LXXXIV. THE MICRO-DETERMINATION OF HYDROXYL GROUPS.

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INTRODUCTION.

DURING the course of other work, it was found necessary to estimate the hydroxyl groups in a substance believed to be a dihydroxy-alcohol [Marrian, 1929], of which only a small amount was available. A search of the literature failed to reveal any suitable micro-method. Experiments carried out on the hydrolysis of acetates of known purity led us to conclude that methods based on this were entirely unreliable when carried out on small quantities. The method based on the volumetric determination of the methane evolved when an alcohol reacts with Grignard's reagent as used by Tschugaeff [1902], Hibbert and Sudborough [1904] and Zerewitinoff [1907] seemed to be the most suitable for adaptation to a micro-scale.

Preliminary experiments made it quite clear that, unless certain precautions were most rigidly observed, entirely irregular results were obtained.

Hibbert and Sudborough emphasise the necessity of working in an atmosphere of nitrogen, since oxygen is absorbed by Grignard's reagent. Zerewitinoff, on the other hand, states that, since oxygen is only slowly absorbed by the reagent, it is unnecessary to work in an atmosphere of nitrogen. In our first experiments, when we did not observe the precaution of filling the apparatus with nitrogen, we were troubled with a rapid and prolonged contraction in volume of the gas in the burette, making it quite impossible to obtain a steady initial reading.

After blowing out the apparatus with dry nitrogen, this contraction was decreased greatly in magnitude, making it possible to obtain a steady initial reading after the lapse of some hours.

Very small variations in temperature and pressure should result in only a very slight error in macro-determinations when the volume of the gas burette is large relative to the total gas volume of the apparatus. In our micro-apparatus, the total gas volume was relatively enormous compared with the changes in volume which were being measured. Consequently small changes in temperature and pressure were observed to introduce appreciable errors. By suitable means the temperature was kept sufficiently constant, but variations in barometric pressure have constantly caused trouble. In unsettled weather, the pressure may change 3-4 mm. during the course of half an hour, making it

impossible to obtain steady readings. We found it necessary to observe the height of the barometer constantly during the course of an experiment, and if necessary to abandon the experiment for the day. The only alternative would be to reduce the total gas volume of the apparatus considerably. This would necessitate working with a much smaller reaction flask, which in many ways would be inconvenient.

The usual method of carrying out macro-determinations is to dissolve the alcohol which is being examined in some inert solvent, preferably the ether with which the Grignard reagent is made, and to allow this solution to react with the reagent. The difficulty of drying and keeping dry such a solvent suggested to us that for micro-determinations it would be preferable to allow the reagent to react with the solid alcohol. This method has been found to work excellently provided the alcohol is readily soluble in the ether of the Grignard reagent.

Preliminary determinations showed us that high results were invariably obtained unless the reaction flask and the substance being examined were dried with extreme care. Even so, blank determinations have always given an appreciable volume of gas. The magnitude of the blank seems to vary with the exact technique of cleaning and drying the flask, and also with the size and nature of the flask itself. With the same flask and with standard conditions of cleaning and drying, the blanks have been reasonably constant for a series of experiments. It has been our practice to do at least one blank determination to every two alcohol determinations.

A number of determinations on some of the common higher fatty alcohols were carried out satisfactorily using amyl ether and anisole Grignard reagents. Attempts were then made to estimate the hydroxyl groups in substances insoluble in these ethers or having only a very slight solubility. Determinations carried out on glucose and the dihydroxy-alcohol from the urine of pregnancy [Marrian, 1929] yielded the rather surprising result that no gas at all was evolved.

It was obviously necessary to dissolve such substances in some solvent that does not itself react with the reagent. Pyridine has been used by several workers in the past for this purpose, but there is some difference of opinion as to its suitability. Tanberg [1914], who was unable to obtain consistent results, criticises the use of pyridine. Zerewitinoff [1907] on the other hand claims that pyridine is well suited to the macro-method, giving consistent and accurate results if a few necessary precautions are observed in drying the pyridine and preparing the Grignard reagent. He suggests that the gas evolved when these precautions are not observed is ethane, produced according to the following equations:

and, therefore, if the Grignard reagent is quite free from methyl iodide, no gas is evolved.

With regard to the reaction between pyridine and Grignard's reagent we are in agreement with Zerewitinoff. When the reagent was prepared in the ordinary way, a large volume of gas was evolved when it was mixed with dry pyridine. If, however, the excess methyl iodide was first removed from the reagent by heating under reduced pressure, only a very small amount of gas was evolved with pyridine. The white solid compound that the reagent formed, even with dry pyridine, reacted completely but slowly with alcohols.

In spite of this the method proved to be a failure for micro-estimations, as the variations in the blanks caused by the introduction of another possible "wet factor" were so enormous. We thought at first that the method might be of use in distinguishing between the number of hydroxyl groups present in an alcohol, but even this is not too reliable, having an error up to 24 %.

Fig. 1.

EXPERIMENTAL.

Apparatus.

The apparatus used was essentially the same as that previously described by Zerewitinoff (Fig. 1). The burette had a capacity of 5 cc., was 50 cm. long and graduated to hundredths of a cc. The flasks used were of two types (Fig 1 *A* and *B*). In all those experiments where pyridine was not employed, a flask of type *A* was used. Since, in the pyridine experiments, it was desirable to reduce the volume of the flask as much as possible to keep the blank to reasonable dimensions, a flask of type *B* was used having a capacity of about

one-half of that of type *A*. This second type of flask has been found to be very satisfactory and is probably preferable for all determinations.

The apparatus was kept as far as possible at a constant temperature by running a rapid stream of tap water through the jacket surrounding the burette, and through the water-bath in which the reaction flask was placed. In order to have as much as possible of the apparatus at a constant temperature, the rubber connections between the flask and the burette were made as short as possible.

A three-way tap connected the circuit with a large flask (*C*) which was kept filled with dry nitrogen, so that if for any unforeseen reason, *e.g.* change in barometric pressure, the mercury level in the burette was too high when the initial reading was to be taken, more nitrogen could be run into the burette.

Preparation of Grignard's reagent.

(a) *Amyl ether reagent.* The amyl ether was dried by standing over sodium for at least a week. *P* was then redistilled. 4.5 g. pure magnesium ribbon were then heated in a dry flask on a sand-bath with 25 g. methyl iodide, 140 cc. amyl ether and a small crystal of iodine under a reflux condenser for about 15 minutes to start the reaction. The flask was then removed from the sand-bath and the reaction allowed to proceed by itself for about one hour. When the reaction slowed down, the flask was again heated for about an hour to complete the reaction. In the case of the reagent used for the pyridine experiments, the flask was heated under reduced pressure for about 15 minutes to remove the last traces of methyl iodide. The reagent prepared in this way remained clear and active for 2–3 months if kept in a tightly stoppered flask filled with dry nitrogen.

(b) *Anisole reagent.* The anisole reagent was prepared by exactly the same method as described above, substituting anisole for amyl ether. This reagent was far less stable than the amyl ether reagent. In the course of two or three weeks it deposited a heavy white precipitate and lost its power of reacting with alcohols.

Drying and cleaning of apparatus.

The exact technique of cleaning and drying the reaction flask and the pipettes used for measuring the reagents has been found to affect the magnitude of the blank. As a minimum blank is essential the methods of cleaning and drying have been varied to some extent in the different series of estimations carried out.

In series *A* (Table I), the flask, which was made of ordinary glass, was washed out in succession with hot water, dilute hydrochloric acid, water and finally acetone. It was then dried for at least 3 hours in an oven at 110°. Before use the flask was cooled in a desiccator over calcium chloride for 30 minutes.

In series *B* the flask, which was made of silica, was cleaned first with hot water and then boiled in a solution of chromic acid for at least an hour. The

flask was then washed thoroughly with distilled water, heated to 110° for 30 minutes and finally to 170° for at least 3 hours over a dish of calcium chloride. As in series *A*, the flask was cooled for 30 minutes in a calcium chloride desiccator before use. The latter technique was adopted for series *C* (Table III) and series *D* (Table IV).

In each case the pipettes were cleaned and dried in the same way as the flask.

In preliminary experiments, we found that high results were invariably obtained unless special precautions were taken to ensure complete dryness of the substance under investigation. The bottle of substance was therefore left in a calcium chloride desiccator for several days before weighing the sample for the estimation. The sample, which was weighed into a small tube made by cutting down a hard-glass combustion tube to a length of about $\frac{1}{2}$ ", was then placed in a desiccator over P_2O_5 for at least 24 hours before use.

Drying the pyridine. The pyridine was allowed to stand over anhydrous barium oxide for a week and then distilled into a bottle containing fresh barium oxide.

Method of determination.

The exact method of carrying out the determination with the solid alcohol (without pyridine) was as follows. For 30 min. nitrogen was passed first through a calcium chloride tube and then through the apparatus, entering at the nitrogen reservoir and leaving at the rubber connection to which the flask was to be attached. The mercury in the burette was then adjusted to a suitable level by raising or lowering the reservoir slowly. The flask was then taken from the desiccator and rapidly blown out with a stream of dry nitrogen. The tube containing the substance was dropped into the flask and 3 cc. of Grignard reagent run into the side tube through a pipette and the special funnel shown in Fig 1 (*D*). The flask was then fitted to the greased stopper previously attached to the rubber connection of the burette. This was invariably carried out in the late afternoon, and the apparatus left overnight before taking the initial readings, so that any residual oxygen could be completely absorbed. The following morning, readings of the level of the mercury were observed at intervals of 10 min. after the temperature of the bath had become constant. When at least four consecutive readings were the same, the flask was taken from the bath, the reagent tipped out of the side-tube on to the alcohol, and the flask shaken vigorously for some minutes. Alternate shakings and readings were made until the mercury level was constant. The flask was then replaced in the water-bath to attain the correct temperature and readings were taken every few minutes until constant, the whole operation usually taking about 15 min.

The procedure in the experiments where the alcohol was dissolved in pyridine was different in several details. After removing the flask from the desiccator and blowing it out with nitrogen, one of us (*A*) placed the tube containing the alcohol in one leg of the flask while the other (*B*) measured out

3 cc. of Grignard reagent. While *B* was running the reagent into the other leg of the flask, *A* measured out 1 cc. of pyridine and then ran it into the leg containing the solid alcohol. *B* then connected the flask to the burette.

The following morning we invariably found that a crust of white solid about 2 mm. thick had formed on the surface of the reagent. We suggest that this was due to the withdrawal of final traces of water from the pyridine.

On tipping the Grignard's reagent into the leg of the flask containing the pyridine solution of the alcohol, a precipitate, presumably an addition compound of pyridine and the reagent, at once formed. The thickness of this precipitate caused the evolution of the gas to be extremely slow, and shaking at intervals for at least 40 min. was found to be necessary for the completion of the reaction. By tapping the leg of the flask and then shaking, the curd could be broken and poured from one leg of the flask to the other. This hastened the evolution of gas considerably. The reaction was considered to be complete when three consecutive readings at intervals of 5 min. were the same.

CALCULATIONS AND RESULTS

The volume of methane evolved in each experiment was corrected to 0° and 760 mm. From this the average corrected blank for the particular series was subtracted. The percentage hydroxyl in the compound could then be calculated from the formula:

$$\% \text{ OH} = \frac{17 \times 100 \times V}{22380 \times W},$$

where 17 is the molecular weight in g. of the hydroxyl group, *V* the corrected volume in cc., and *W* the weight in g. of substance taken. The results are shown in Tables I, II, III and IV.

Table I. *Amyl ether reagent.*

Blanks (at N.T.P.) 0.14 cc., 0.11 cc., 0.13 cc., 0.11 cc., 0.10 cc. Mean 0.12 cc.

Substance	Weight g.	cc. CH ₄ at N.T.P.	cc. CH ₄ corrected for blank	% OH		% Error
				Found	Theoretical	
Octadecyl alcohol	0.0113	1.04	0.92	6.18	6.29	-1.7
Octadecyl alcohol	0.0102	0.94	0.82	6.10	6.29	-3.0
Octadecyl alcohol	0.0102	0.96	0.84	6.25	6.29	-0.7
Octadecyl alcohol	0.0100	0.98	0.86	6.53	6.29	+3.8
Batyl alcohol	0.0123	1.75	1.63	10.06	10.88	-7.5
Batyl alcohol	0.0122	1.78	1.67	10.39	10.88	-4.5
Cholesterol	0.0098	0.67	0.55	4.27	4.40	-2.9

Table II. *Amyl ether reagent.*

Blanks (at N.T.P.) 0.09 cc., 0.08 cc., 0.05 cc., 0.07 cc., 0.09 cc. Mean 0.08 cc.

Substance	Weight g.	cc. CH ₄ at N.T.P.	cc. CH ₄ corrected for blank	% OH		% Error
				Found	Theoretical	
Octadecyl alcohol	0.0110	0.97	0.89	6.14	6.29	-2.4
Octadecyl alcohol	0.0101	0.91	0.83	6.24	6.29	-0.8
Hexadecyl alcohol	0.0108	1.06	0.98	6.89	7.08	-2.7
Hexadecyl alcohol	0.0102	1.03	0.95	7.08	7.08	0.0
Hexadecyl alcohol	0.0116	1.20	1.12	7.34	7.08	+3.6
Cholesterol	0.0110	0.68	0.60	4.15	4.40	-5.7
Cholesterol	0.0107	0.73	0.66	4.69	4.40	+6.6

Table III. *Anisole reagent.*

Blanks (at N.T.P.) 0.09 cc., 0.07 cc., 0.04 cc., 0.04 cc., 0.07 cc., 0.04 cc. Mean 0.06 cc.

Substance	Weight g.	cc. CH ₄ at	cc. CH ₄ corrected	% OH		% Error
		N.T.P.	for blank	Found	Theoretical	
Cholesterol	0.0105	0.64	0.58	4.19	4.40	-4.8
Cholesterol	0.0109	0.67	0.61	4.25	4.40	-3.4
Dilauryl alcohol	0.0106	0.72	0.66	4.73	5.00	-5.4
Dilauryl alcohol	0.0099	0.77	0.71	5.44	5.00	+8.8
Octadecyl alcohol	0.0092	0.78	0.72	5.94	6.29	-5.5

Table IV. *Amyl ether reagent. Substance dissolved in 1 cc. pyridine.*Blanks (at N.T.P.) 0.40 cc., 0.17 cc., 0.38 cc., 0.25 cc., 0.27 cc.,
0.09 cc., 0.22 cc., 0.26 cc. Mean 0.26 cc.

Substance	Weight g.	cc. CH ₄ at	cc. CH ₄ corrected	% OH		% Error
		N.T.P.	for blank	Found	Theoretical	
Cholesterol	0.0111	1.00	0.74	5.06	4.40	+15.0
Cholesterol	0.0082	0.81	0.55	5.09	4.40	+15.7
Cholesterol	0.0076	0.75	0.49	4.89	4.40	+11.1
Cholesterol	0.0096	0.69	0.42	3.32	4.40	-24.5
Hexadecyl alcohol	0.0082	0.89	0.63	5.83	7.03	-17.1
Hexadecyl alcohol	0.0110	1.16	0.90	6.21	7.03	-11.7
Hexadecyl alcohol	0.0092	0.99	0.73	6.02	7.03	-14.4
Quinol	0.0128	4.39	4.13	24.49	30.91	-20.7
Quinol	0.0026	1.16	0.90	26.27	30.91	-15.0

SUMMARY.

1. A micro-method for estimating the percentage of hydroxyl in the higher fatty alcohols, based on the macro-method of Hibbert and Sudborough [1904] is described.

2. Results accurate to $\pm 8\%$ are obtained when the alcohol is readily soluble in the ether of the Grignard reagent used.

3. The use of pyridine to dissolve alcohols which are not soluble in the ether of the reagent is not recommended, the results being very unreliable owing to a variable blank.

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