized (disappearance of yellow color) in thirty minutes. The sample with crystalline carotene and added olive oil was oxidized in sixty minutes, whereas the sample which contained crystalline carotene without olive oil remained unchanged for at least three hours. In the presence of fat gaseous oxygen was taken up rapidly when the reaction was measured in the Warburg-Barcroft respirometer. When crystalline  $\beta$ -carotene in mineral oil was employed, however, no oxygen was consumed and the color of the enzyme solution remained yellow.

These results show that the oxidation of carotene, and probably also the oxidation of xanthophyll, is dependent upon the simultaneous oxidation of unsaturated fats. The unsaturated fats are oxidized (probably to peroxides) by the *unsaturated fat oxidase* and the products of oxidation in turn oxidize the carotenoids.

I am grateful to Professor L. Zechmeister of the California Institute of Technology for a generous sample of crystalline  $\beta$ -carotene, and to the Short Milling Company for supplying me with soy meal.

LONG ISLAND CITY, N. Y.

HENRY TAUBER

RECEIVED JUNE 12, 1940

#### IMPROVED SYNTHESIS OF PANTOTHENIC ACID

Sir:

A method of synthesis of pantothenic acid has been described<sup>1,2</sup> in which  $\beta$ -alanine ester was

(1) Woolley, Waisman and Elvehjem, *THIS JOURNAL*, 61, 977 (1939).

(2) Woolley, *J. Biol. Chem.*, 134, 461 (1940).

conjugated with the acid chloride of the acetylated hydroxy acid fragment followed by selective hydrolysis of the ester linkages. This method suffers from the instability of  $\beta$ -alanine ester, and from the need of 1 mole of the rather expensive ester merely to neutralize the hydrochloric acid formed in the reaction. In work on the isolation of the acid fragment from liver,<sup>3</sup> the use of excess ester was justified by the rarity of the acid chloride. Since it has been shown<sup>4</sup> that the acid fragment of pantothenic acid is a readily obtainable compound,<sup>5</sup> it would be desirable to eliminate the use of  $\beta$ -alanine ester in the synthesis of this vitamin.

The sodium salt of  $\beta,\beta$ -dimethyl- $\alpha,\gamma$ -dihydroxybutyric acid<sup>5</sup> was refluxed for thirty minutes with excess acetic anhydride, and the solution was concentrated under reduced pressure to dryness. The residue was dissolved in dilute hydrochloric acid and extracted with ether for six hours. The extract was dried and evaporated, and dissolved in a large excess of thionyl chloride. After one hour, excess thionyl chloride was removed under reduced pressure. The acid chloride may be used directly, or purified by distillation (b. p. 140–142° at 13 mm.) with equal success.

8.9 g. (0.1 mole) of  $\beta$ -alanine was dissolved in 50 cc. of 2 *N* sodium hydroxide and cooled in ice and salt. During the next thirty minutes 50 cc. of 2 *N* sodium hydroxide and 25 g. of the acid

(3) Woolley, *Science*, **91**, 245 (1940).

(4) Williams and Major, *ibid.*, **91**, 246 (1940).

(5) Kuhn and Neustadter, *Monatsh.*, **39**, 293 (1918).

chloride were added alternately in small portions. The reaction flask was shaken vigorously during these additions. The solution was adjusted to pH 7.0, concentrated under reduced pressure to dryness, and the residue extracted with ethanol. To the concentrated ethanol solution was added 9 g. of sodium hydroxide in 100 cc. of methanol. After one hour concentrated hydrochloric acid was added until the solution was acid to thymol blue. Sodium chloride was filtered off and the filtrate was concentrated under reduced pressure to a sirup. This was extracted thrice with ethyl acetate, and the extracts freed of solvent and dissolved in water. Sodium bicarbonate was added until pH 7.0 was reached, when the solution was extracted with ether for six hours and then evaporated under reduced pressure. The cake of sodium salt was dissolved in absolute ethanol and 6 volumes of ethyl acetate added. Sodium pantothenate was obtained as a white, hygroscopic powder; yield, 10–12 g. Calcd. for  $C_9H_{16}NO_5Na$ : Na, 9.5. Found: Na, 9.4. Half-maximum effect in promoting growth of *Lactobacillus casei* was obtained<sup>6</sup> when 0.02 microgram per cc. was added. For analyses the barium salt was more convenient than the sodium salt because it was not hygroscopic. It was precipitated from alcohol solution by acetone or ether. Calcd.: Ba, 23.9. Found: Ba, 23.8.

(6) Snell, Strong and Peterson, *THIS JOURNAL*, **60**, 2825 (1938).

THE HOSPITAL OF THE ROCKEFELLER INSTITUTE  
FOR MEDICAL RESEARCH  
NEW YORK, N. Y. D. W. WOOLLEY

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## NEW BOOKS

**Quantitative Analysis.** By HAROLD SIMMONS BOOTH, Ph.D., Professor of Chemistry, and VIVIAN RICHARD DAMERELL, Ph.D., Assistant Professor of Chemistry, Western Reserve University. McGraw-Hill Book Company, Inc., 330 West 42nd Street, New York, N. Y., 1940. xi + 246 pp. 48 figs. 15.5 × 23.5 cm. Price, \$2.25.

This book is designed for the usual college course in elementary quantitative chemical analysis. It appears to fulfill this purpose admirably.

A typical chapter in the book is devoted to the determination of some one radical or to a volumetric method. There is first a summary of the method, discussing the

principles involved, and sources of error in the results. Then follow detailed directions and finally a series of questions to test the students' knowledge. Theory and principles are introduced as they are needed in the course of the work. The very inadequate treatment of quantitative calculations, particularly in volumetric methods, is intentional as the authors believe this subject should be treated in a separate volume.

The book is perhaps a little over-extended on the subject of balances, weights and weighing (24 pages) and, on the other hand, the number of determinations does not allow the student or instructor a very wide choice. For instance, determination of silica in an insoluble silicate is given in