A CONVENIENT SYNTHESIS OF 2-THIOPHENALDEHYDE[1]

Sir:

During the preparation of some biologically important thiophene compounds we found that the best reported methods[2,3,4] for the synthesis of 2-thiophenealdehyde are tedious and give low yields. We have found that the reaction described by Sommelet[5] for the preparation of benzaldehyde from benzyl chloride is applicable to the preparation of 2-thiophenealdehyde. By this method 2-thiophenealdehyde was prepared in good yield from 2-thenyl chloride (2-chloromethylthiophene) which is easily obtained from thiophene by a method described by Blicke and Burckhalter.[6]

Experimental.—In a typical experiment, 13.75 g. (0.104 mole) of 2-thenyl chloride and 14.5 g. (0.104 mole) of hexamethylenetetramine in 50 cc. of chloroform were refluxed for one hour. The finely crystalline addition product was filtered by suction and washed thoroughly with ether. The air-dried product was dissolved in 100 cc. of hot water and steam-distilled rapidly. The distillate was acidified with dilute hydrochloric acid and extracted with ether. Evaporation of the solvent yielded 5.9 g. (51%) of 2-thiophenealdehyde; b. p. 187° (630 mm.). The 2,4-dinitrophenylhydrazone derivative of this product melted at 242°. A sample of the aldehyde, obtained by decarboxylation of thienylglyoxylic acid,[4] was converted into the 2,4-dinitrophenylhydrazone; mixed m. p. 242°.

(1) The authors wish to thank Mr. W. M. Holaday of the Socony-Vacuum Laboratories for a gift of thiophene.

(2) Grishkevich-Trokhimovskii, *J. Russ. Phys.-Chem. Soc.*, **43**, 204 (1911); *C. A.*, **6**, 223 (1912).

(3) Barger and Easson, *J. Chem. Soc.*, 2100 (1938).

(4) du Vigneaud, McKennis, Simmonds, Dittmer and Brown, *J. Biol. Chem.*, **159**, 387 (1945).

(5) Sommelet, *Compt. rend.*, **157**, 852 (1913).

(6) Blicke and Burckhalter, THIS JOURNAL, **64**, 477 (1942).

DEPARTMENT OF CHEMISTRY FLOYD W. DUNN
UNIVERSITY OF COLORADO THOMAS D. WAUGH
BOULDER, COLORADO KARL DITTMER

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THE ACTIVITY OF MODIFIED PROTEINS AS ANTIGENS IN CULTURES OF A YEAST[1]

Sir:

Production of specific precipitins in a yeast, *Saccharomyces cerevisiae*, has been observed. This observation was made during a search for simpler procedures for antibody production which are needed in studies in immunochemistry[2,3,4] and therapy.[5,6]

(1) Journal Paper No. J-1380 of the Iowa Agricultural Experiment Station. Project No. 897, in coöperation with the Veterinary Research Institute.

(2) Landsteiner, "The Specificity of Serological Reactions," Revised ed., Harvard University Press, Cambridge, Mass.

(3) Harington, *J. Chem. Soc.*, 119 (1940).

(4) Pauling and Pressman, THIS JOURNAL, **67**, 1003 (1945).

(5) Bacon, *Arch. Int. Med.*, **72**, 581 (1943).

(6) Deutschmann, British Patent 239,302 (1924).

The antigens tested were 4-sulfobenzeneazo casein, 4-carboxybenzeneazo casein, nitrocasein, 3,5-diiodo-4-hydroxybenzeneazo casein, and the iodo and 4-arsonobenzeneazo derivatives of casein, egg albumen, blood albumen, and crystalline plasma albumin. The antibody production methods used employed the incubation procedure of Bacon[5] (similar to that of Ostromuislenskii[7]), rabbits,[8] and yeast. Yeast was tested because of its rapid synthesis of protein.[9] In all three methods the most powerful antigens were those containing arsenic or iodine.

In the method employing *S. cerevisiae*, inoculae of Strain No. 567 of the Northern Regional Research Laboratory (kindly furnished by Dr. L. A. Underkofler) were incubated in 100 cc. of 15 or 20% molasses for twenty-four hours at 30°, aliquots containing approximately 6×10^5 cells were transferred to fresh medium (100 cc.) containing the antigen in 0.1% concentration and reincubated for forty-eight hours. The cells were centrifuged, washed with water, and cytolyzed with sand and ether. The cytolyzates were each extracted with 25 cc. of 5% sodium chloride solution, and the extracts were cleared by centrifugation, and stirring with a little Filter-Cel. In the majority of twenty-five of such preparations tested by a microprecipitin test,[10] homologous precipitin reactions were positive.

Precipitin extracts which gave macro tests were obtained by transferring cells each forty-eight hours to fresh medium containing the same antigen. The precipitates of Table I were centrifuged, washed with water, dried, and weighed. Iodo egg albumen[11] and 4-arsonobenzeneazo casein[12] were used in this set of tests.

TABLE I

PRECIPITIN TESTS OF EXTRACTS FROM YEAST

Antigen in solution (1 cc.)	—Antibody extract[a] (5 cc.)— Control,[b] mg.	Antiiodo egg albumen, mg.	Anti-arsonobenzene-azo casein, mg.
None (1% NaCl)	0.3	0.4	0.4
Iodo egg albumen	.0	11.6	0.4
4-Arsonobenzeneazo casein	.0	0.2	9.7

[a] Two transfers in this set. [b] Cells grown in absence of any antigen. Tests were incubated three hours at 37°, stood overnight in icebox, centrifuged eight hours later. All figures are averages of duplicates.

The strength of precipitin extracts varied considerably in experimentation during twelve months; differences in simultaneous cultures suggested that the variability was primarily biological.

A widening in outlook on the occurrence of the antibody mechanism in nature (*e. g.*, in yeast and

(7) Ostromuislenskii, *J. Russ. Phys.-Chem. Soc.*, **47**, 263 (1915).

(8) Hawk and Bergeim, "Practical Physiological Chemistry," 11th ed., The Blakiston Co., Philadelphia, Penna., p. 400.

(9) Fink, *Vorratspflege u. Lebensmittelforsch.*, **1**, 52, 107 (1938).

(10) Hanks, *J. Immunol.*, **28**, 95 (1935).

(11) Shahrokh, *J. Biol. Chem.*, **151**, 659 (1943).

(12) Boyd and Mover, *ibid.*, **110**, 457 (1935).