[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF OREGON STATE COLLEGE]

Organic Oxidation Equivalent Analysis. I. Theory and Applications

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In connection with the problem of analysis of very small samples of biological materials, the possible importance of being able to determine the amount of oxygen required for their complete combustion was noted. Further study revealed the fact that in the case of the vast majority of pure compounds this determination offers an alternative to the familiar "combustions" for carbon and hydrogen.

A search of the literature shows that apparently no one has approached the subject from exactly this angle. Strebinger,[1] using potassium iodate as the oxidizing agent, determined the oxygen required for the complete combustion of organic compounds and from this and the known carbon and hydrogen contents he calculated the oxygen contents of the compounds. Simon and Guillaumin[2] oxidized certain (non-nitrogenous) organic compounds with sulfuric acid and silver dichromate and from the carbon dioxide formed and the oxidizing agent used up calculated the hydrogen content of the compounds. Stanek and Nemes[3] developed a complicated micro method (using iodate) for the quantitative determination not only of the "oxygen consumed" but also carbon, nitrogen and halogens. The determination of oxygen consumed along with the other values made possible with rather complicated equations the calculation of hydrogen content. They pointed out that the amount of oxygen required for complete oxidation of an organic compound is itself a very characteristic quantity, but did not deal experimentally with this determination except as a part of their general set-up for complete micro analysis.

Sample Calculation.—The way in which the oxidation equivalent value may be used as a substitute for "combustion" data in determining the formula for a compound may best be illustrated by a specific case in which we will use the following designations

$C_xH_yO_z$ = formula of unknown
M = its molecular weight
E = its oxidation equivalent (grams oxidized by one gram atom of oxygen; 17.90 in the case cited below)

(1) Strebinger, Z. anal. Chem., **58**, 97–114 (1919).
(2) Simon and Guillaumin, Compt. rend., **176**, 1065 (1923).
(3) Stanek and Nemes, Z. anal. Chem., **95**, 244 (1933).

$M/E = N$ = the number of atoms of oxygen required to oxidize one molecule of the compound.

Suppose that one gram of an "unknown" containing only carbon, hydrogen and oxygen (gluconic acid) is found experimentally to require 0.894 g. of oxygen (calcd. 0.898) for complete combustion. From this it can be calculated that one gram atom of oxygen will completely oxidize 17.90 g. (17.82 calcd.) of the unknown.

The molecular weight M must be an even integer (except in the case of odd molecules) and a multiple of E. Which multiple of E it is, can be determined cryoscopically or better yet, in this case, by titration. If $N = 10$, $M = 178$; if $N = 11$, $M = 196$; if $N = 12$, $M = 214$. Experimentally it is easily ascertained that N is 11.

Of the above values x, y, z, M and N are all integers (of these y and M are *even* numbers) and it is possible to set up the simple Diophantine equations (1) and (2) from which may be derived (3), (3a) and (4).

$$12x + y + 16z = M \tag{1}$$
$$2x + 0.5y - z = N \tag{2}$$
$$y = (6N - M + 22z)/2 \tag{3}$$
$$z = (M - 6N + 2y)/22 \tag{3a}$$
$$x = (M - 2N - 18z)/8 \tag{4}$$

Substituting 196 and 11 for M and N, respectively, in equations (3) and (4) we obtain (5) and (6)

$$y = (-130 + 22z)/2 \tag{5}$$
$$x = (174 - 18z)/8 \tag{6}$$

Below are given the values of y and x derived from equations (5) and (6) by substituting various integral values of z. From these it is evident that the formula of the unknown can be nothing other than $C_6H_{12}O_7$.

Integral values of z	Corresponding values of y	Corresponding values of x
5	−10	10.5
6	1	8.25
7	12	6
8	23	3.75
9	34	1.5

Calculations for N and S Compounds.—In order to calculate in a similar way the formula for a compound containing reduced nitrogen, $C_xH_yO_zN_b$, M is used to represent the weight of the residue after b equivalents of NH_3 have been subtracted. For calculating in case of a compound contain-

ing nitrogen and sulfur (both in reduced condition), $C_xH_yO_zN_bS_c$, M is used to represent the residue after $3c$ moles of water have been *added* and b molecules of NH_3 and c molecules of SO_3 subtracted. This is on the assumption that ammonium and sulfate ions are the end products of the oxidation, respectively. Separate analyses for nitrogen and sulfur must, of course, be made.

Applicability to Different Types.—The question naturally arises as to whether the value of the molecular weight of an unknown can be determined with sufficient accuracy so as to make the calculation valid. This problem has been given careful study and there appears to be no difficulty except in the case of certain compounds of carbon, hydrogen and oxygen which have a high molecular weight and/or low value of E, and the molecular weights of which it is impossible to determine by titration or otherwise, with reasonable accuracy. These, however, can be handled as indicated below. In the case of many simpler compounds such as glycerol or oxalic, tartaric and succinic acids the molecular weight determinations as well as those of the oxidation equivalents can be very inexact and yet lead inevitably to the correct results. Nitrogen compounds in general offer no difficulty provided the nitrogen can be determined accurately since the value obtained along with the oxidation equivalent data, is basis for a sufficiently accurate value for the molecular weight. If, in the case of carbon–hydrogen–oxygen compounds which offer difficulty because of uncertainty regarding the molecular weight, one additional fact is known, namely, the degree of unsaturation, then the difficulty disappears. These are illustrated by the type $C_8H_{10}O_4$ and $C_9H_8O_4$, which, in this case, have exactly the same oxidation equivalent value, and molecular weights which differ from each other by less than 6%. They differ markedly in the number of double bonds and/or rings present, however, and could in general be easily differentiated, without a carbon and hydrogen analysis.

With regard to compounds of high molecular weight, calculation of the molecular formula has limitations in accuracy similar to those which hold for traditional combustion analyses. In either case unless the analyses are very accurate, the data may fit several formulas equally well.

Notes on Calculations.—There are several points regarding the calculations which need to be clarified. It is not infrequent that more than one

mathematical solution to the equations can be found, but that only one falls within the limits possible for a chemical formula. Thus, the same data fit approximately the formulas $C_8H_2O_6$, $C_6H_{12}O_7$ and $C_4H_{22}O_8$, but this should not cause any uncertainty, because the two extremes are either impossible as compounds or else would have very unusual properties. In the case of higher molecular weight compounds two solutions may arise as above, both of which represent *possible* compounds. In such cases the two are so far apart in their hydrogen content that they could be distinguished easily without analysis because of differences in chemical behavior.

It should be emphasized that in order to have a valid calculation, the value of N (which is an integer) *must be correctly assigned*. If this is done, then the value of M is obtained with considerable accuracy ($N \times E$) and by substituting reasonable values[4] of y in equation (3a), z can be established with certainty. These values of z, if substituted in (3) and (4) then lead to integral values of x and y unless the molecular weight value is slightly in error. If it is high or low by two integers the value of x will differ from an integer by $1/4$, and y will be an odd number. If M is high or low by 4 integers (but this should not happen if the oxidation equivalent value is obtained with satisfactory accuracy) then the value of x will differ from an integer by $1/2$ and y will be even.

Use in Checking Composition.—In practical experience there is seldom an occasion to analyze an organic compound which is entirely unknown. Usually the analysis serves as a check to avoid false assumptions.

Here the usefulness of the oxidation equivalent analysis is apparent when the data in the case are studied. In order to study this problem a table was prepared giving the calculated oxidation equivalent values for all of the C_8 and C_9 compounds (containing C, H, O and reduced N) which are listed in Richter's "Handlexicon." The total number of molecular formulas in the sample was 547 and should be representative.

The oxidation equivalent values in the sample ranged from 4.56 to 99, and it is a striking fact that the formulas represented are very evenly spread through a wide range. The distribution

(4) The possible values must lie at 11 unit intervals. Various relationships exist which may be helpful in determining reasonable values of y, *e. g.*, for any compound containing carbon, hydrogen, oxygen and reduced nitrogen $y = 2x + 2 - 2\alpha + b$, where α is the number of rings and/or double bonds and the other terms have the meanings previously assigned.

is approximately even from the values 6 to 16 inclusive and about 27% of the formulas lie outside this range. In all cases observed throughout the whole table where the oxidation equivalent values lie close together the formulas represented differ materially in molecular weight, nitrogen content or in some cases in degree of unsaturation as previously explained.

Use in Determining Hydrogen Content.—Whether a sample constitutes a pure compound or not, if it contains only carbon, hydrogen and oxygen (plus other elements that may be determined and corrected for), its hydrogen content can be determined accurately by analysis for carbon and for its oxidation equivalent. Strebinger,[1] from the oxidation requirements of the elements, calculated (using slightly different notation and uncorrected values) that $8/3 \cdot \%$ C $+ 7.93 \cdot \%$ H $- \%$O $= \%O_R$ where $\%O_R$ is the oxygen requirement in grams per hundred grams of sample. Combining this with the relationship that for a compound or residue under consideration the sum of the carbon, hydrogen and oxygen percentages is equal to 100, we obtain

$$\%H = (\%O_R + 100 - 11/3 \cdot \%C)/8.93$$

The molecular formulas of certain compounds (particularly sterols) have been in doubt because the hydrogen percentage could not be determined with sufficient certainty so as to make possible a definite choice between two values which lie 4–5 parts per hundred apart. Fisher[5] indicates the "allowed" error for a hydrogen analysis in macro combustions to be 2 parts and never over 3 parts per hundred.

Carbon can be determined much more accurately than this[5] without undue complication in

(5) Fisher, "Laboratory Manual of Organic Chemistry," John Wiley and Sons, Inc., New York, 1931, p. 286.

apparatus. It should be possible by careful work to determine both the carbon percentage and the oxidation equivalent value with an error of one part per thousand. This would allow a calculation of the hydrogen content (e. g., of cholesterol) with an error of not more than 5 parts per thousand even if the errors in analysis were in opposite directions thus producing the maximum error in the value for hydrogen percentage.

Other Applications.—Very small samples of tissue, bacteria, etc., can be subjected to a proximate analysis for "protein," carbohydrate and fat by combining an oxidation equivalent analysis with a determination of nitrogen on a micro scale. For carbohydrate (starch or glycogen) the oxidation equivalent value is 13.5; for a protein (egg albumin) it is 10.47; for a fat (triolein) it is 5.53. The proportions in a mixture can be calculated readily from the data mentioned above. The method of oxidation equivalents also has a possible application in the determination and characterization of organic matter in soils and in water.

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Summary

Equations are given by which the molecular formula of a compound can be calculated from its molecular weight and the amount of oxygen required for its complete combustion. The use of oxidation equivalent data as a substitute for that obtained from carbon and hydrogen combustions is discussed.