The Determination of the Isotopic Composition of the Oxygen in Alcohols and Related Compounds.

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Two general methods for the isotopic analysis of oxygen in organic compounds are described briefly. A simpler method, but more limited in its application, is described fully. This is based upon the establishment of an isotopic equilibrium between the oxygen atom of an alcohol and carbon dioxide when the two are heated in a sealed tube with a little sulphuric acid. The conditions necessary for the analysis of various classes of alcohols are indicated, and the application of the method to 25 compounds is described.

One of the main difficulties in the use of oxygen-labelled compounds in the study of organic reactions has been the isotopic analysis of the oxygen in the reactants and products. Some compounds may be analysed by introducing them directly into a mass spectrometer, but only in a few cases has such a procedure been attempted (*e.g.*, Long and Friedman, *J. Amer. Chem. Soc.*, 1950, **72**, 3692). In addition to the trouble caused by the absorption of the sample in the inlet and other parts of the mass spectrometer, the considerable fragmentation, which occurs upon ionisation, makes the interpretation of the mass spectrum difficult. It is, therefore, always preferable to transfer the oxygen atoms of the organic molecule to another simpler molecule, such as carbon dioxide or oxygen, which can be purified before introduction into the mass spectrometer.

In an adaptation of Ter Meulen's procedure for the analysis of oxygen in organic compounds (Elving and Ligett, *Chem. Reviews*, 1944, **34**, 129; Russell and Fulton, *Ind. Eng. Chem., Anal.*, 1933, **5**, 384), the sample is pyrolysed over a catalyst in a stream of hydrogen, and the cracked material is then passed over a hydrogenation catalyst. The water produced is collected and analysed for its isotopic oxygen content (Dostrovsky and Klein, *Analyt. Chem.*, 1952, **24**, 414). The main difficulty with this method lies in the considerable " memory " effect (*i.e.*, contamination of one sample by traces of a previous sample) which occurs mostly in the pyrolysis chamber. By replacing this chamber by one made of platinum and filled with platinum gauze, and introducing a liquid-air trap between pyrolysis and reduction sections of the apparatus, a considerable reduction in the " memory " effect is obtained. Nevertheless the procedure is time-consuming and requires relatively large amounts of material, particularly since, in order to minimise " memory " effects, several samples have to be processed before a final analysis is made.

An adaptation of Unterzaucher's procedure for oxygen determination (cf. Harris, Smith, and Mitchell, *ibid.*, 1950, **22**, 1297) was investigated. Pyrolysis was carried out in a vacuum-system without a carrier, the condensable compound frozen out with liquid air, and the hydrogen removed by pumping through a heated palladium tube. The remaining carbon monoxide was either introduced directly into the mass spectrometer, or converted catalytically over nickel into carbon dioxide. This method is again rather lengthy, and the isotopic contamination due to the silica surfaces and the granulated carbon is considerable (see, however, Doering and Dorfman, *J. Amer. Chem. Soc.*, 1953, **75**, 5595).

Numerous other attempts by the authors to develop a method of general applicability for the isotopic analysis of oxygen in organic compounds have not been quite satisfactory. However, a convenient and simple procedure applicable mainly to alcohols has been devised. Since alcohols themselves are often products of reactions where oxygen isotopes can be used, or may easily be formed from a number of other materials, the procedure described below has a wide field of application.

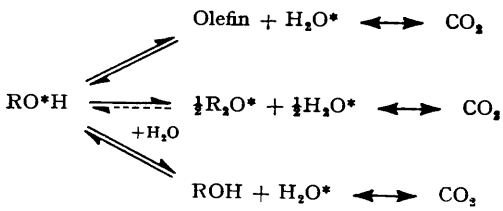
The procedure is based on the isotopic equilibration of carbon dioxide and the oxygen in the material tested, through the agency of water. The water may either be a product of decomposition of the material tested, or be in isotopic equilibrium with it. It follows that the method is limited to those substances which can undergo either partial reversible

or complete and irreversible dehydration, and also those which can exchange oxygen with water directly. These classes include alcohols, ketones, aldehydes, and carboxylic acids.

Although water exchanges its oxygen readily with carbon dioxide even at room temperature (Mills and Urey, *J. Amer. Chem. Soc.*, 1940, **62**, 1019; Reid and Urey, *J. Chem. Phys.*, 1943, **11**, 403) the dehydration reactions of organic compounds, and the oxygen-exchange reaction between them and water require higher temperatures and the presence of catalysts. The equilibration is therefore carried out by heating the sample (about 1 mmole) in a small sealed tube together with a trace of sulphuric acid (0·001 ml.) and carbon dioxide (0·05 mmole). After the mixture has been heated for a few hours at 150—200°, depending on its nature, the tube is transferred to the gas inlet system of the mass spectrometer, and the carbon dioxide analysed for its ^{18}O content.

Alcohols vary greatly in their ease of dehydration and exchange of oxygen with water. These differences are closely related to differences in the mechanism of the reactions and will be reported elsewhere (Dostrovsky and Klein). Thus the tertiary alcohols listed in the Table (p. 159) gave complete equilibrium in 1 hr. at 150°. The secondary alcohols listed gave good results after 3 hr. at 170°. Primary aliphatic alcohols, heated to 200° for 1 hr., tend to form ethers which undergo further reaction very slowly. For this reason, only half the oxygen atoms of such alcohols are readily available for exchange. Exceptions are ethyl and benzyl alcohol, which undergo almost complete exchange in 3 hr. at this temperature, and *neo*pentyl alcohol, which does not undergo ether formation but requires heating at 230° for 3 hr. for complete dehydration.

The various possible modes of reaction of an alcohol are:



In most cases, the carbon dioxide reaches isotopic equilibrium with the water, produced by any of the mechanisms described above, much faster than either the rate of formation of the water or its rate of exchange with the alcohol. Exceptions are those cases which require high temperatures for decomposition. At a temperature above 200° and with the size of sealed tube used, the small amount of water formed is almost completely volatilised. In addition, the solubility of carbon dioxide in water at this temperature is very small. Since the exchange between carbon dioxide and water under these conditions proceeds only in the liquid phase (Klein, unpublished results), the rate of the equilibration reaction may become very slow and limiting. For this reason, in all analyses involving heating above 180°, a second heat treatment is given at a lower temperature, which ensures complete isotopic equilibrium between the water and carbon dioxide. For the same reason the size of the sealed tube should be as small as possible. Sealed tubes of 1·2 ml. volume were used successfully in this work.

Because of the high ratio of the amounts of alcohol to carbon dioxide taken for the analysis, the final concentration of ^{18}O in the latter material is not very sensitive to the extent of exchange of the alcohol. Thus, if only half the oxygen atoms of an alcohol undergo exchange (as in complete ether formation), the ^{18}O concentration of the carbon dioxide is 92% of its value for complete statistical equilibrium. If the extent of oxygen exchange is now raised to 60% by further heating, the ^{18}O concentration in the carbon dioxide rises to 94% of its maximum possible value. Therefore, when an accuracy of a few units % is sufficient in the isotopic analysis of a primary alcohol, it is immaterial what the extent of alcohol oxygen exchange is, provided it is over 50%. The method can be made even less sensitive to the extent of alcohol decomposition or exchange, by using larger samples and smaller quantities of carbon dioxide, though such a procedure increases the danger of spurious results caused by impurities in the alcohol.

In most tracer work, where a precison of only a few units % is required, it is sufficient to compute the isotopic composition of the alcohol by using the ordinary dilution formula and ignoring isotope effects; thus,

$$N - N_0 = (n_1 - n_0)(m_1 + m_2 + m_3)/m_1 \quad . \quad . \quad . \quad . \quad . \quad (1)$$

where $N - N_0$ is the atom fraction excess of ^{18}O in the alcohol and $n_1 - n_0$ is that in carbon dioxide after equilibration, and m_1, m_2, m_3 are the number of equivalents (with respect to oxygen atoms) of alcohol, carbon dioxide, and sulphuric acid respectively. The validity of equation (1) for various alcohols may be judged from the data of col. 4 in the Table.

For accurate work it is necessary to take into account, not only the dilution of the isotope, but also the equilibrium constants of the carbon dioxide and water exchange reactions at various temperatures and the equilibrium constants which may be involved in the water–alcohol exchange. In addition, allowance must be made for incompleteness of equilibrium, particularly in the case of primary alcohols. Rather than estimating these correcting factors separately, an experimental procedure was developed which eliminates all possible errors of this kind. It is only essential that the conditions of the measurements are accurately reproducible. For each alcohol two calibrating experiments are necessary where alcohol of normal isotopic composition is subjected to the analytical procedure described above, using in one case carbon dioxide of known composition n_L and in the second of known composition n_H.

The general equations relating to these experiments are :

$$N_0m_1 + n_Lm_2 + N_3m_3 = (f_1m_1 + m_2 + f_3m_3)n_1 \quad . \quad . \quad . \quad . \quad (2)$$
$$N_0m_1 + n_Hm_2 + N_3m_3 = (f_1m_2 + m_2 + f_3m_3)n_2 \quad . \quad . \quad . \quad . \quad (3)$$

Subtracting (2) from (3), we obtain

$$(n_H - n_L)m_2 = (f_1m_1 + m_2 + f_3m_3)(n_2 - n_1) \quad . \quad . \quad . \quad . \quad (4)$$

where N_0 and N_3 are the atom fractions of ^{18}O in the alcohol and sulphuric acid respectively, and n_1 and n_2 are the atom fractions of ^{18}O in the carbon dioxide from the two calibration experiments; f_1 and f_2 are correcting factors which take into account isotope effects, incomplete exchange, and lack of equilibrium.

If now an alcohol of isotopic composition N_x is analysed under identical conditions, using carbon dioxide of composition n_L, we have :

$$N_xm_1 + n_Lm_2 + N_3m_3 = (f_1m_1 + m_2 + f_3m_3)n_x \quad . \quad . \quad . \quad . \quad (5)$$

Subtracting again (2) from (5) and dividing the result by equation (4) we obtain :

$$N_x - N_0 = m_2(n_x - n_1)(n_H - n_L)/m_1(n_2 - n_1) \quad . \quad . \quad . \quad . \quad (6)$$

Equation (6) expresses the atom fraction excess of ^{18}O in the unknown alcohol in terms of easily measurable quantities, m_1, m_2, the number of equivalents of alcohol and carbon dioxide used, and the composition of the carbon dioxide before and after equilibration; n_L is usually tank carbon dioxide and n_H a standard sample of " heavy " carbon dioxide and therefore $n_H - n_L$ is, in practice, a constant. It follows that $n_2 - n_1$ is also a constant for any given alcohol. The atom fraction of ^{18}O in the carbon dioxide may be calculated from the relation $n = r/2(r + 1)$, where r is the ratio of mass 46 to 44 as obtained from the mass spectrometer and corrected for instrumental and other factors.

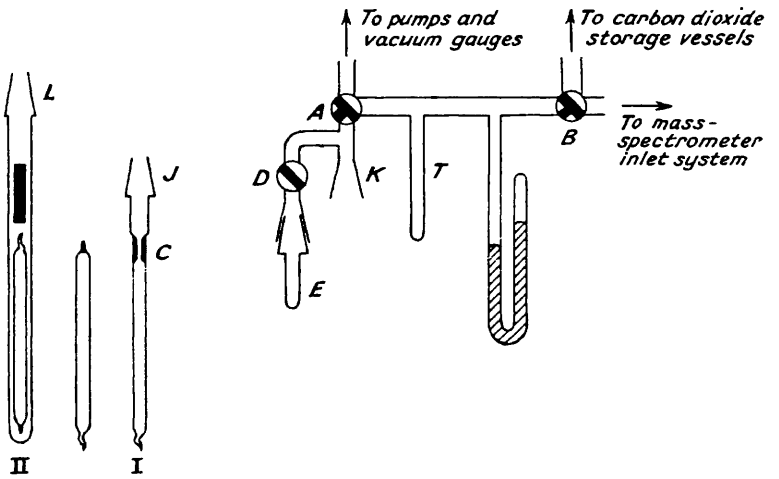
In addition to the monohydroxylic alcohols mentioned above, a number of more complicated alcohols and other oxygen-containing organic compounds were tested. Glycerol could be analysed under the usual conditions for primary alcohols, but mannitol gave only 50% exchange at 200° in 3 hr. and cholesterol only 30% under the same conditions. Phenol did not exchange its oxygen even in 3 hours at 200°. Methanol could not be analysed satisfactorily by this procedure for, although it forms an ether very readily, it is difficult to separate the dimethyl ether from the carbon dioxide. Since this ether gives a strong peak at mass 46 when introduced into a mass spectrometer, even traces of this

material can completely vitiate the result of the isotopic analysis. Methanol has been analysed very satisfactorily by Ter Meulen's procedure described above (Dostrovsky, unpublished results). Bunton and Frei (*J.*, 1951, 1872) have used the same procedure for the analysis of phenol.

Triphenylmethanol failed to give good results even when heated for 3 hr. at 200°. This compound can, however, be readily analysed for its oxygen isotope content by conversion of a small sample in benzene solution into triphenylmethyl chloride and water by means of a stream of dry hydrogen chloride (for experimental details see Anbar, Dostrovsky, Samuel, and Yoffe, *J.*, 1954, 3603).

Acetone was decomposed after 3 hr. at 180° and gave almost complete oxygen exchange with the carbon dioxide. Acetonylacetone behaves similarly when heated to 200° for 3 hr. Aldehydes and ketones which are not pyrolysed so readily can be analysed by adding a known amount of water to the contents of the sealed tube. Alternatively, such substances may be reduced to the corresponding alcohols by means of lithium aluminium hydride (Samuel, unpublished results) without change in the isotopic content.

Di-*n*-butyl ether does not equilibrate with carbon dioxide during 1 hr. at 200°. *n*-Butyl acetate does not exchange under the same conditions, but carboxylic esters and acids can be reduced to alcohols by lithium aluminium hydride and analysed in the usual way.



Experimental

Filling of Tubes.—The tubes, made from borosilicate glass tubing of 5 mm. inner diameter, have the shape shown as I in the Fig. A known quantity of concentrated sulphuric acid (0·001 ml.) is introduced into a tube by means of a micropipette and is followed by 1 mmole of the alcohol to be analysed, delivered from a micrometer syringe. Solid alcohols are weighed in the tubes before constriction *C* is made. The tube is then attached to a vacuum-line by means of the standard joints *J* and *K*, and the contents are frozen by cooling with liquid air. The tube is pumped out and, after the stopcock *A* has been closed, the contents are allowed to warm. The tube is again cooled in liquid air and evacuated once more. A small amount of pure carbon dioxide (0·05 mmole) is measured between stopcocks *A* and *B* and is then condensed in the tube by opening *A*. The tube is subsequently sealed off at *C*.

Analysis.—The sealed tube is heated at the temperature and for the time indicated in the Table in a multibarrel oven and then is placed in a tube II under an iron breaker and attached to the mass spectrometer inlet system by means of ground joints *L* and *K*.

After pumping out, the stopcock *A* is closed, the break-off tip smashed by operating the iron breaker with a magnet, and tube II is then cooled in liquid air. The carbon dioxide which is evolved when the tube is allowed to warm, is collected in another trap *T*, cooled in liquid air. The gas is purified by a further sublimation into the space behind the leak of the mass spectrometer. As a check on the purity of the gas the mass spectrum on either side of the

carbon dioxide peaks (44, 45, 46) is examined. If excessive peaks are observed when using this simple procedure, the samples may be purified more effectively as follows. The sealed tube is broken open in II, into which quinoline (0·5 ml.) has been added. The gas is condensed into bromine (0·5 ml.) in tube *E* by means of liquid air. Stopcock *D* is again closed, and the bromine is allowed to warm to room temperature and then cooled again to −80°. The gas is condensed back into tube II by means of liquid air, and the mixture with quinoline allowed to warm to room temperature. After a few minutes the quinoline is cooled to −80°, and the purified carbon dioxide transferred to the mass-spectrometer sample line.

Tests of Applicability.—The applicability of the procedure to various compounds was tested by determining (*a*) the extent to which the composition of the carbon dioxide approaches its theoretical value (ignoring isotope effects), (*b*) the degree of exchange of the alcohol oxygen atoms. Dividing equation (4) (p. 157), obtained for each alcohol from the calibrating experiments, by $m_1 + m_2 + m_3$, we obtain :

$$(f_1m_1 + m_2 + f_3m_3)/(m_1 + m_2 + m_3) = m_2(n_H - n_L)/(n_2 - n_1)(m_1 + m_2 + m_3)$$

Now the left-hand term is a measure of the deviation from ideal conditions, *i.e.*, complete equilibrium and absence of isotope effects. In the Table, col. 4, are presented values for various alcohols of the quotient :

$$100m_2(n_H - n_L)/(n_2 - n_1)(m_1 + m_2 + m_3) \quad . \quad . \quad . \quad . \quad . \quad . \quad . \quad (7)$$

The extent of exchange of the alcohol oxygen may also be derived from equation (4). Assuming that the sulphuric acid reaches complete statistical isotopic equilibrium with water ($f_3 = 1$), we obtain the following expression for f_1 :

$$f_1 = [m_2(n_H - n_L)/m_1(n_2 - n_1)] - (m_2 + m_3)/m_1 \quad . \quad . \quad . \quad . \quad (8)$$

Values of f_1 for various alcohols, expressed as percentages, are given in col. 5.

Percentage of equilibrium attained by carbon dioxide and the extent of exchange of oxygen atoms of organic compounds.

Compound	Temp.	Time, hr.	% equilibrium value of CO_2 (eqn. 7)	% of exchange of alcohol oxygen (eqn. 8)
Ethyl alcohol	200°	3	99	90
n-Butyl alcohol	200	3	95	65
n-Octyl alcohol	200	3	94	60
n-Dodecyl alcohol	200	3	94	60
Benzyl alcohol	200	1	99	90
*neo*Pentyl alcohol	230	3	97	77
*iso*Propyl alcohol	170	3	100	100
sec.-Butyl alcohol	170	3	100	100
Diphenylmethanol	170	3	100	100
2 : 2-Dimethyl-1-phenylpropanol	170	3	100	100
*cyclo*Hexanol	170	3	100	100
1-Phenylethanol	170	3	100	100
tert.-Butyl alcohol	150	1	100	100
tert.-Pentyl alcohol	150	1	100	100
3-Methylpentan-3-ol	150	1	100	100
Triphenylmethanol	200	3	70	13
1 : 1 : 2-Triphenylethanol	180	3	100	100
Cholesterol	180	1	31	17
Glycerol	200	3	100	100
Mannitol	200	3	91	48
Phenol	200	3	0	0
Acetone	180	3	96	70
Pentane-2 : 4-dione	200	3	99	90
Di-*n*-butyl ether	200	1	0	0
n-Butyl acetate	200	1	0	0

Effect of Tube Size.—*tert.*-Butyl alcohol was analysed under identical conditions (150° and 1 hr.) in tubes of 4·5, 1·3, and 0·4 ml. volume. The percentage of equilibrium attained by the carbon dioxide in these experiments was 90, 99, and 100, respectively.

Effect of Second Heat Treatment.—*neo*Pentyl alcohol was analysed by treatment at 230° for 3 hr. Without a second heat treatment the carbon dioxide reached 76% equilibrium, while after a further heating at 160° for 1 hr. the value was 99%.

Effect of Ratio of Alcohol to Carbon Dioxide.—Changing the ratio of *n*-butyl alcohol to carbon dioxide by a factor of two (alcohol : CO_2 = 1 : 0·065 and 2 : 0·065, in mmole) under identical conditions had little effect on the percentage exchange of equilibrium (94 and 95%).

Effect of Quantity of Acid.—The quantity of acid was reduced in one experiment with *tert.*-butyl alcohol by a factor of 10 (from 0·02 to 0·002 mmole) without affecting the percentage exchange of equilibrium by more than 1% (99% to 98%).

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