Determination of Deuterium in Organic Compounds

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The method of Graff and Rittenberg⁽¹⁾ is the basis of the commonly applied deuterium analysis. The water obtained by the combustion of an organic sample is reduced with the aid of heated zinc. The resulting gaseous hydrogen is subjected to mass spectrometric analysis.

Several attempts have been reported to improve this method by reducing the memory effect of the apparatus used for the combustion and the reduction[2-6]. The present paper describes a modification by which both combustion and reduction are carried out in small, sealed glass tubes. The new method is simple, and one can analyze more than twenty samples a day without interference by the memory effect.

Experimental

Apparatus and Procedure.—Figure 1a shows a combustion tube made of Pyrex glass. The tube is dried in an electric furnace at 300°C for 60 min. under vacuum. After dried air is introduced into it, the tube is connected to a magnesium perchlorate tube. An organic sample, which will give about 2.5 mg. (±20%) of water on combustion, is taken into the tube (Fig. 1b). The capillary end of the tube is cut off, and dried oxygen is passed through to replace the air. The tube is then sealed at both ends as shown in Fig. 1c (9 ml. in volume) and placed in an electric furnace of 620°C for 30 min. This is the highest temperature which the glass will stand. When the combustion is completed, the furnace is allowed to cool. The water produced is collected in the capillary end by this procedure (Fig. 1d). One may centrifuge the tube with a vacuum rubber tube as a cap at 1,500 r.p.m. for 5 min. if the water contains bubbles. The water vapor remaining in the main part of the tube must contain less deuterium than the water. The amount of the vapor is, however, too small to affect the results.

Figure 2a shows a reduction tube with a breakable seal on one end. This is essentially similar

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[1] J. Graff and D. Rittenberg, *Anal. Chem.*, **24**, 878 (1952).
[2] C. A. Dubbs, ibid., **25**, 828 (1953).
[3] F. P. Chinard and T. Enns, ibid., **25**, 1413 (1953).
[4] T. Titani, S. Horibe and M. Kobayakawa, *Mass Spectroscopy* (*Tokyo*), **No. 6**, 27 (1956).
[5] N. Tamiya, *Anal. Chem.*, **32**, 724 (1960).
[6] T. Titani, S. Horibe and S. Koda, *J. Chem. Soc. Japan, Pure. Chem. Sec.* (*Nippon Kagaku Zasshi*), **81**, 1624 (1960).

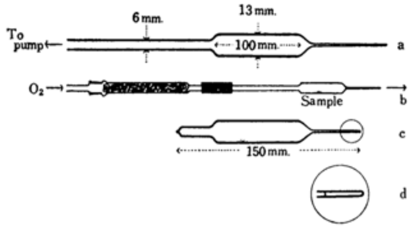

Fig. 1. Combustion tube for organic samples.
- a: A combustion tube.
- b: A combustion tube with a sample, being flashed with dried oxygen.
- c: A sealed combustion tube.
- d: The top of a combustion tube with deuterium containing water produced in it.

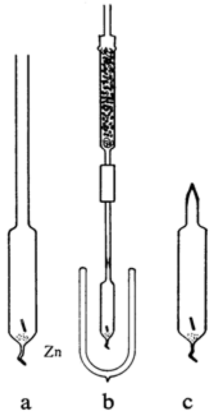

Fig. 2. Reduction tube for water.
- a: A reduction tube with zinc granules and a water sample in a capillary in it.
- b: A reduction tube chilled with solid carbon dioxide.
- c: An evacuated and sealed reduction tube.

to that described by Chinard et al.[3] The tube is dried at 300°C for 60 min. with 0.25 g. of 20∼30 mesh zinc granules in it. The capillary end of the combusition tube, which contains the water, is cut off and thrown into the reduction tube. The reduction tube is attached to a magnesium perchlorate tube and made thinner, with a flame at a part of its neck, as shown in Fig. 2b. The tube is then chilled with solid carbon dioxide to freeze the water, evacuated and cut by a flame under vacuum (9 ml. in volume, Fig. 2c). The reduction of the water is carried out at 620°C for 60 min. in an electric furnace. The hydrogen produced is taken out from the breakable seal and subjected to mass spectrometry. A Process and Instruments Model M-60 mass spectrometer was used. The reduction procedure is essentially similar to that described previously[8], except that a larger tube is used and that a higher temperature is applied. The addition of platinum catalyst to zinc, described in the previous paper, is no longer necessary, because the higher temperature is applied.

Results

The results are summarized in Table I. The standard water was prepared by diluting commercially available 99.65 mol.% deuterium oxide.

TABLE I. EXAMPLES OF DEUTERIUM ANALYSIS

Sample		Deuterium found Atom %	Deuterium found Atoms per mole
10% (v/v) D_2O	2.5 μl.	9.95 10.01 9.89	
DL-Alanine-2d	3.5 mg.	14.15 14.3 13.5	0.990 1.00 0.945
L-Alanine-2d, $3d_3$	3.5 mg.	54.0	3.78
Glycine-$2d_2$	7.0 mg.	34.8 36.3	1.75 1.82
L-Aspartic acid-2d		14.8	1.04
L-Aspartic acid-3d		13.6	0.95
L-Aspartic acid-2d, 3d		28.9	2.02
DL-Aspartic acid-2d, $3d_2$		39.7	2.78

DL-Alanine-2d was prepared by an electrolytic reduction of pyruvic acid oxime in deuterium oxide in the presence of deuterated sulfuric acid[7]. A rough estimation of the deuterium content at the α- and β-positions of the preparation was made by comparing the infrared absorption peaks at 1308 cm^{-1} (α-CH deformation) and 1451 cm^{-1} ($-CH_3$ degenerating deformation) with that at 1410 cm^{-1} ($-COO^-$ symmetric stretching), which was taken as the standard. According to this estimation, the deuterium content of the α- and β-positions is 90 and 7 atom %, respectively[8,9]. L-Alanine-2d, $3d_3$ was prepared by treating L-alanine with transaminase in deuterium oxide; the infrared absorption spectrum of the preparation showed an almost complete disappearance of the $-CH_3$ and α-CH groups[8,9]. Glycine-$2d_2$ was prepared by treating glycine with salicyl aldehyde and cupric ion in deuterium oxide[10]. The results of the mass spectrometric analysis are in good agreement with the infrared absorption data.

Deuterium derivatives of L-aspartic acid were synthesized enzymically, and their infrared absorption spectra showed that there was almost no contamination of each other[10] and of hydrogen compound.

Summary

A device to simplify the deuterium analysis of organic compounds was described. The

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[7] S. Suzuki, T. Oshima, N. Tamiya, K. Fukushima, T. Shimanouchi and S. Mizushima, *Spectrochim. Acta*, **11**, 969 (1959).

[8] T. Oshima and N. Tamiya, ibid., **17**, 384 (1961).

[9] T. Oshima and N. Tamiya, *Biochem. J.*, **78**, 116 (1961).

[10] N. Tamiya, *J. Biochem.*, **51**, 78 (1962).

combustion of organic samples and the reduction of deuterium containing the water produced were both carried out in sealed glass tubes. By this procedure one could completely avoid the "memory effect" and get satisfactory results, as shown by the examples of amino acid analysis.

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