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).

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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).

marine eggs[13]) has a number of theoretical connotations in the chemistry of proteins and their biological manifestations.

(13) Tyler and Fox, *Biol. Bull.*, **79**, 153 (1940).

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ELEMENTARY ISOTOPIC ANALYSIS. DETERMINATION OF OXYGEN

Sir:

At present *oxygen* in organic compounds is usually determined by difference, that is, after deducting the percentage of carbon, hydrogen and any other constituents from 100. The ter Meulen catalytic method,[1] proposed in recent years, is the only direct method, but it is complicated and requires careful manipulation.

In view of the availability of mass spectrographs and heavy oxygen, it occurred to us to develop an isotopic method. It is a very simple adaptation of the isotope dilution principle originally introduced by G. v. Hevesy and F. Paneth. A known weight (a) of the substance to be analyzed ($x\%$ O) is equilibrated with a known volume of oxygen gas ($= b$ g.), containing a known amount of O^{18} ($= m\%$) in *excess* of the normal concentration. The excess of O^{18} in the mixture after equilibration ($= n$) is determined by mass spectrograph. Thus a, b, m and n being determinable, it follows simply from the mixture rule that

$$x = \frac{b(m - n)100}{an} \% \text{ Oxygen} \qquad (1)$$

The equilibration takes place in a platinum test-tube of 80 ml. volume, at 600–800°, connected to a vacuum system. The time required is about one-half hour. The following oxygen determinations show results obtainable by this method:

Detn. no.	1	2	3	4	5
Substance analyzed	Formic acid HCOOH (excess of O_2)	Formic acid HCOOH (deficiency of O_2)	Acetic acid CH_3COOH	1-Nitroethane $CH_3CH_2NO_2$	Ethyl ether $(C_2H_5)_2O$
a, *i. e.*, mg. sample	38.2	60.4	26.0	20.9	52.70
b, mg. oxygen-18 gas taken (using at. wt. of O = 16.00) (ml. at N. T. P.)	20.0 (14.0)	22.2 (15.5)	36.5 (25.5)	31.4 (22.0)	12.03 (9.4)
m, mole % O^{18}-O^{16} in b above normal concn. (−0.40%)	2.00	2.00	2.07	2.10	2.145
n, mole % O^{18}-O^{16} of equilibrated mixt. (−0.40%)	0.85	0.69	1.50	1.64	1.15
x, % oxygen in substance, calcd. from formula (1)	70.8	69.8	53.4	40.3	19.8
% oxygen, theoretical.	69.5	69.5	53.3	42.6	21.6

These experiments, particularly 2 and 3, show even better agreement than is to be expected. With the comparatively small O^{18} concentration available to us, the average probable error is ±3.0% of the oxygen content.

Since $(m - n)$ and n enter into our equation, the accuracy is highest when the amount of isotopically labeled element added approximately equals the element content of the sample. The precision of oxygen determinations can be increased simply by production of O^{18} in high concentration. With about 20 at. per cent. O^{18} an average precision of ±0.1% of the oxygen content of the sample analyzed should be attainable.

The two experiments with formic acid show that it is not necessary to completely combust the sample to CO_2 and H_2O. As in Experiment 1, equilibrium is attained, with CO and CH_4 in the gas mixture.

The most reliable O^{18} figures are obtained from the CO_2-peaks. The same method may be used for solid organic compounds, and of course can be extended to inorganic compounds which would equilibrate with an O^{18}-containing molecule.

Description of our equipment and our detailed results will be published elsewhere. The method is now being extended to the determination of carbon, hydrogen and nitrogen.

We are greatly indebted to Dr. Harry Thode of Toronto, Canada, for a generous supply of H_2O^{18}, without which this investigation would not have been possible.

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(1) For a comprehensive and critical review of this and other less developed methods see: P. J. Elving and W. B. Ligett, *Chem. Rev.*, **34**, 129–156 (1944).

BUBBLE FORMATION FROM CONTACT OF SURFACES

Sir:

If commercial bottled soda water is poured carefully into a specially *cleaned wet* glass container the liquid remains free of bubbles and supersaturated with carbon dioxide, in contrast with soda water in an ordinary tumbler, where gas phases (gas nuclei) are present to start bubble formation. A clean wet quartz rod in the soda water will also form no bubbles. However, if the quartz rod is drawn over the surface of the glass so as to produce a scratch, bubbles arise at the contact of the two surfaces and a chain of bubbles may continue to form at points along the scratch for a considerable time period.

The above observations are widely known. We have, however, now been able to show that bubbles