milk independently confirm those recently reported by Peterson, Haig and Shaw.[1] Preliminary experiments have also indicated that the destruction by radiation increases with temperature, as previously reported by Williams and Cheldelin.[2]

Samples of so-called "raw" milk (I), homogenized raw milk (II), pasteurized milk (III), homogenized pasteurized milk (IV), and irradiated vitamin D-containing milk (V), were freshly obtained. All samples were stored in the dark in the usual capped quart bottles and were refrigerated until the experiment was begun. Exposures were made within twenty-four hours of bottling to direct mid-morning spring sunshine in the open air for periods up to two hours during which the atmospheric temperature was 16.7 to 20.6°.

TABLE I

Sample	Riboflavin concentration,[a] γ/ml.	Destruction of riboflavin after 1 hr. exposure,[a] %	2 hr. exposure[a] %
I	2.07	36	59
II	2.05	27	54
III	1.97	26	54
IV	1.86	39	68
V	1.89	32	66

[a] All riboflavin assays were made in triplicate and corrected for the values of the blank.

Riboflavin assays were made by the microbiological method of Snell and Strong[3] and frequent recovery experiments were made by adding known amounts of the pure crystalline vitamin to samples of milk to check the accuracy of the method. Recoveries amounted to 94–102%. In addition, samples of milk were withdrawn at the same time as those for assay, made alkaline to *p*H 10 with 1 *N* sodium hydroxide, and exposed to the light from a 750-watt bulb at 50 cm. for twenty-four hours in order to destroy all riboflavin present. The photolyzed milk was then neutralized to *p*H 6.8 and added separately to the basal medium in identical amount to the samples being assayed. The results of these blanks were subtracted from the values of the actual assays to give the riboflavin concentrations reported in Table I.

Since milk after delivery to the consumer frequently remains on an open porch in direct sunlight, it is apparent that during this period from one-third to two-thirds of the riboflavin may be destroyed.

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RECEIVED APRIL 24, 1944

(1) W. J. Peterson, F. M. Haig and A. O. Shaw, THIS JOURNAL, **66**, 662 (1944).

(2) R. R. Williams and V. H. Cheldelin, *Science*, **96**, 22 (1942).

(3) E. E. Snell and F. M. Strong, *Ind. Eng. Chem., Anal. Ed.*, **11**, 346 (1939).

COMMUNICATIONS TO THE EDITOR

THE TOTAL SYNTHESIS OF 2,3,4,5-TETRADEHYDROBIOTIN

Sir:

Since the announcement of the structural elucidation of biotin [du Vigneaud, *et al.*, *J. Biol. Chem.*, **146**, 475, 487 (1942)], the quest for a practical synthesis of the vitamin in this Laboratory has led to the development of a seventeen-step synthesis of 2′-keto-3,4-imidazolido-2-thiophenevaleric acid, the aromatic analog of biotin. Experiments are now in progress on the nuclear reduction of this compound to biotin.

The starting materials were trimethylene chlorobromide and ethyl malonate, from which was obtained ethyl 3-chloropropylmalonate by the method of Fischer and Bergmann [*Ann.*, **398**, 120 (1913)]. Hydrolysis and decarboxylation followed by esterification gave ethyl 5-chlorovalerate, described by Mellor [*J. Chem. Soc.*, **79**, 132 (1901)]. This compound reacted with ethyl malonate to produce ethyl pentane-1,1,5-tricarboxylate (b. p. 165–170° (4 mm.), for $C_{14}H_{24}O_6$—Calcd.: C, 58.32; H, 8.37. Found: C, 58.49; H, 8.54). Hydrolysis yielded pentane-1,1,5-tricarboxylic acid (m. p. 82°, for $C_8H_{12}O_6$—Calcd.: C, 47.05; H, 5.92. Found: C, 47.11; H, 5.88). Treatment with sulfuryl chloride followed by decarboxylation gave 2-chloropimelic acid (m. p. 89–90°, for $C_7H_{11}O_4Cl$—Calcd.: C, 43.25; H, 5.70. Found: C, 43.27; H, 5.78) which after reaction with β-mercaptopropionic acid and esterification yielded 2-carbethoxyethyl 1,5-dicarbethoxyamyl sulfide (b. p. 210–213° (3 mm.), for $C_{16}H_{28}O_6S$—Calcd.: C, 55.14; H, 8.10. Found: C, 55.14; H, 8.15). Cyclization by the Dieckmann reaction gave ethyl 4-carbethoxy-3-keto-2-tetrahydrothiophenevalerate (for $C_{14}H_{22}O_5S$—Calcd.: C, 55.60; H, 7.33. Found: C, 55.53; H, 7.36). The oxime of this keto ester was converted to ethyl 3-amino-4-carbethoxy-2-thiophenevalerate (m. p. 43–44°) by means of dry hydrogen chloride followed by decomposition of the resulting amine hydrochloride with a suitable base. Selective hydrolysis produced 3-amino-4-carbethoxy-2-thiopheneval-

eric acid (m. p. 97–97.5°, for $C_{12}H_{17}O_4NS$—Calcd.: C, 53.12; H, 6.32. Found: C, 53.14; H, 6.32) which was converted to the N-benzoyl derivative (m. p. 126.5–127.5°, for $C_{19}H_{21}O_5NS$—Calcd.: C, 60.77; H, 5.63. Found: C, 60.70; H, 5.53). The Curtius degradation through the hydrazide (m. p. 140–141°, for $C_{17}H_{19}O_4N_3S$—Calcd.: C, 56.49; H, 5.30. Found: C, 56.40; H, 5.57), and the azide (dec. 99–100°, for $C_{17}H_{16}O_4N_4S$—Calcd.: C, 54.83; H, 4.33. Found: C, 55.11; H, 4.53) gave 3-benzoylamino-4-carbethoxyamino-2-thiophenevaleric acid (m. p. 156.5–157.5°, for $C_{19}H_{22}O_5N_2S$—Calcd.: C, 58.44; H, 5.68. Found: C, 58.88; H, 5.72) when the azide was refluxed with absolute alcohol. The final product, 2′-keto-3,4-imidazolido-2-thiophenevaleric acid (m. p. 253–254° with decomposition, for $C_{10}H_{12}O_3N_2S$—Calcd.: C, 49.98; H, 5.03. Found: C, 50.33; H, 5.25) was prepared from the urethan by hydrolysis to the diamine and immediate treatment with phosgene.

Ultraviolet absorption curves prepared by Dr. J. M. Vandenbelt show that the compound has strong absorption in the region 250 to 270 mμ, with the peak value at 260 mμ having a molar extinction coefficient of approximately 17×10^{-3}. The curve closely corresponds with the curves for 2′-keto-3,4-imidazolido-2-γ-phenoxypropylthiophene (m. p. 174–174.5°), 2′-keto-3,4-imidazolido-2-γ-benzyloxypropylthiophene (m. p. 127–127.5°) and 2′-keto-3,4-imidazolido-2-γ-hydroxypropylthiophene (m. p. 138–139°), all of which have been prepared and characterized during this work.

The authors gratefully acknowledge the assistance of A. W. Spang who did the microanalyses. Details for the preparation of all compounds and the results of biological tests for biotin and antibiotin activity will be forthcoming.

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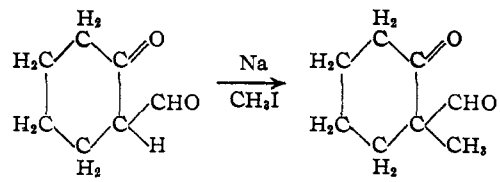
RECEIVED MAY 20, 1944

ATTEMPTED REPETITION OF A REPORTED TOTAL ASYMMETRIC SYNTHESIS

Sir:

In a recent letter to the editors of *Nature*, Paranjape, Phalnikar, Bhide and Nargund[1] report the synthesis of several optically active substances from inactive starting materials without the use of any asymmetric reagents or catalysts. They report that the asymmetric synthesis occurred at the following step in their series of reactions



(inactive) $[\alpha]_D$ −26.22

The reported activity was determined on the crude material, since the methylated formylcyclohexanone was reported to be an unstable liquid, impossible to purify by distillation.

An attempt has been made in these Laboratories to repeat this extraordinary result, which would not be predicted on the basis of any known theory of optical activity.

Inactive formylcyclohexanone was prepared according to the method of Rupe and Klemm,[2] and the methylation was carried out using two different techniques.

In the first trial, formylcyclohexanone was added to a 10% ethanolic solution of sodium ethylate and refluxed with methyl iodide. This yielded, after evaporation of the solvent and washing with water, a reddish liquid, a portion of which darkened and resinified when an attempt was made to distill it. The crude material had a specific rotation of zero, with an error of ±0.7° due to the difficulty of taking readings on such a comparatively strongly colored material.

In the second synthesis, the sodium salt of the formylcyclohexanone was suspended in toluene and allowed to react with methyl iodide. This procedure yielded a much clearer product after removal of the solvent, and it was possible to determine that the material was totally inactive within the 0.01° accuracy of the Zeiss polarimeter. Readings were taken on the material itself as well as on chloroform solutions of it as reported by the Indian authors.

Considerable speculation on possible sources of their reported results has yielded no reasonable explanation.

CONTRIBUTION NO. 970 FROM THE
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RECEIVED MAY 17, 1944

(1) K. D. Paranjape, N. L. Phalnikar, B. V. Bhide and K. S. Nargund, *Nature*, **153**, 141 (1944).

(2) H. Rupe and Otto Klemm, *Helv. Chim. Acta*, **21**, 1539 (1938).