UNSOLVED PROBLEMS IN THE PRESERVATION OF FOOD: THE INFLUENCE OF CULTURAL CONDITIONS ON THE QUALITY AND PRESERVATION OF FRUITS AND VEGETABLES *

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THE problems in the production of fruits and vegetables that face the agriculturalist are those of developing methods for obtaining maximum yields of produce of the right 'quality'.

The problems that face the processor and the person who stores fruit are concerned with the assessment of the 'quality' and the 'suitability' of samples for some particular purpose, and the recognition of characteristics that lead to susceptibility to various processing or storage disorders.

At present, quality is assessed mainly on visual characteristics, such as size, shape, uniformity, colour, freedom from blemish and, to a less extent, on flavour and texture. Such assessments of quality presuppose that external appearances give some indication of internal composition and physiological characteristics, and to some extent this is probably true. With experience it is often possible to guess from its appearance the conditions under which fruit has been grown, or whether it is from young or mature trees. Further, it is often apparent that quality and appearance are greatly affected by weather and cultural conditions, and that storage is also affected.

But the methods of assessing quality on visual appearances cannot be considered satisfactory. Objective methods for assessing certain aspects of quality are needed and before these can be devised it will be necessary to discover how quality is related to composition and physiological characteristics. Then, when the effect of quality on storage behaviour has been established, we ought to be able to predict storage behaviour from a knowledge of chemical composition, and adjust our storage condition accordingly. We might also hope at this stage to be able to produce fruit particularly suitable for specific purposes.

The present methods of handling and preserving fruits and vegetables are based largely on experience rather than on exact scientific knowledge, and are briefly as follows: Many varieties of fruits and vegetables are available. By trial, individual growers discover what varieties can best grow under their conditions, and they produce as far as possible a variety that they can grow and sell most readily. The person who stores fruit knows approximately from past experience how long he can keep fruit of different varieties, and the conditions of temperature, concentration of carbon dioxide and oxygen at which to store to get good results. But in storing he always takes risks, for he cannot predict what losses he may suffer. Processors have discovered by experience or by variety-trials the varieties most suited to their needs, i.e. what varieties are best for jam-making or for quick-freezing or for drying. They assume that, on the whole, varieties behave in much the same way from year to year, though occasionally they may experience some serious departures from the normal.

Let me therefore briefly outline more specifically some of the problems of the chemical composition and physiological activities of fruits and vegetables that arise in preserving fruits and vegetables. We can then look at examples of the ways in which cultural factors have been shown to influence storage behaviour, and consider whether these examples suggest any new ways in which we can approach the study of the chemistry of fruits. Finally, we can consider whether, by deliberately modifying cultural conditions, we can hope to control variability in the types of fruits and vegetables that are wanted.

Ripening and factors affecting it

The first problem that arises in the handling of any crop is the time of picking. This may be very simple in some instances, and is soon solved by sheer practical necessity. With fruit that undergoes marked ripening-changes there is always some doubt, because later changes have to be anticipated, i.e. pears, apples and other deciduous fruit have to be picked before

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they reach a certain stage of ripeness if they are to be kept for the maximum period possible. The criteria that have been used to judge the maturity of apples include the ease of removal from the tree, the number of days from petal fall, the starch pattern as shown by staining with iodine, the change in the ground colour, or hardness as measured by a penetrometer. None of these criteria is entirely satisfactory. By rule-of-thumb methods or the choice of some date (say the last week in September, the first week in October), practical requirements are met. Tomatoes are gathered at the stage of ripeness that allows them to be got to some particular market before they are over-ripe, and peaches, bananas and other tropical fruit are picked in a sufficiently unripe condition to allow them to be transported to the U.K. and then ripened.

Fruit that has been picked continues to ripen. What is ripening ? There is a change in colour, a change in flavour and a change in texture. Chlorophyll is lost, but what happens to it or its breakdown products is unknown. They may well catalyse or inhibit further changes During ripening, small amounts of ethylene are produced, but it is not known by what reaction, nor why exposure to small amounts of ethylene may stimulate ripening and the production of more ethylene. This is one of the most conspicuous unsolved problems of the preservation of fresh fruits.

Many of the chemical changes that occur in the ripening of apples have been studied in detail.[1] Starch rapidly disappears. The concentration of cane sugar, which may rise somewhat with the disappearance of starch, then falls rapidly. Acids disappear slowly but continuously. The soluble pectin in the juice increases and the insoluble pectin is lost from the cell walls. The data so far available, although of the greatest interest, do not provide a clear picture of what happens during ripening, nor serve to explain variations in storage life or susceptibility to physiological diseases.

During ripening, the rate of respiration changes in a characteristic way and exhibits what is termed the climacteric rise. This provides a stage in the process of ripening to which other changes can be related, and the terms post- or pre-climacteric are now quite frequently used by growers.

A physiological explanation of the nature or cause of the climacteric has not yet been provided. Hulme[2] has shown that an increase in protein is associated with the rise. More recently, Millerd, Bonner & Biale[3] have shown that the respiratory activity of mitochondrial preparations from avocadoes in the preclimacteric stage of ripening is increased by the presence of 2 : 4-dinitrophenol (DNP), but that of similar preparations from avocadoes in the post-climacteric stage is not affected by DNP. They deduce that, before the climacteric rise, respiration is coupled to the oxidative production of adenosine triphosphate (ATP), and that the rate of production of ATP limits the rate of respiration ; but during the climacteric some substance is produced that is similar in its action to DNP, and, in consequence, the synthesis of ATP ceases and no longer limits the rate of respiration.

Though we cannot explain ripening we can influence its rate and can slow it down or speed it up by varying conditions. Fruit can be rapidly brought to the best stage of maturity for processing, or can be held back in an immature condition for purposes of storage and preservation in a fresh condition. Factors known to affect the rate of ripening are temperature and concentration of oxygen and carbon dioxide.

Ripening is also stimulated by the presence of ethylene, and oranges that are often ' ripe ' while still green are often coloured by exposure to ethylene. Bananas and certain varieties of plums are similarly treated. The temperature must be suitable, and 60–70° F is usually best ; higher temperatures may cause abnormal ripening, lower temperatures delay ripening.

The retarding influence of low temperature on the ripening of fruit is made use of in cold storage, and that of low temperatures and of carbon dioxide and low concentrations of oxygen in refrigerated gas-storage.

Exactly how oxygen and carbon dioxide affect ripening is not known, but it has been established by experiment that the storage life of certain fruits is considerably prolonged if they are stored not in air but in an atmosphere in which the oxygen concentration is reduced and the concentration of carbon dioxide is increased. This is the basis of gas-storage of fruit, which was discovered and developed by Kidd & West.[4]

The conditions of temperature and concentrations of oxygen and carbon dioxide suited to a number of varieties of apples and pears have been determined and these are widely used. But are they the ' best ' conditions ? Probably not, for they are the conditions which, when held constant over the whole storage period, give the best results, and it is probable it will be found that still better results could be obtained by varying conditions at suitable intervals during the storage period. Certain varieties of plum exported from South Africa to England are stored at 31° F for 14 days and then at 45° F or 50° F, for by this means the maximum storage-life is

attained.[5] With English plums, brief exposure to high temperature during the storage period has been shown to give longer storage-life at 31° F.[6]

The effects of changing the temperature during the storage period will no doubt be discovered by experiment, but this will require much work. Even more work will be required to discover the changes in temperature, concentration of carbon dioxide and of oxygen required to give maximum storage-life. But provided a sufficient number of model gas-stores were available the results could be obtained. We certainly cannot work out these conditions from theory but in using the direct method to find them we shall get much information of theoretical interest, such as the stages of ripeness at which fruits are most susceptible to carbon dioxide or low-temperature injury.

The restriction of ventilation, which is the method used to obtain an increase in the carbon dioxide and a reduction of oxygen within a gas-store, results in the accumulation of certain volatile compounds also produced by the fruit. Some of these, e.g. ethylene, may have a stimulating effect on ripening, others may have other, and adverse, effects. Methods of removing some of these products and the effects of doing so are being explored

The use of activated charcoal in stores used for apples has been advocated by Smock[7] in America as a means of removing certain volatile products and thereby prolonging storage-life, but the value of the use of activated-charcoal filters has, however, not been confirmed in experiments carried out in England by Fidler.[8]

Physiological problems of storing fresh fruit

It is not only ripening that has to be considered in storage: care has to be taken to avoid certain specific disorders. Storage-life may be terminated by attacks of micro-organisms or by physiological troubles. The microbiological problems of the preservation of fruit and vegetables in the fresh state have been discussed on a previous occasion[9] and will not be considered now.

If fruits are kept too long at low temperatures they are injured. Bananas fail to ripen, the flesh of apples may turn brown. Why are fruits damaged in this way? It is not known. Haynes & Archbold[10] suggested that breakdown at low temperatures occurred when the rate of hydrolysis of sucrose failed to keep pace with the rate of loss of sugar. Haynes[11] had previously shown by analysis that breakdown in apples is associated with an increased loss in acid. The loss of acid can be shown by the application of a pH indicator to the cut surface of an apple. Incidentally, the discovery of an indicator that changes colour in the region of pH 3·0–3·2 would be of great value in studying ripening changes of many fruits.

The time of exposure to a low temperature required to produce low-temperature injury varies greatly from variety to variety and from year to year. If we could predict liability to low-temperature injury we could make far better use of the preservative effects of low temperatures, since now, in order to avoid injury, we often store at 37° F when, did we but know it, better results might at times be obtained by storing at 34° F.

Another storage trouble of apples is 'scald', a disease in which the skin turns brown, presumably because the surface cells die prematurely. It has long been believed that 'scald' is due to the accumulation in the surface layers of toxic volatile products, but so far we have not been able to find ways of adjusting storage conditions to produce scald at will. We know that some varieties are more susceptible than others, that scald is influenced by time of picking, and that it can be reduced by the use of oiled wraps. But scald does not occur regularly every season, and usually only about one season in five or six. Is a grower therefore to bear the expense of wrapping his fruit every year in order to guard against scald? At present he has to be advised to do so. Hence a method of predicting the likely occurrence of scald would be of the greatest value.

Enough has been said to indicate that storage practice is empirical and attended with risks and consequent losses. Yet we still want to know how to store all products with less wastage, and some products much longer, than is at present possible. We cannot, for example, distribute soft fruits to all parts of England without serious losses. We cannot store Cox's Orange Pippin apples for more than 4–5 months, and, with increasing production, growers want to store for longer periods.

Some problems of preservation by canning, freezing and drying

There are some problems that arise in the preservation of fruits and vegetables by canning, freezing and drying. During the preliminary preparation, fruits and vegetables have to be peeled and may undergo enzymic browning. Certain practical steps, such as immersion in

water to exclude air, and the addition of salt, acid (to reduce the pH), sugar, ascorbic acid and, in some instances, sulphur dioxide, are used to retard enzymic browning. But these steps are not always effective. Further, in some tissues the enzymes appear to be particularly resistant to destruction, and colour changes may occur on exposure to air after processing. Materials from different sources vary greatly in the readiness with which they undergo enzymic browning.

The significance of the phenol-oxidase system in the physiology of the plant is at present unknown, but active work on this subject is in progress[12] so it is possible that the estimation of peroxidase activity and of the concentration of substrate and of inhibitors of the reaction may provide much information about the quality of fresh material, and its behaviour in storage and in processing.

Another example of a colour change in plant tissue, which may cause serious trouble, is the non-enzymic stem-end blackening of potatoes. The black pigment that is formed in potatoes after cooking has not been identified, though Wager[13] has shown that it is a compound of iron with a colourless precursor. Much work has been done on the cultural factors affecting liability of potatoes to blacken. It has been correlated with potash deficiency, with potash deficiency associated with high nitrogen-level and with other mineral deficiencies, with certain soil types, and with cool weather during the maturation of the tubers, and it would appear to depend on many factors. It arises most sporadically and is a serious cause of loss.

During scalding or blanching—the thermal killing process that is an essential preliminary in preservation by freezing and drying—and during sterilization in canning, the tissues of fruits and vegetables are softened. Similar changes occur in cooking. The nature of the changes in the cell walls that occur in processing (or in ripening) are imperfectly understood. Various techniques for measuring firmness have been evolved, and methods such as control of pH or the addition of calcium salt for preventing softening during processing have been discovered. The ease with which cell walls are softened by ' pectinase ' enzymes or by heat treatment is probably one of the characteristics of the greatest importance in assessing ' quality ' and ' suitability ' for some purpose. There is, however, no recognized test for measuring the softening of cell walls.

Drying is a method of preservation that offers many advantages but presents so many technical difficulties that it is not as widely used as might be expected. The tissues must first be killed, and, in scalding, solutes may be lost and yield of dried product reduced. To remove the water the tissues have to be finely divided. Even so the removal of most of the water is difficult, for drying must be carried out within fairly narrow limits, for otherwise scorching may occur. The dried products do not always reconstitute easily and unless they have been sufficiently scalded before drying may be tough. They have also a limited storage-life. At temperatures in excess of 25° they may undergo non-enzymic browning. This can be reduced by the use of sulphur dioxide, and to some extent by the selection of certain varieties and by suitable pretreatments. But browning still remains a major problem. When stored at lower temperatures dried vegetables may develop rancid or ' off ' flavours. These changes, which also occur with frozen foods, can be reduced by storage in nitrogen. Some ' off ' flavours are due to the oxidation of carotene, and some to the oxidation of fats or lipids[14] but the chemistry of their production is not fully understood. Hence for a variety of reasons drying has not been successfully established as a peace-time method of preserving fruits and vegetables.

The effect of cultural conditions on quality and preservation

In outlining some of the problems mentioned above we have stressed, on the one hand, the constancy of behaviour of fruits and vegetables in storage and in processing, and the reliability we can place on past experience as a guide to action, and, on the other, the differences that occur within the same variety from season to season and from locality to locality. We can say on sight that an apple is a Cox, a Bramley or some other variety, and we can expect it in consequence to have certain characteristics ; nevertheless there can be a wide degree of variation within a variety. The composition may be roughly the same in regard to water content, carbohydrate, acidity and nitrogen content, but there may be differences in colour, flavour or texture, and this being so we may ask which are the characteristics that make so much difference in quality. Unfortunately it is impossible to give an answer, and it is perhaps simpler to consider some of the variations in physiological characteristics, such as rates of respiration, liability to wilting, and behaviour in storage of samples drawn from different sources.

The rate of respiration of individual apples can vary appreciably, and there can be variations in the time of onset of the climacteric, and in the increase in the rate of respiration at the climacteric.[15a]

Examples of the ways in which orchard conditions affect storage diseases have been given in an article recently published by Wallace.[16] Other examples have been published by Kidd & West in the Annual Reports of the Food Investigation Board.[15b, c]

In their earlier work, in which they compared the keeping quality of Bramley's Seedling apples grown on different types of soil in different parts of the country, Kidd & West[15b] found that the respiratory activity of individual apples was related to their nitrogen content, and that storage-life was inversely related to the rate of respiration. They also noted that good keeping quality (more especially freedom from low-temperature breakdown) was directly correlated with the available potash in the soil. They found that the time of picking affected the incidence of low-temperature breakdown, and that good keeping quality was associated with warm dry weather during the few weeks before the date of gathering.

Haynes & Archbold[10] also found that long storage-life was associated with low nitrogen-content and low rate of respiration, and further suggested that it was also associated with a high content of sucrose.

In later experiments with Cox's Orange Pippin apples, Kidd & West[15c] found that the application of potash to the soil resulted in apples less liable to fungal attack but more liable to low-temperature breakdown.

It is probable that the amounts of fertilizers now applied to fruit trees are very much greater than those applied 20 years or so ago ; this may account for some of the apparent differences in the effect of fertilizers.

The differences in storage behaviour of fruit taken from trees on neighbouring but differently treated plots are often most striking and apparently quite definite. Unfortunately, as Wallace[16] has shown, the same treatment may produce entirely different results in different seasons or in different areas. Hence although we have evidence of changes being produced we cannot produce these changes at will. The reason is, of course, that though the same treatments are apparently applied they may not in fact be the same nor, in any case, do they necessarily directly affect the composition of the fruit. The nutrients absorbed into the plant from the soil may be used to produce leaves, shoots or fruits. Moreover, the amounts absorbed may depend in part on soil moisture, and therefore on rainfall and on methods of cultivation. Some primary treatment may produce one effect in one year and another effect in another year because of differences in rainfall or temperature. Hence we cannot assume that any particular treatment has had any particular effect on the composition of fruit. The effect can only be discovered by analysis not only of the leaves, which may indicate the nutritional status of the tree, but also of the fruit itself. Further, the application of a fertilizer to the soil does not necessarily mean that the concentration of that element will be increased in the plant. The addition of nitrogen to the soil may not increase the concentration of nitrogen in the plant but only allow more growth, the concentration's in the plant being thereby kept more or less constant.

'High nitrogen' fruit is a term often used by growers to describe the type of large green fruits that are reputed not to keep well, and which are produced by young trees given large amounts of nitrogenous manures. It is possible that such fruits have a high nitrogen content but it appears from such data as are available that the protein nitrogen is increased far less readily than the alcohol-soluble nitrogen. Hence if high levels of nitrogen fertilizers result in fruit of poor keeping quality is it because higher protein content results in higher enzyme activity and therefore higher metabolic activity ? Or is it that the higher content of soluble nitrogen allows easier attack of micro-organisms or leads to more physiological disorders ? Both possibilities need to be considered. Presumably if we had data on total soluble and insoluble nitrogen, and data on the storage-life of samples of fruit, we might see whether there was any relation between the various nitrogen fractions and length of storage life or the various disorders.

Effect of fertilizers containing potassium

The results obtained by adding potassium to the soil have been most variable, but it is perhaps possible to understand why this is so. Two facts must be considered. One, well known, is that potassium is the major base in the plant ; and the other, not well known, that the pH of the tissue of fruits drops to its characteristic value at a very early stage of development.[17] Why, we may ask, is the pH of the flesh of an apple fruit say 3·0 (though that of the skin may be 3·5–4·0) whereas that of the leaves and other parts of the plant is 5·5 ? An explanation cannot be offered but if we regard the developing apple as an absorbing system into which pass carbohydrates and potassium and other compounds, and assume there is some mechanism regulating pH, then the amount of potassium entering the fruit determines the amount of acid formed from carbohydrate, the amount being sufficient to bring the pH to the characteristic

value. Large uptake of potassium would normally result in high concentrations of titratable acid, and lower concentrations of sugars, and low uptake of potassium would result in low concentrations of acid and higher concentrations of sugars. There is evidence that in oranges the amount of acid produced depends on the potassium taken up, and that, if the concentration of acid is low, the concentration of glucose is higher.[18] However, excessive amounts of potassium combined with low supplies of carbohydrate might lead to failure to achieve complete pH equilibrium, and we could have a high concentration of acid but a pH higher than normal. This might result in a higher rate of some metabolic activities or a lower rate for others, such as the acid hydrolysis of cane sugar. On the other hand, with very low potassium-uptake a slight excess of acid formation might lead to a low pH value, though the titratable acid was still low.

Much of the available data on the composition of apples gives values for titratable acid only and make no mention of potassium or pH. We cannot, therefore, decide the extent to which the loss of acid during ripening is likely to affect the change in pH. It can, however, be quite easily demonstrated that a Cox apple loses its quality when its pH rises from 3·2 (the value when on the tree) to about 3·6, i.e. when bromophenol blue applied to the cut surface shows signs of turning blue.

Although moderate amounts of potassium would therefore appear advantageous, excessive amounts might cause damage, particularly in wet years when absorption would be high and photosynthesis low. With oranges, low potassium-uptake can, in some instances, be advantageous, since this may result in a low concentration of acid and more sugar, and maturity is judged by the sugar/acid ratio.

Potassium may also affect the uptake of magnesium or calcium and if applied in excess might reduce the uptake of these minerals.[18, 19] Texture might thereby be affected, since these metals, by combining with pectic acid, are believed to have an effect on cell-wall structure.

It would seem likely that differences in cultural conditions affect mineral-uptake more than carbohydrate metabolism and that it is to differences in mineral content and the effects of these differences that we must look for differences in physiological behaviour.

The problem is, however, not solely one of chemical analysis. Water supply has a very obvious effect on amount of growth and on the character of fruit. Fruit grown under wet conditions may, when held in store, lose water more rapidly than fruit grown under dry conditions. Is this due to dry conditions having produced a thicker cuticle and less open stomata? And if water loss is reduced may not the diffusion of oxygen and carbon dioxide be restricted and respiration and metabolic activity be decreased? If so, would not some measurement of the openness of the stomata and of the thickness of the cuticle give a useful index of storage behaviour? Respiration has, in the past, always been considered a useful index of physiological activity. Is it not possible that phenol-oxidase activity may likewise give some measure of storage behaviour?

The scope and practical objectives of future investigations

A sufficient number of problems have been mentioned to indicate the very wide field that exists for the investigation into the chemistry of plant products, and those who have had experience with the preservation of fruit and vegetables will have encountered many other problems. In discussing how progress can best be made the view is often expressed that only by large teams undertaking mass analyses are we likely to make advance. It is, however, possible that progress is likely to be just as rapid if some of the physiological aspects of growth and development are considered, and if chemical investigations are planned to throw light on these. In this connexion investigation of the differences produced by environmental conditions give ample scope for theory and experiment, and a field in which theory can soon be put to the test.

The practical objective of such investigations should be the devising of simple tests whereby we can assess quality more accurately than is possible at present, and the discovery of the storage conditions or the processing conditions that suit particular batches of fruit. When this can be done we may even be able to look forward to a time when we can grow products more suited to our particular requirements by suitable adjustment of the manurial and cultural conditions.

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ANALYTICAL STUDIES ON THE CARBOHYDRATES OF GRASSES AND CLOVERS. IV.*—Further developments in the Methods of Estimation of Mono-, Di- and Oligo-saccharides and Fructosan

By CLARE B. WYLAM

Methods for the determination of the water-soluble carbohydrates in both fresh and ensiled materials are discussed. A technique has been developed for the estimation of the oligosaccharides in grasses, after their separation from the other free sugars by paper chromatography. Their fructose content is determined by a colorimetric method, and fructose, glucose and galactose are estimated after their complete hydrolysis with 0·5N-sulphuric acid.

Enzymic hydrolysis of sucrose and fructosan has been found to occur after the cutting of grass samples, and inactivation of the enzyme systems in the living material by immediate immersion in hot ethanol has therefore been advocated.[1] After the adoption of this technique for the analysis of wilted grass and silage, it became evident that certain modifications of the analytical procedures described by Laidlaw & Reid in Part I of this series[2] were necessary. By this method, dried grass samples were extracted with 80% aqueous ethanol in a Soxhlet apparatus, the extract was evaporated, clarified with cadmium hydroxide and de-ionized, and the glucose, fructose and sucrose present were estimated after being separated by paper chromatography. The residue was then hydrolysed with 1% oxalic acid and the fructose produced was determined, after being separated from arabinose by paper chromatography.

General modifications of the method

The first problem concerned the extraction of 20-g. samples of freshly-cut ethanol-immersed grass. In preliminary experiments the grass was lacerated with scissors and exhaustively extracted under reflux in a flask by frequent decantation and renewal of the ethanol. This was very tedious and difficulty was experienced in removing the last traces of sugars from the grass residue. Therefore the procedure that has now been adopted is to macerate the sample in ethanol, transfer it to a cellulose thimble and extract it in a Soxhlet apparatus for seven hours, with 80% ethanol in the reservoir flask.

If the direct-extraction method is used, it is advisable to extract with 90% ethanol, since

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the composition of the vapour from 80% ethanol, used in the Soxhlet method, is 89% (v/v) In a control experiment, samples of a grass were extracted in three different ways. After extraction in a Soxhlet apparatus with 80% ethanol the residue contained 8·6% of fructosan; after direct extraction with 90% ethanol the residue contained 8·4% of fructosan; after direct extraction with 80% ethanol the residue contained only 5·2% of fructosan. The fructosan is probably synthesized in the plant by enzymic transfructosidation of sucrose,[3, 4] and it is reasonable to expect that there will be present in the plant a large range of fructosan molecules of varying chain lengths, which will have different solubilities. In the above-mentioned experiment it would appear therefore, that 40% of the fructosan that is insoluble in 90% ethanol consists of shorter-chain molecules, which are soluble in 80% ethanol. Such a division is purely arbitrary, and the fructosan that is extracted by heating directly with 80% ethanol would appear in the oligosaccharide fraction. It follows that when the sugars in the grass are being estimated by determination of total reducing power, before and after hydrolysis,[5] this fructosan may be included in the sucrose figure, and considerable errors may result.

After paper-chromatographic separation of the free sugars it has been found time-saving to combine elution and hydrolysis of sucrose by suspending the paper strips over boiling 1% oxalic acid under reflux for 75 minutes. This has been found to give satisfactory results provided paper blanks are treated concurrently. The monosaccharides are eluted with cold water.[6] The Somogyi iodometric method[7] has been found to give reliable results for the determination of sugars after chromatographic separation, and in acid hydrolysates after careful neutralization with potassium hydroxide.

A method has been developed by Harwood[8] for the determination of hemicelluloses and cellulose in grasses. When this method is applied to the analysis of an alcohol-extracted residue the determination of the fructosan by hydrolysis of the residue with 1% oxalic acid is unsatisfactory, since this treatment hydrolyses some of the arabinose-containing hemicellulose. The procedure we now use is to extract the fructosan by shaking the ethanol-extracted grass with cold water for 12 hours. This extracts only a negligible quantity of araban, and effects complete removal of the fructosan, which is estimated by the colour reaction with resorcinol, glycerol and hydrochloric acid.[9] This method is specific for fructose and may be carried out in the presence of arabinose, as well as glucose or galactose. Fructosan has been found to give a 90% recovery of fructose under the conditions of the reaction.

Although there is no starch in the leaf or stem of grasses, it may sometimes be necessary to estimate starch in the presence of fructosan, e.g. in the analysis of the seed of the plant, or of a mixture of grasses and legumes such as are often used for silage. In this event a hot-water extraction of the alcohol-extracted sample is carried out. The fructosan in the extract is then determined by the colorimetric method mentioned above, and the starch by hydrolysis with 1·5N-sulphuric acid for two hours,[10] and estimation of the glucose after separation from other components by paper chromatography. It has been found, however, that fructose is decomposed to the extent of 35% under these conditions, so that a correction must be applied if the fructose is determined in the hydrolysate. Other sugars are unaffected. It should be pointed out that this method can give only a rough estimate of the starch present, since the glucose produced on hydrolysis of such an extract may not be due entirely to starch. McIlroy[11] has isolated from perennial rye-grass a water-soluble polysaccharide containing glucose and galactose residues, and confirmatory evidence of this has been obtained by Harwood (private communication).

Estimation of oligosaccharides

Laidlaw & Reid[2] obtained evidence from paper chromatography that the ethanol extracts of grasses and clovers contained oligosaccharides, and a preliminary examination of a mixture of oligosaccharides obtained from cocksfoot grass suggested that it might contain stachyose, raffinose and melibiose.

A subsequent investigation of the oligosaccharides present in a sample of perennial rye-grass has shown them to be a complex mixture of compounds containing fructose, glucose, galactose and traces of xylose and arabinose. The presence of raffinose, and probably melibiose and stachyose, has been established,[12] and the mixture also contains short-chain fructosans and compounds that are probably intermediates in the biosynthesis of hemicellulose polysaccharides.

It has become obvious that the estimation of these oligosaccharides is of primary importance in carrying out detailed studies of the water-soluble carbohydrates in grasses, since they almost certainly play a large part in the metabolism of the plant. They are probably subject to considerable seasonal variation, and are of special importance in studying the transformation of

carbohydrates during the ensilage process, when a rapid decomposition of fructosan into molecules of shorter chain-length has been found to occur.[1]

Because of the complex nature of the oligosaccharide mixture it is impossible to separate and estimate the individual compounds in the routine analysis of grasses, but a method has been developed for the determination of the total mixture.

The main difficulty encountered in work on rye-grass was that the oligosaccharides were usually found to contain a preponderance of labile glucose- and fructose-containing compounds, with small amounts of the raffinose type, in which the glucose–galactose linkage is more resistant to acid hydrolysis. Fructose suffers considerable decomposition under the conditions required for the hydrolysis of compounds of the raffinose type, and the problem has been to find conditions that will effect complete hydrolysis of the mixture with minimum decomposition. Various methods have been tried and the most successful procedure is the following. The oligosaccharides, which do not move from the starting line of the chromatogram in the solvent used, are extracted by suspending the paper strip over boiling water under reflux for 90 minutes (elution with cold water was found to be ineffective). The extract is made up to 5 or 10 c.c. and the fructose determined in a portion by the colorimetric method mentioned above.[9] Another portion is made 0·5N with respect to sulphuric acid, heated for four hours on a boiling-water bath, neutralized, and the total reducing power determined by the Somogyi method. By applying a correction factor for the decomposition of fructose, which is 28% under these conditions, the total weight of oligosaccharides on the paper strip can be calculated.

It is also possible to obtain some idea of the proportion of galactose-containing compounds in the mixture by separating the oligosaccharides in larger quantity on thick filter paper. They are then hydrolysed with 0·5N-sulphuric acid, and the ratio of the sugars in the hydrolysate is determined after separation by paper chromatography.

Results obtained for the oligosaccharide contents of samples of perennial rye-grass, collected during the growing season of 1953, have shown that the amount of oligosaccharides present is often appreciable. For example, the oligosaccharide content of a stem sample cut on 27 July was 8·0% of the dry weight, and on complete hydrolysis the oligosaccharide mixture yielded fructose, glucose and galactose in the ratio 100 : 10 : 1. Results have indicated that the amount of galactose-containing compounds is always small, and that high total figures are caused by an unusually high proportion of short-chain fructosans.

The analysis of ensiled materials

In the application of these methods to the analysis of silages two additional problems emerged. First, there is the possibility that, in a mature silage, decomposition of hemicellulose may have occurred, and some free xylose and arabinose may be present.[1] This rules out the addition of xylose to the ethanol extract as a standard sugar, and ribose has been used. In this event the solution should not be de-ionized by electrodialysis, since preferential migration of ribose has been observed during electrodialysis of sugar mixtures.

The second problem concerned the acid nature of the silage. The organic acid can be removed by thorough washing with 80% ethanol before extraction, but it was thought possible that some hydrolysis of sucrose or fructosan might occur at a later stage in the treatment of the combined extracts, e.g. during evaporation or clarification. This is not the case, however, as was shown by the addition of sucrose and xylose to a silage extract that contained no sucrose. The extract was evaporated, clarified and electrodialysed, and the relative amounts of xylose and sucrose were estimated. A good recovery of sucrose was obtained, showing that the alcohol extract of a silage does not contain a sufficiently high hydrogen-ion concentration to cause hydrolysis of the labile constituents. It may therefore be treated in the same way as a grass extract.

A complete scheme based on these observations is given in the experimental section for the estimation of mono-, di- and oligo-saccharides and fructosan in fresh and ensiled materials.

Experimental

Alcohol extraction of grass samples.—Laidlaw & Reid[2] achieved complete removal of the free sugars from 1-g. samples of dried, milled grass by Soxhlet extraction with 80% aqueous ethanol for two hours. After the changeover to the technique of immersion of the grass in alcohol after cutting, the samples were at first extracted directly in a flask with 80% ethanol. However, it was suspected that this treatment might remove some fructosan and therefore three 1-g. samples of freeze-dried milled S24 rye-grass (cut 12/11/51) were extracted as follows: (*a*) in a Soxhlet extractor for two hours with 80% ethanol in the reservoir flask; (*b*) in a flask for two hours with 80% ethanol; and (*c*) in a flask for two hours with 90% ethanol.

In (*b*) and (*c*) the residue was filtered off after extraction and thoroughly washed with ethanol. In all instances, ribose (about 50 mg.) was added to the residue after extraction and the fructosan determined after hydrolysis with 1% oxalic acid for one hour, to give : (*a*) 8·6% ; (*b*) 5·2% ; (*c*) 8·4% (per cent. dry matter of grass).

After this, it was decided to adopt the Soxhlet extraction method, which gives a more complete extraction and is less time-consuming. The ethanol-immersed grass (20 g.) is macerated in alcohol and then completely transferred to a cellulose thimble (27 mm. × 250 mm.) and extracted for seven hours in a Soxhlet apparatus, with fresh 80% ethanol in the reservoir flask (paper-chromatographic studies have revealed that extraction is complete after 5½ hours).

Estimation of fructosan.—A 20-g. sample of rye-grass was macerated and extracted with 80% ethanol in a Soxhlet apparatus for seven hours. The residue was dried in the oven at 80° and portions were treated as follows.

(1) The material (1 g.) was shaken with cold water (100 c.c.) for three hours, filtered, washed and shaken with 100-c.c. portions of water for further 3-hour periods. Hydrolysis of the filtrates, followed by chromatographic examination, showed that extraction of the fructosan was complete in 12 hours. Only a very slight trace of araban was extracted. Hydrolysis of the residue after 12 hours' extraction gave no fructose.

(2) The residue (1 g.) was heated with 100 c.c. of water on a water bath at 50° for 30 minutes. Hydrolysis of the extract gave fructose, galactose and arabinose. No further fructosan could be extracted by continued heating with water.

(3) The residue (0·69 g.) was hydrolysed at 100° for one hour with 1% oxalic acid, ribose (50 mg.) was added and the fructose and ribose were determined after separation on a paper chromatogram. The residue was found to contain 2·69% of fructosan.

(4) The residue (0·66 g.) was shaken with cold water (100 c.c.) for six hours, filtered, washed and shaken with a further 100 c.c. of water for six hours. The combined extracts were diluted to 500 c.c. and the fructosan, in 2-c.c. portions, was determined by the colour reaction with glycerol, resorcinol and hydrochloric acid.[9] A pure sample of the fructosan isolated from *Lolium perenne* was found to yield 90% of fructose under the conditions of the reaction.

Estimation of fructose in the presence of other sugars.—Fructose was determined in synthetic mixtures containing equal quantities of fructose and (1) glucose, (2) galactose and (3) arabinose by the colorimetric method.[9] This gave results within 3% of the known weights of fructose in the mixtures over the range of concentration used in the method (0·03–0·15 mg. of fructose).

Conditions for hydrolysis of oligosaccharides.—A sample of stachyose isolated from soya beans[13] and a mixture of oligosaccharides isolated from perennial rye-grass[11] were used for hydrolysis experiments. Heating with oxalic acid solutions of concentration up to 5% for five hours did not effect complete hydrolysis ; in both instances examination of the hydrolysate on a paper chromatogram showed the presence of a disaccharide, which travelled at the same rate as melibiose in benzene–butanol–pyridine–water (1 : 5 : 3 : 3) and in amyl alcohol–pyridine–water (7 : 7 : 6).

Examination by paper chromatography of stachyose after heating on a boiling-water bath with 0·5N-sulphuric acid for varying times showed that hydrolysis was complete in four hours. Hydrolysis of the oligosaccharide mixture was similarly found to be complete after four hours under the same conditions.

In a control experiment, 20·0 mg. of raffinose pentahydrate and 25·2 mg. of pure fructosan, isolated from perennial rye-grass as described by Laidlaw & Reid,[14] were dissolved in 250 c.c. of water. The fructose determined in 1-c.c. portions by the colorimetric test was 0·117 mg. Allowing for 90% recovery of fructose from the fructosan, this indicates an error of − 2·9%. Portions (5 c.c.) were made 0·5N with sulphuric acid and hydrolysed for four hours on a boiling-water bath. Determination of the total reducing power after hydrolysis gave a result equivalent to 0·770 mg. of sugar. After being corrected for 28% decomposition of fructose under these conditions (see below), the total weight of sugars produced on hydrolysis is 0·956 mg., whereas theoretically 5 c.c. of this solution should yield 1·004 mg. (error − 4·8%).

Decomposition of sugars by acid treatment.—In order to determine the stability of various sugars under hydrolytic treatment, the sugar (8 mg.) was dissolved in acid (50 c.c.) and 5-c.c. portions of the solution were heated under reflux in tubes on a boiling-water bath for different times. The solutions were cooled, neutralized drop by drop with 4N-potassium hydroxide and the sugar was estimated by the Somogyi iodometric method.[7] Glucose, galactose, fructose, xylose and arabinose were treated with (*a*) 0·5N-sulphuric acid, (*b*) 1·5N-sulphuric acid. In both instances only fructose was found to suffer decomposition, and the recoveries are shown in Table I.

Table I

(a) 0·5N-H_2SO_4		(b) 1·5N-H_2SO_4	
Time of heating, h.	Recovery, %	Time of heating, h.	Recovery, %
1	94	1	87
2	90	1·5	76
3	81	2	65
4	73	2·5	55
5	65		
6	60		

These are the results of one experiment only, but the average decomposition of fructose from several experiments was (*a*) 28% in four hours in 0·5N-sulphuric acid, (*b*) 35% in two hours in 1·5N-sulphuric acid.

Electrodialysis of sugar solutions.—A synthetic mixture of sucrose, glucose, fructose, xylose and ribose was dissolved in water and the solution divided into two parts. One part was evaporated to small volume and the sugars were determined after separation on a paper chromatogram. A little sodium sulphate was dissolved in the other part, which was then electrodialysed for 30 minutes and the sugars were determined in the same way. The results are shown in Table II.

Table II

	Composition without electrodialysis	Composition after electrodialysis	Error, %
Sucrose	119	122	+ 2·5
Glucose	87	85	− 2
Fructose	82	84	− 2
Xylose	100	100	standard
Ribose	124	105	− 15

These results confirm the finding of Laidlaw & Reid[2] that there is no preferential migration in a mixture of sucrose, glucose, fructose and xylose, but they indicate that there is a preferential migration of 15% of ribose in relation to these sugars. Preferential migration of ribose in other sugar mixtures has been observed by Harwood (private communication), and therefore its use as a reference sugar is not to be recommended when solutions are electrodialysed.

Effect of organic acids on treatment of silage extracts.—A sample of silage (pH 3·5) was extracted with 80% ethanol. Analysis of an aliquot of the combined extracts, by addition of xylose followed by the usual procedure, showed that it contained glucose, galactose and fructose and no sucrose. To another aliquot were added weighed quantities of sucrose and xylose; the solution was evaporated, clarified and electrodialysed, and the sugars were estimated in the usual way. As shown by the results in Table III, the same ratio of xylose/sucrose was recovered as was added by weight, with no increase in the glucose and fructose contents.

Table III

	Known composition	Recovery	Error, %
Sucrose	3·9 (added)	4·1	+ 5
Glucose + galactose	3·0	2·9	− 3
Fructose	13·6	12·9	− 5
Xylose	3·8 (added)	3·8	standard

These figures are all expressed as a percentage of the dry matter of the material. This shows that the hydrogen-ion concentration of the organic acids in silage extracts is not sufficient to cause hydrolysis of sucrose or other labile substances under the conditions used for analysis of alcoholic extracts.

General scheme for the analysis of glucose, fructose, sucrose, oligosaccharides and fructosan in fresh or ensiled grass samples

Determine the moisture content by heating to constant weight at 80°.

Decant most of the ethanol from the alcohol-immersed material (20 g.), cut the material into small pieces (1–2 in.) with scissors and macerate in ethanol. Transfer the macerated material to a Soxhlet thimble (27 mm. × 250 mm.) and extract with 80% ethanol (350 c.c.) for seven hours. Combine the ethanol extracts, make up to a specific volume (about 1000 c.c.)

and take an aliquot equivalent to about 1 g. of dry material. To this add about 25 mg. of xylose (or ribose if a silage) and evaporate to small volume at 40°. Add 100–150 c.c. of water and clarify by maintaining the solution at 100° for two minutes after the simultaneous addition of equimolecular volumes of cadmium sulphate and barium hydroxide solution (10 c.c., 0·36N). Cool, filter and de-ionize the filtrate by means of electrodialysis, or by passage through ion-exchange resins if ribose has been added. Evaporate at 40° to a syrup, dissolve in about 1 c.c. of water and spot about 0·04 c.c. on duplicate quantitative chromatogram papers by means of a micro-pipette. Irrigate the papers with the upper layer of ethyl acetate–acetic acid–water (3 : 1 : 3) for 40 hours at 23°, dry in air and locate the sugars by spraying the side strips with aniline oxalate solution. Elute the glucose, fructose and xylose into tubes with 5 c.c. of cold water[6] and estimate by the Somogyi method. Extract the sucrose by suspending the paper strips over 5·5 c.c. of boiling 1% oxalic acid under reflux for 1½ hours, neutralize drop by drop with 4N-potassium hydroxide and estimate by the Somogyi method. Extract the oligosaccharides, located near the starting line of the chromatogram, by suspending the strips over 3·5 c.c. of boiling water for 1½ hours. Make the extract up to 5 or 10 c.c., according to the amount present. Determine the fructose in a 2-c.c. portion of this solution.[9] Take another portion, and make it 0·5N with respect to sulphuric acid, heat on a boiling-water bath for four hours, neutralize with potassium hydroxide and determine the total reducing power by the Somogyi method.

[In order to determine the ratio of the sugar components of the oligosaccharides, spot most of the remaining 1 c.c. of solution containing the mixture of free sugars on a chromatogram of Whatman 3MM paper, and irrigate with the upper layer of ethyl acetate–acetic acid–water (3 : 1 : 3) for at least 40 hours. Cut out the oligosaccharide portion and extract exhaustively with water on a boiling-water bath. Filter the extracts, evaporate at 40° to a syrup and heat with 0·5N-sulphuric acid for four hours on a boiling-water bath. Cool, neutralize with barium carbonate, filter and evaporate to small volume. Filter and spot the solution on quantitative paper-chromatograms. Irrigate with the upper layer of benzene–butanol–pyridine–water (1 : 5 : 3 : 3) for 40 hours and estimate the glucose, fructose and galactose in the usual way.]

Knowing the weight of fructose in the oligosaccharide portion from the colorimetric determination, calculate the total weight of the oligosaccharides on the paper from the total reducing power after hydrolysis, or from the ratio of sugars obtained, correcting for 28% decomposition of fructose during the hydrolysis. The weights of the sugars on the paper can then be related back to the dry weight of grass by means of the xylose used as reference sugar.

Allow the ethanol-extracted grass to drain thoroughly, transfer to a flask and shake for six hours with cold water. Filter, and shake the residue with cold water for a further six hours. Combine the filtrates and make up to one litre. For samples of leaf take 2-c.c. and 1-c.c. portions and determine the fructosan by the colorimetric method.[9] For samples of stem it is first necessary to make a further dilution of the solution, depending on the season and fructosan content anticipated. Correct for a 90% yield of fructose from the fructosan by this method.

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THE FLUORINE AND CHROMIUM CONTENTS OF TREATED ALUMINIUM SURFACES

By R. HOLT

'Alocrom' treatment deposits a film containing incidental quantities of fluorine and chromium on an aluminium surface. Fluorine and chromium in the films have been estimated. The results indicate that lacquered food-cans made from aluminium treated by this process, and with which experiments are now proceeding, would not be dangerous to health.

Introduction

'Alocrom' is the name given to a patented process[1] that is intended to improve the adhesion of paints, lacquers, varnishes etc. to aluminium and its alloys by the formation of a surface film on the metal before application of the lacquer. The bath used for this treatment contains fluoride and chromate ions as essential components, and both elements become incorporated in the surface film. Compounds of both fluorine and chromium are toxic in varying degrees and, because 'Alocrom'-treated aluminium cans may be used for food preservation, the work described in this paper was undertaken to determine the quantities of fluorine and chromium in the coatings formed by immersing plate in the bath at 90° F for (*a*) 15 seconds and (*b*) two minutes. Samples were obtained in the form of thin sheets about 6 inches square and weighing about 20 g. each.

Experimental

Determination of fluorine

It was decided to base the method for the determination of fluorine on that recommended by the Sub-Committee of the Society of Public Analysts.[2] Aluminium retards the distillation of fluorine[3] and it was therefore necessary to separate the 'Alocrom' film as completely as possible from the base metal, and to show that the quantity of aluminium that inevitably dissolved did not interfere with the final determination. Sodium hydroxide (20% solution) was first used for dissolving the film (procedure A) and gave satisfactory results with the heavier 'Alocrom' coatings. With the lighter coatings this method gave a blank that was too high relative to the fluorine in the sample (Table I). A different stripping agent was therefore sought; N-sulphuric acid was eventually chosen (procedure B) because it gave low blanks and its attack on the metal underneath the coating was relatively slight. A larger sample could therefore be used for fluorine determination without the solution's becoming overburdened with aluminium. One sample of each type was examined by both procedures. The results are shown in Table I, and the agreement between them was considered satisfactory. The amounts of fluorine found in three coatings of each type are shown in Table II.

Table I

Comparison of results obtained by means of the two methods for determination of fluorine

Procedure	Type of coating	μg. of F/sq. in. of surface (blank deducted)
A	Heavy	29·0
B	Heavy	29·0
A	Light	6·5
B	Light	7·3

Table II

Quantities of fluorine found in the coatings (blank deducted)

Sample	Type of coating	Procedure	μg. of F/sq. in. of sample	μg. of F/sq. in. of surface (mean)
A	Heavy	A	48, 51, 50	25·0
B	Heavy	A	58, 57	29·0
C	Heavy	A	57, 55	28·0
1	Light	B	14, 14·5	7·1
2	Light	B	14·5	7·3
3	Light	B	14·5, 14·5	7·3

Procedure A.—The sample was weighed, its surface area was measured, and it was cut into small pieces each weighing approximately 20 mg. Sodium hydroxide solution (5N; 5 ml.)

was then added to a weighed sample of about 0·5 g. in a small beaker. The beaker was rotated during one minute and the liquid was then decanted into a 50-ml. Claisen flask. The residual metal was washed in the beaker with a mixture of 15 ml. of 60% perchloric acid and 7 ml. of water, in three portions, which were transferred successively to the flask. The methods recommended by the S.P.A. Sub-Committee for the determination of fluorine were used both for the distillation of the perchloric acid solution and for the determination of fluorine.

Procedure B.—A piece of the sample having an area of 20 sq. in. was boiled under a reflux condenser for three minutes with 50 ml. of N-sulphuric acid. The acid solution was cooled and poured through the condenser to wash down any traces of fluorine that might have collected there. A volume of 5 ml. of this solution was then added to a previously distilled mixture of 15 ml. of 60% perchloric acid and 7 ml. of water, and the fluorine determined as before.

Various factors in the procedures were then checked as follows:

Blank.—Duplicate blank-determinations on the reagents used gave 0·6 and 0·7 μg. of fluorine by procedure A and 0·1 and 0·2 μg. of fluorine by procedure B, calculated in each instance from the reagents used per square inch of surface. Figures of 0·4 and 0·8 μg. of fluorine per square inch of surface were obtained by following procedure A on aluminium of the type on which the 'Alocrom' coatings were deposited. This indicated that no detectable fluorine was obtained from the base metal itself.

Efficiency of the distillation.—To find whether or not the aluminium in the distillation medium was retarding distillation of the fluorine, a second fraction of 150 ml. of distillate was collected and the fluorine in it estimated (Table III). A comparison of the figures obtained from these second fractions with the figures from the blank determinations shows that distillation of fluorine is almost completed in the first 150 ml.

Table III

Efficiency of distillation of fluorine (blank included)

Procedure	μg. of F/sq. in. of surface	
	From first 150 ml.	From second 150 ml.
A	24·0	0·7
A	25·0	0·8
A	25·0	0·3
B	14·0	0·6
B	14·5	0·1

Efficiency of dissolution of the coating.—The residues from two samples whose coatings had been removed by procedure A, and from one sample stripped by procedure B, were subjected to exactly the same processes again. The results, when compared with the blank figures, indicate that the residual fluorine was negligible (Table IV).

Table IV

Fluorine obtained by re-stripping (blank included)

Procedure	μg. of F/sq. in. of surface	
	First stripping	Second stripping
A	25·5	0·6
A	29·5	0·4
B	29·0	0·3

Recovery.—After two fractions of 150 ml. were collected from the solutions obtained by treating the samples as described above, quantities of sodium fluoride solution equivalent to 40 μg. of fluorine were added to the residues, and a further 150 ml. was distilled. The fluorine in the distillate was then estimated as before. The recoveries of added fluorine are recorded in Table V.

Determination of chromium

N-Sulphuric acid was used to dissolve the coatings as in procedure B above. Chromium in the solution was then determined colorimetrically as chromate by use of the method described by Sandell[4] as a basis.

Procedure.—A solution of the film was prepared as described in procedure B above. Aliquots of this solution, in a conical flask, were neutralized with 5N-sodium hydroxide solution, oxidized by boiling with 0·5 g. of sodium peroxide until all the oxygen had been evolved, and made up to 50 ml. with water. The colour of the filtered solution was measured in an absorptio-

Table V

Recovery of added fluorine (blank deducted)

Procedure	Per cent. recovery of 40 μg. of fluorine added as sodium fluoride
A	103, 103, 101, 97
B	96, 98, 99, 99

meter with a violet filter. The chromium content of the solution was then found from a standard curve, prepared by treating the base metal on which the coating was deposited in the same way, and adding known quantities of tervalent chromium in a standard solution before oxidizing. The results are shown in Table VI.

Table VI

Quantities of chromium found in the coatings

Sample	Type of coating	mg. of Cr/sq. in. of sample	mg. of Cr/sq. in. of surface (mean)
D	Heavy	0·245, 0·22	0·12
E	Heavy	0·245, 0·25	0·13
F	Heavy	0·245, 0·255	0·13
1	Light	0·060, 0·062	0·031
2	Light	0·061, 0·068	0·032
3	Light	0·058, 0·060	0·030

Efficiency of dissolution of the coating.—In order to test this, the stripping procedure was repeated in each instance on the residue from the first stripping. An aliquot of the solution thus obtained was neutralized, oxidized and filtered as above. The filtrate was made 0·5N with sulphuric acid and tested with a 0·25% solution of diphenylcarbazide in equal parts of acetone and water. Only a pale-lilac colour was obtained, indicating that the residual chromium was negligible.

Discussion

Aluminium surfaces treated by the 'Alocrom' process at 90° F contain about 7·3 μg. of fluorine and 0·031 mg. of chromium, and 27 μg. of fluorine and 0·13 mg. of chromium, per square inch of surface, after immersion in the bath for 15 seconds and two minutes respectively. Aluminium treated by the shorter process is at present being used in the manufacture of internally lacquered cans for experimental purposes. The object of the work described in this paper was to find what quantities of fluorine and chromium might be introduced into foods packed in such cans.

The internal area of an A2 can is 61·5 sq. in. Such a can, therefore, made from aluminium treated by the shorter process, would have 0·45 mg. of fluorine and 1·9 mg. of chromium on its internal surface. This quantity of fluorine is equivalent to that in one pound of food or water containing 1 p.p.m. of fluorine, or in one-third of a pound of self-raising flour containing the recommended limit of 3 p.p.m.[5]

Any food-cans made from 'Alocrom'-treated aluminium will be lacquered internally because the whole aim of the treatment is to improve the adhesion of the lacquer. It is unlikely that a large proportion of the coating will be dissolved, and also unlikely that the whole of the fluorine in the contents will be ingested by one person. From a consideration of the amount of fluorine present, therefore, there appears to be little or no danger to health in the use of cans made from aluminium treated by the shorter process described above.

The quantity of chromium found is about four times as much as that of fluorine, but it is probably present as the tervalent cation and this is relatively harmless.[6]

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THE PROPERTIES OF THE ALGAL CHEMICALS. II.*—Some Derivatives of Laminarin

By W. A. P. BLACK and E. T. DEWAR

The solubilization of the cold-water-insoluble form of laminarin from *Laminaria cloustoni* frond has been investigated. The hydroxyethyl, hydroxylpropyl and carboxymethyl ethers of laminarin have been prepared in good yield, and solutions of these in water show no sign of precipitation after being set aside for several weeks. The benzyl ether is water-insoluble. Laminarin triacetate, tribenzoate and tricarbanilate have also been prepared; these esters are insoluble in water but soluble in a wide range of organic solvents. A series of laminarin sulphates, containing from 2·15 to 0·20 sulphate groups per glucose unit, have been prepared, and the more highly sulphated esters are water-soluble.

Introduction

Laminarin prepared from *Laminaria cloustoni* frond[1, 2] is insoluble in cold water, and this property has been found to have many disadvantages when attempting to find uses for this polysaccharide. The present investigation, therefore, has been directed chiefly at the problem of solubilizing laminarin, although several water-insoluble derivatives have also been prepared. A few compounds of laminarin have been prepared by previous workers in their investigations into the structure of laminarin, e.g. the triacetate and trimethyl ether have been prepared by Barry,[3] and Connell *et al.*[1] prepared a monobenzoate in addition. Because of the similarity in chemical composition between laminarin and starch, most of the methods used in this paper are modifications of those that have been applied successfully for preparing the derivatives of starch, and these have been reviewed by Degering.[4]

Experimental and discussion of results

The laminarin used in these investigations was the insoluble form extracted from *L. cloustoni* frond;[2] it contained 0·7% of ash and gave $[\alpha]_D^{16}$ − 12·7° in water (*c*, 2·134). All rotations were measured in a 2-dm. tube.

Effect of sodium hydroxide on laminarin

Laminarin (1·49 g.) was dissolved in water (19 ml.) at 70°, cooled, 2N-sodium hydroxide (15 ml.) was added, and the solution was set aside at room temperature for 6 hours; the solution slowly turned yellow but no precipitate was formed. Hydrochloric acid (2N) was then added to give pH 4 and the solution was stirred, when the typical white precipitate of insoluble laminarin was slowly deposited. Sodium hydroxide was, therefore, ineffective as a means of solubilizing laminarin. Recently, some doubt has been thrown on the stability of laminarin towards alkali by Corbett *et al.*,[5] who have shown that insoluble laminarin is degraded by lime-water to a monobasic acid and a residue that is resistant to attack by lime-water. We have found that laminarin is attacked by 0·1N-sodium hydroxide, and these results will be discussed later.

Oxidation of laminarin

(1) *With hypoiodite.*—The method of Blair & Reeves[6] for converting the terminal aldehydic glucose units in hydrocellulose into gluconic acid was employed. Laminarin (3·065 g.) was

* Part I: *J Sci. Fd Agric.*, 1952, **3**, 542

dissolved in water (30 ml.) at 70°, the cold solution was buffered to pH 10·6 by adding sodium carbonate (0·716 g.) and sodium bicarbonate (0·036 g.), and treated immediately with 0·25N-iodine (15 ml.) containing 40 g. of potassium iodide per litre. A precipitate began to form within an hour. After 24 hours at room temperature, the solution was acidified with 2N-hydrochloric acid, set aside overnight, and the oxidized laminarin A was centrifuged, washed with water (10 ml.), ethanol (3 × 70 ml.) and ether (70 ml.), and dried *in vacuo* over phosphorus pentoxide to a white powder (2·632 g.). Analytical results are given in Table I. Oxidation of the reducing groups in laminarin, therefore, did not induce solubilization.

(2) *With hypochlorite.*—An amount equivalent to 0·5 atom of available oxygen per glucose unit was employed initially. Laminarin (2·719 g. ; 16·78 mg. mol. of $C_6H_{10}O_5$) was dissolved in water (25 ml.) at 70°, the solution was cooled, 0·1N-sodium hydroxide (25 ml.) and 0·33N-sodium hypochlorite (50 ml., i.e. 8·25 mg. atoms of available oxygen) were added, and the solution was allowed to stand at room temperature in a stoppered flask for 2¼ hours, with occasional shaking. The specific rotation showed little change : $[\alpha]_D^{17}$ − 2·0° (initial) → − 2·6° (after 2 hours). The solution (pH 10·8) was then acidified with 2N-hydrochloric acid and dialysed for 2 days, when a precipitate was slowly deposited. The oxidized laminarin B was finally centrifuged and isolated, as above, as a white powder (1·203 g.). Analytical results are shown in Table I.

Table I

Analysis of oxidized laminarin

Fraction	Yield from laminarin, %	Ash, %	$[\alpha]_D^{16}$ in water (*c*, 2·0)
A	85·9	0·4	− 13·4°
B	44·2	0·4	− 13·3°

Craik[7] found that starch, on oxidation with hypochlorous acid for several days, was largely broken down into alcohol-soluble products which were strongly reducing.

In a second experiment, more drastic conditions were used in an attempt to effect solubilization. A quantity equivalent to 1·0 atom of available oxygen per glucose unit was employed, and the solution was heated at 40–45°. The reaction was followed polarimetrically by withdrawing 25 ml. of the solution at intervals, centrifuging to remove a small precipitate, and measuring the rotation. Solubility was then tested by acidifying the rotation solutions with 2N-hydrochloric acid, adding sufficient sodium sulphite ($Na_2SO_3,7H_2O$) to react with the chlorine liberated, and allowing the solutions to stand. The results are recorded in Table II.

Table II

Oxidation of laminarin with sodium hypochlorite at 40–45°

Time of oxidation, h.	$[\alpha]_D^{17}$ (*c*, 2·724)	Solubility on standing at room temperature
0·0	− 1·3°	—
1·0	− 4·2°	Copious white precipitate overnight
2·25	− 7·9°	Copious white precipitate overnight
4·0	− 16·3°	Copious white precipitate overnight
6·5	− 17·3°	Precipitation started after 2 days

These results show that laminarin is not readily solubilized by hypochlorite, and, as with starch,[7] the polysaccharide is probably undergoing considerable degradation.

Reduction of laminarin

Abdel-Akher *et al.*[8] have shown that insoluble laminarin from *L. cloustoni* can be transformed into a non-reducing polysaccharide, 'laminaritol', by reduction with 'sodium borohydride', a reagent that converts the aldehydic reducing group in sugars into the corresponding alcohols in aqueous solution. The following experiment, however, shows that reduction of laminarin did not induce solubilization.

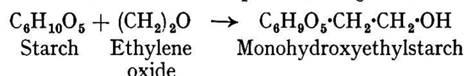
Laminarin (0·502 g.) was dissolved in water (25 ml.) at 70°, the solution was cooled, sodium borohydride (0·177 g.) added, and the solution set aside at room temperature for 47 hours. The alkaline solution was then acidified with 2N-acetic acid, and, on being set aside, a precipitate began to form. After 3 days, the insoluble laminaritol was centrifuged and isolated as a dull white powder. Yield, 84·5% ; $[\alpha]_D^{13}$ − 7·6° in water (*c*, 2·03).

These oxidation and reduction experiments indicate that laminarin cannot be solubilized by simply modifying the terminal reducing group in the chain, and results recorded below show that a major chemical reaction involving more or less every glucose unit is necessary to render the polysaccharide soluble.

Ethers of laminarin

Dr. A. G. Ross (unpublished work) has shown that laminarin can be solubilized by partial methylation with 40% sodium hydroxide and dimethyl sulphate, and the methyl ethers with a methoxyl content of more than 6% were soluble in water at a concentration of 6% to give solutions that were stable over a period of many weeks. In view of the rather excessive quantities of alkali required in this process, and of the need for dialysis to remove the large amount of sodium acetate after neutralization, the possibility of preparing other ethers of laminarin has been investigated.

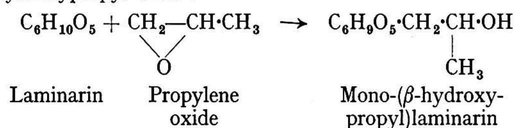
(1) *Hydroxyethyl-laminarin.*—Ziese[9] has shown that starch readily reacts with ethylene oxide in aqueous alkaline solution at room temperature to give the monoglycol ether:



Lamarin was found to react similarly: Laminarin (2·997 g.) was dissolved in water (38 ml.) at 70°, cooled, 2N-sodium hydroxide (30 ml.) and ethylene oxide (3·0 ml.) were added, and the solution was kept at room temperature in a stoppered flask for 6 hours with occasional shaking; after 2 hours, a further 1·5 ml. of ethylene oxide was added. The yellow solution was neutralized by passing it through a Zeo-Karb 225 column, the effluent was adjusted to pH 7·6 with 0·1N-sodium hydroxide, and evaporated *in vacuo* at 50° to 36 ml. Acetone was then added to 85% (v/v) concentration, and the hydroxyethyl-laminarin was centrifuged, washed with ethanol and ether, and dried *in vacuo* over phosphorus pentoxide to a white powder (3·367 g.). Yield, 88·3% (assuming a monohydroxyethyl ether); $[\alpha]_D^{17}$ — 11·2° in water (*c*, 2·018) [Found: ash, 0·4%; C, 45·1; H, 7·31%. These results from the elementary analysis did not correspond too well with a monoglycol ether (calc. for $C_6H_9O_5{\cdot}CH_2{\cdot}CH_2{\cdot}OH$: C, 46·6; H, 6·84%), but this may be due to the presence of a small amount of water that has not been removed on drying *in vacuo* over phosphorus pentoxide].

This product dissolved readily in cold water to give a clear solution, and a 6% solution gave no precipitate when set aside for 26 days. A quantity of 148 g. of this derivative has been prepared, and is now under test as a possible substitute for blood plasma. In the large-scale preparation, the bulk of the sodium hydroxide was neutralized with Amberlite resin IRC-50-H, which is a weak acid resin with a high capacity, and the last traces were removed with Zeo-Karb 225. The alkali can also be eliminated, after neutralization, by dialysis, but the yield is less by this method.

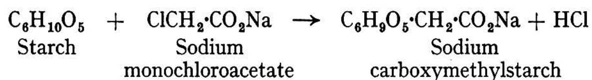
(2) *Hydroxypropyl-laminarin.*—Laminarin has also been successfully solubilized by conversion into the hydroxypropyl ether:



When laminarin (3·002 g.) was treated with sodium hydroxide and propylene oxide exactly as described under (1), hydroxypropyl-laminarin was isolated as a white powder (3·094 g.). Yield, 75·9% (assuming a monohydroxypropyl ether); $[\alpha]_D^{15}$ — 11·8° in water (*c*, 2·04). Found: ash, 0·4%. Again the results of elementary analysis did not correspond exactly with those for a monoether. The product dissolved in cold water to give a clear solution, and a 6% solution gave no precipitate after being set aside for 36 days. A quantity of 66 g. of this material has been prepared in a larger-scale experiment, and this derivative is now under test as a possible substitute for blood plasma.

One advantage of these hydroxyalkyl ethers is that the number of hydroxyl groups per glucose unit remains unaltered, so that these derivatives can presumably still form trimethyl ethers and triacetates in the same way as the original laminarin.

(3) *Sodium carboxymethyl-laminarin.*—Chowdhury[10] has shown that various polysaccharides, including starch, react with chloroacetic acid in strongly alkaline solution to give the carboxymethyl (or glycollate) ether:



$$C_6H_{10}O_5 + ClCH_2{\cdot}CO_2Na \rightarrow C_6H_9O_5{\cdot}CH_2{\cdot}CO_2Na + HCl$$

Starch — Sodium monochloroacetate — Sodium carboxymethylstarch

The conditions employed to give the monocarboxymethyl ether of starch were used for laminarin. The polysaccharide (2·973 g.) was dissolved in water (12 ml.) at 70°, cooled, 50% (w/w) sodium hydroxide (37 g.) was added slowly with shaking and cooling, followed by monochloroacetic acid (18·0 g.) added slowly with cooling, and the curdy, white mass was thoroughly shaken in a stoppered flask. After 19 hours at room temperature, the mixture was diluted to 200 ml., neutralized with glacial acetic acid, dialysed against running water for 4 days, and the solution was evaporated *in vacuo* at 50° to 45 ml. Saturated sodium chloride solution (0·5 ml.) was then added, followed by ethanol to 85% (v/v) concentration, and the sodium carboxymethyl-laminarin was centrifuged, washed with ethanol and ether, and dried *in vacuo* over phosphorus pentoxide to a dazzling white powder (4·084 g.). Found: ash (as Na_2SO_4), 27·6%, i.e. Na, 8·94%. This represents 0·91 sodium carboxymethyl grouping per glucose unit, and gives the formula weight of the repeating unit, $C_6H_9O_5(CH_2{\cdot}CO_2Na)_{0{\cdot}91}$, as 235. Yield, 94·7%; $[\alpha]_D^{20}$ − 7·0° in water (*c*, 2·004).

The material was readily soluble in cold water, giving a stable solution, and was insoluble in organic solvents. A 2% aqueous solution formed insoluble salts with copper sulphate, silver nitrate, lead acetate and aluminium sulphate solutions, but gave no precipitate with calcium, barium, magnesium or manganous chloride solutions.

Dr. A. G. Ross (unpublished work) has found, by using different amounts of chloroacetic acid, that products with a much lower carboxymethyl content can be prepared, and the material containing 0·22 sodium carboxymethyl grouping per glucose unit gives a 6% aqueous solution which is stable for at least 21 days. Less-substituted products are unstable and are precipitated from aqueous solution within a few days. Sodium carboxymethyl-laminarin containing 0·22 side chain per glucose unit has been tested as a substitute for blood plasma.

Unsuccessful attempts have been made to carboxymethylate laminarin with less alkali, as in the preparation of the hydroxyethyl and hydroxypropyl ethers. Thus when laminarin (2·974 g., i.e. 18·34 mg. mol. of $C_6H_{10}O_5$, in 10 ml. of water), 2N-sodium hydroxide (30 ml., 60 mg. mol.) and monochloroacetic acid (2·848 g., 30·14 mg. mol.) were allowed to stand at room temperature for 19 hours, and the solution was neutralized and dialysed, a white precipitate began to be deposited in the dialysis bag within a few hours. After 2 days, the precipitate was centrifuged and isolated in 70·7% yield.

(4) *Benzyl-laminarin.*—The benzyl ethers of various carbohydrates, including starch, have been prepared by Gomberg & Buchler,[11] although complete benzylation was not readily achieved in the presence of only a slight excess of benzyl chloride. The object of preparing benzyl-laminarin was not an attempt to effect solubilization, for a benzyl ether would be expected to be water-insoluble, but simply to characterize the derivative. More drastic conditions than those employed by Gomberg & Buchler[11] were used.

Laminarin (2·502 g.; 15·44 mg. mol. of $C_6H_{10}O_5$) was dissolved in 20% (w/w) sodium hydroxide (18·5 g.; 93 mg. mol.), benzyl chloride (11·7 g.; 92 mg. mol.) was added, and the mixture heated under reflux at 80–85° for 4 hours with occasional shaking. After 17 hours at room temperature with mechanical shaking, the excess of benzyl chloride was removed by steam distillation (20 minutes), the solution was diluted to 80 ml., centrifuged, and the gummy product washed thoroughly with hot water containing a small quantity of sodium chloride. During the washing process the gum gradually solidified, and the resulting solid was dried *in vacuo* over phosphorus pentoxide (1·295 g.). This was purified by solution in glacial acetic acid (100 ml.) and precipitation with ether (200 ml.), and the benzyl-laminarin was centrifuged, washed with ether until free from acetic acid, and dried at 105° for one hour to a white powder (1·146 g.). $[\alpha]_D^{16}$ − 16·0° in pyridine (*c*, 2·0) (Found: C, 55·7; H, 6·80%. Calc. for 0·5 benzyl group per glucose unit, $C_6H_{9{\cdot}5}O_5(CH_2{\cdot}C_6H_5)_{0{\cdot}5}$: C, 55·1; H, 6·33%).

The properties were very similar to those of benzylstarch.[11] Benzyl-laminarin was soluble in glacial acetic acid, pyridine and ethylene chlorohydrin, and insoluble in water, ether, ethanol, acetone, tetrahydrofuran, ethyl acetate, chloroform, benzene and light petroleum.

Esters of laminarin

(1) *Laminarin acetate.*—Acetyl-laminarin was first prepared by Barry,[3] who used both the sodium acetate/acetic anhydride procedure and the acetic acid/acetic anhydride/chlorine/sulphur dioxide method of Barnett. Connell *et al.*[1] dispersed the laminarin, which had been freshly precipitated by ethanol, in pyridine and acetylated with acetic anhydride in the cold, and this

method has been adopted in the experiments described below. Percival & Ross[12] have also used this method for acetylating soluble laminarin from *Laminaria digitata.*

As pointed out by Barry,[3] it is essential that the laminarin is in a finely divided state before acetylation by dissolving in water and precipitating with ethanol. Laminarin that has not been freshly precipitated gives a poor yield, as shown in the following experiment. Anhydrous laminarin (5·15 g.) was heated at 60° for 10 minutes with pyridine (52 ml.), cooled, and acetic anhydride (16 ml.) was added slowly with shaking; the polysaccharide swelled up but did not dissolve, and no heat was evolved. After 48 hours at room temperature the mixture was poured into water (500 ml.), and the acetate A was centrifuged, washed thoroughly with water (5 × 100 ml.), and dried *in vacuo* over phosphorus pentoxide to a white powder (3·19 g.). Yield (assuming a triacetate), 34·8%, $[\alpha]_D^9$ — 71·7° in chloroform (*c*, 1·018) (Found: Ac, 48·8%. Calc. for laminarin triacetate: $C_6H_7O_5Ac_3$: Ac, 44·8%). Acetyl content was determined by treating the acetate (0·1 g.) with A.R. methanol (10 ml.) and 0·1N-sodium hydroxide (25 ml.) at 60° for 20 minutes, in order to dissolve the acetate completely. After being set aside overnight in a stoppered flask, the solution was back-titrated with 0·1N-hydrochloric acid and phenolphthalein; a control was carried out simultaneously. Since the acetyl value was appreciably higher than the theoretical for the triacetyl derivative, a blank was run with laminarin itself; when 86·7 mg. was treated with methanol and sodium hydroxide exactly as described in the method, the consumption of alkali corresponded to 14·7% Ac or to an equivalent weight of 292. This result supports the findings of Corbett *et al.*,[5] who have found that laminarin is degraded by lime-water with the formation of a monobasic acid.

A good yield of triacetyl-laminarin was obtained in the following manner: Laminarin (2·434 g.) was dissolved in water (15 ml.), ethanol (200 ml.) was added, and the precipitate was centrifuged and washed thoroughly with ethanol and ether. The solid, still wet with ether, was heated with pyridine (33 ml.) at 60° for 10 minutes, cooled, and treated with acetic anhydride (10 ml.), when the solid dissolved completely to give an almost colourless solution. After being set aside for 48 hours at room temperature, when a certain amount of precipitation occurred, the mixture was poured into water, and acetate B was isolated as before, as a white powder (3·821 g.). Yield (assuming a triacetate), 88·3%. $[\alpha]_D^{17}$ — 63·6° in chloroform (*c*, 1·014) (Found: C, 49·3; H, 5·79; Ac, 47·4%. Calc. for $C_6H_7O_5Ac_3$: C, 50·0; H, 5·60; Ac, 44·8%).

Barry[3] obtained — 52°, and Connell *et al.*[1] — 60°, for the specific rotation of the triacetate in chloroform. Percival & Ross[12] found — 62° and — 65° for the acetates from insoluble and soluble laminarin respectively. Triacetyl-laminarin can be purified by solution in acetone and precipitation with light petroleum, but this treatment is not normally required. The product was soluble in chloroform, acetone, pyridine, ethyl acetate and glacial acetic acid; less soluble in benzene and tetrahydrofuran; and insoluble in water, ether, ethanol, carbon tetrachloride and light petroleum. The melting-point range was 190–220°.

A product with a lower acetyl content was obtained when the laminarin was dispersed in pyridine by the method employed by Pacsu & Mullen[13] for starch. The polysaccharide (5·01 g.) was dissolved in water (25 ml.), pyridine (150 ml.) was added, and the pyridine–water azeotrope distilled off at 50°/15 mm. until the volume of distillate was 125 ml., to eliminate the water; on being cooled, the solution gave a white gel. This was treated with pyridine (10 ml.) and acetic anhydride (15 ml.) to give a yellow solution, which was kept for 3 days at room temperature before being poured into water to yield acetate C as previously described (5·55 g.). $[\alpha]_D^9$ — 1·0° in chloroform (*c*, 1·01) (Found: Ac, 35·0%).

(2) *Laminarin benzoate.*—A monobenzoate of laminarin has been prepared by Connell *et al.*[1] by treating laminarin containing about 10% of moisture with pyridine and benzoyl chloride for 12 days at room temperature. Attempts have, therefore, been made to obtain a derivative with a higher benzoyl content. Preliminary experiments indicated that the conditions used for acetylation, particularly the reaction time, were unsuitable for benzoylation. The tribenzoate has been prepared as follows.

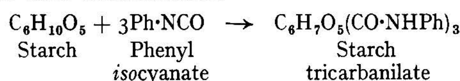
Laminarin (2·499 g.; 15·41 mg. mol. of $C_6H_{10}O_5$) was dissolved in water, precipitated with ethanol, and dispersed in pyridine (33 ml.) as described for acetate B. Benzoyl chloride (14·6 g.; 104 mg. mol.) was then added with cooling, when the solution turned pink and a precipitate formed. After 8 days at room temperature with mechanical shaking during 5 hours each day, the mixture was poured into water (250 ml.), when the benzoate separated as a dark red-brown oil. After being set aside overnight, the viscous syrup was centrifuged, stirred with hot water (3 × 150 ml.), and dried *in vacuo* over phosphorus pentoxide to a red-brown solid (6·952 g.). Yield, 95·0% assuming a tribenzoate (tribenzoyl derivative), $C_6H_7O_5Bz_3$, has been formed. The removal of colour from this crude product proved a difficult problem; solution in chloroform and precipitation with light petroleum removed very little colour, and fractionation of an acetone

solution with ether and then light petroleum was unsuccessful. Eventually, most of the colour was removed by dissolving the benzoate (6·537 g.) in acetone (130 ml.) and precipitating with water (160 ml.) containing a small quantity of sodium chloride to prevent the precipitate's becoming colloidal. This procedure was repeated three times, and the precipitate was finally dried *in vacuo* over phosphorus pentoxide to a cream-coloured powder (6·276 g.). Yield (from laminarin), 91·2%. $[\alpha]_D^{17}$ − 97·1° in chloroform (*c*, 1·01) (Found : C, 67·0 ; H, 4·78 ; Bz, 65·3%. Calc. for $C_6H_7O_5Bz_3$: C, 68·3 ; H, 4·67 ; Bz, 66·4%).

In determining the benzoyl content, tribenzoyl-laminarin was found to be unattacked by the conditions used for acetyl estimation. A slight modification of Mullen & Pacsu's method[14] for determining the acetyl value of starch acetate was employed. The benzoate (1 g.) was dissolved in acetone (25 ml.), 2N-sodium hydroxide (10 ml.) was added, and the solution was heated under reflux at 55–60° for one hour with frequent shaking. The brown solution was then diluted with water (500 ml.) and back-titrated with 0·5N-hydrochloric acid and phenolphthalein ; a control was also carried out. Even this drastic treatment sometimes failed to decompose the benzoate completely, and small particles of unchanged material were still visible.

Tribenzoyl-laminarin was soluble in chloroform, acetone, pyridine, benzene, tetrahydrofuran and ethyl acetate ; less soluble in glacial acetic acid ; and insoluble in water, ethanol, ether and light petroleum.

(3) *Laminarin carbanilate.*—Wolff & Rist[15] have shown that various polysaccharides, including corn and potato starch, glycogen and dextran, react with phenyl *iso*cyanate in the presence of pyridine to form tricarbanilates :



$$C_6H_{10}O_5 + 3Ph{\cdot}NCO \rightarrow C_6H_7O_5(CO{\cdot}NHPh)_3$$

Starch — Phenyl *iso*cyanate — Starch tricarbanilate

Laminarin readily formed a tricarbanilate in quantitative yield. Anhydrous laminarin (2·501 g.) was heated with pyridine (40 ml.) and phenyl *iso*cyanate (10 ml.) at 100° for 7½ hours with occasional stirring. After being set aside overnight at room temperature, the brown solution was centrifuged to remove a small residue, the supernatant liquid was poured into ethanol (200 ml.) to give a clear solution, and water (250 ml.) was added, when the ester appeared as a colloid. This was coagulated with saturated sodium chloride solution (6 ml.), and the precipitate was centrifuged and washed with water. The ester was purified by dissolving in acetone (50 ml.), adding ethanol (200 ml.) and precipitating with water (250 ml.) containing a small amount of sodium chloride. The tricarbanilate was centrifuged, washed with 50% (v/v) ethanol (2 × 100 ml.), and dried *in vacuo*, first over calcium chloride and finally over phosphorus pentoxide (8·219 g.). Yield (assuming a tricarbanilate), 103·5%. $[\alpha]_D^{20}$ − 6·9° in chloroform ; − 52·4° in pyridine (*c*, 1·01) ; m.p. 175–185° (without charring) (Found : ash, ± 0·0 ; C, 63·0 ; H, 5·30 ; N, 8·37%. Calc. for $C_6H_7O_5(CO{\cdot}NH{\cdot}Ph)_3$: C, 62·4 ; H, 4·85 ; N, 8·09%).

The product, which formed an almost white powder, was soluble in chloroform, pyridine, acetone, glacial acetic acid, ethyl acetate and tetrahydrofuran ; slightly soluble in ethanol ; and insoluble in water, ether, benzene, carbon tetrachloride and light petroleum.

(4) *Laminarin sulphate.*—Recently, Ricketts & Walton[16] have shown that low-molecular-weight dextran sulphates are active blood-anticoagulants. High-molecular-weight dextran was degraded by acid hydrolysis, and various dextran preparations containing a range of molecular weights were obtained by fractional precipitation. Dextran sulphate, with a molecular weight of about 7000, was found to be non-toxic, and maximum blood-anticoagulant activity was attained when the number of sulphate groups exceeded an average of 1·3 per glucose unit. It was considered that laminarin, in view of its low molecular weight, might prove successful as an anticoagulant, and consequently a series of laminarin sulphates have been prepared by the methods of Astrup *et al.*[17] and of Ricketts.[18] These are at present under test for their heparin activity. Sulphation is also an effective means of solubilizing insoluble laminarin, but these sulphates, in view of their possible anticoagulant activity, have not been investigated as substitutes for plasma.

By varying the proportion of chlorosulphonic acid in the reaction mixture, a series of laminarin sulphates have been prepared. A typical preparation is as follows : Chlorosulphonic acid (4·0 ml.) was added drop by drop to pyridine (40 ml.), which was cooled in ice–salt, and the mixture was then heated to 65°, when most of the white solid dissolved. Anhydrous, finely powdered laminarin (4·999 g.) was then added, and the mixture was heated at 65–70° for 30 minutes, with frequent stirring. After being cooled, the syrupy solution was dissolved in water (300 ml.), precipitated with ethanol (1000 ml.), and the viscous syrup centrifuged. The product was redissolved in water (150 ml.), neutralized to a phenolphthalein end-point with

2N-sodium hydroxide to decompose the pyridine salt, saturated sodium chloride solution (20 ml.) was added, and reprecipitation was carried out with ethanol (400 ml.). The syrup was dissolved in water (180 ml.), dialysed against running water until free from sulphate (2 days), and the solution was adjusted to pH 7·6 with sodium hydroxide and then concentrated *in vacuo* at 45° to 60 ml. Ethanol was added to give a concentration of 85% (v/v), and the precipitated syrup was centrifuged, washed with ethanol and ether, ground to a white powder, and dried *in vacuo* over phosphorus pentoxide (8·030 g.) [Found: ash (as Na_2SO_4), 32·3; i.e. SO_4, 21·8; total sulphate on hydrolysis (as SO_4), 43·5%].

The sulphate in the ash should be exactly one-half of the total sulphate for a polysaccharide sulphuric ester, for one-half of the sulphur is lost during ignition:

$$2(C_6H_9O_5{\cdot}SO_3Na) + 4H_2O \xrightarrow[\text{hydrolysis}]{\text{acid}} 2C_6H_{12}O_6 + H_2SO_4 + Na_2SO_4$$

Sodium laminarin sulphate — Glucose

$$2(C_6H_9O_5{\cdot}SO_3Na) \xrightarrow[\text{ashing}]{} Na_2SO_4 + SO_3\nearrow + \text{products of combustion}$$

The number of sulphate groups per glucose unit is calculated from the total sulphate content as follows:

$$\text{Let the repeating unit} = C_6H_{10-n}O_5(SO_3Na)_n$$

where n = number of sulphate groups per glucose unit.

Then the weight of repeating unit $= 162 - n + 103n = 162 + 102n$

and the total sulphate (as SO_4) $= \dfrac{96 \times n \times 100}{162 + 102n} = 43{\cdot}5$

Therefore $n = 1{\cdot}37$, and the weight of repeating unit = 302.

$$\%\ \text{Yield} = \frac{8{\cdot}030 \times 162 \times 100}{4{\cdot}999 \times 302} = 86{\cdot}2$$

A series of laminarin sulphates, with varying sulphate contents, have been prepared, and the results are recorded in Table III.

Table III

Analysis of various sodium laminarin sulphates

Laminarin sulphate	Chloro-sulphonic acid per 5 g. of laminarin, ml.	Reaction time at 65–70°, min.	Ash (as Na_2SO_4), %	Sulphate in ash (as SO_4), %*	Total sulphate on hydrolysis (as SO_4), %	No. of sulphate groups per glucose unit	Yield, %	Stability of 6% aqueous solution	$[\alpha]_D^{18}$ in water (c, 2·0)
A	8·8	75	41·6	28·1	54·1	2·15	99·4	no pptn. after 32 days	− 9·8°
B	6·0	30	41·7	28·2	52·9	2·04	104·6	—	—
C	4·0	30	32·3	21·8	43·5	1·37	86·2	no pptn. after 32 days	− 3·7°
D	2·0	30	19·6	13·2	26·1	0·61	87·1	18·2% pptd. after 11 days	− 8·2°
E	1·0	15	7·4	5·0	10·5	0·20	78·2	47·7% pptd. after 6 days	—

* Calculated from the ash

The results show that, for most samples, the total sulphate is very nearly twice the sulphate in the ash. Sulphates A, B, C and D are being tested as anticoagulants (patent applied for).

It is interesting to note that the insoluble material from sample D, which was precipitated from 6% aqueous solution after 11 days, contained an ash content of only 4·9%, although the ash in the original product was 19·6%. This suggests that sulphation is a random process

giving a series of molecules of widely varying sulphate content, and the calculated number of sulphate groups is merely an average; molecules with least sulphate attached have been precipitated from solution.

Summary

The solubilization of the cold-water-insoluble form of laminarin from *Laminaria cloustoni* frond has been investigated. Laminarin cannot be rendered soluble by modification of the terminal reducing groups at the end of the chains, either by oxidation with hypoiodite or hypochlorite or by reduction with sodium borohydride.

Various ethers of laminarin, however, have been prepared, aqueous solutions of which show no sign of precipitation on being set aside at room temperature for a period of several weeks. Hydroxyethyl- and hydroxypropyl-laminarin have been obtained in 88·3% and 75·9% yields by treating an alkaline solution of the polysaccharide with ethylene and propylene oxides respectively. Both derivatives are ash-free, like the original polysaccharide, the specific rotations are very little different from that of laminarin, and, being ethers, they should be just as stable to the action of acids and alkalis as laminarin itself. These are being tested as possible substitutes for blood plasma. Sodium carboxymethyl-laminarin, containing almost one carboxymethyl group per glucose unit, has been prepared in 94·7% yield. Its aqueous solution is stable at room temperature, but yields insoluble copper, silver, lead and aluminium salts. A water-insoluble benzyl ether, containing 0·5 benzyl groups per glucose unit, has also been prepared.

A number of esters of insoluble laminarin have been prepared, most of which are water-insoluble. Triacetyl-laminarin, $[\alpha]_D^{17}$ − 63·6° in chloroform, has been obtained in 88·3% yield by treating the freshly precipitated polysaccharide with acetic anhydride and pyridine in the cold for 2 days. An acetate with a lower acetyl content, $[\alpha]_D^{9}$ − 1·0° in chloroform, was obtained when the laminarin was dispersed in pyridine by the Pacsu & Mullen method.[13] Benzoyl chloride and pyridine, after 8 days at room temperature, gave the tribenzoyl ester in 91·2% yield, and phenyl *iso*cyanate and pyridine at 100° for 7½ hours gave the tricarbanilate in quantitative yield. Laminarin triacetate, tribenzoate and tricarbanilate are insoluble in water, but are soluble in a wide range of organic solvents.

Laminarin has been sulphated with chlorosulphonic acid and pyridine, and a series of sulphates, containing from 2·15 to 0·20 sulphate groups per glucose unit, have been prepared. Those containing 2·15 and 1·37 sulphate groups per glucose unit gave 6% aqueous solutions which showed no sign of precipitation after being set aside for 32 days. It was considered that laminarin sulphate might possess anticoagulant properties similar to those of dextran sulphate and other sulphated polysaccharides, and these esters are being tested for heparin activity.

Acknowledgment

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Inveresk
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SOIL FUMIGATION. I.—The Sorption of Ethylene Dibromide by Soils*

By PETER WADE

Sorption of ethylene dibromide by soils is rapid, the greater part of the total amount sorbed being taken up within half an hour. Equilibrium was established by the end of 24 hours. Sorption isotherms on the air-dried and moist soils have been plotted. Over the usual field range of moisture content the amount of ethylene dibromide held by the soil remains constant ; the total amount sorbed increases slightly with increasing moisture content owing to solution of the fumigant in the soil water. In this range of moisture content the amount sorbed is proportional to the content of organic carbon of the soils. Very little evidence for any irreversible sorption or decomposition of ethylene dibromide by the soils has been found over the range 10–25°.

Introduction

The use of toxic volatile chemicals as soil fumigants for the large-scale control of various plant parasites is becoming of increasing economic importance, particularly since the introduction of relatively cheap fumigants. An extensive literature on the results of field trials of various fumigants has accumulated, but relatively few investigations of the soil–fumigant relationship have been made.

The amount of a fumigant that must be introduced into the soil in order to produce a lethal concentration in the intergranular space depends upon the proportion of fumigant rendered unavailable by sorption by the soil constituents. The general term sorption is here used to include adsorption (reversible and irreversible), absorption, solution of the fumigant in the soil water, and biological decomposition by the soil micro-organisms. Measurements of the sorption of various fumigants on soils have been published,[1–7] and Clegg[8] and Pauwels[9] have determined the decomposition of carbon disulphide in soils, but no systematic study of factors affecting the sorption of a fumigant by soils at moisture contents in the field range appears to have been made.

The fumigant used in the present investigation, ethylene dibromide, is one of the more recently introduced. It was chosen because it is readily available in a substantially pure form, and it seemed probable that it could be determined conveniently by a modification of the method developed in this Laboratory by Lubatti & Harrison,[10] for the determination of methyl bromide.

Soils used in the investigation

Samples of three types of soil were obtained for the investigation. The first was a sandy soil taken from the grounds of the Field Station and known as ' Ashurst Lodge '. The second was a soil with a high content of organic matter, known as ' Black Fen ', and the third was a Hertfordshire clay with 10% of added sand, known as ' Bones Close '. The soils were first dried on open trays for one week (the clay when received was already very dry) and then screened through a 2-mm.-mesh sieve. The material passing the sieve was retained and stored in dust-bins, which had been rustproofed by coating with aluminium paint. The soils were characterized by various chemical and physical analyses, the results of which are given in Table I. In order to be able to compare the soils at corresponding moisture contents a study of the soil-moisture relationships of the three soils was made by using the pF scale[11] to express the state of the soil water.

Methods

Determination of ethylene dibromide

The ethylene dibromide used in the investigation was a laboratory sample, which was purified by washing with sodium bicarbonate solution and water. The sample was then dried over anhydrous sodium sulphate and distilled twice, and the fraction boiling at 130·5–131·1° during the second distillation was retained.

Ethylene dibromide was determined by the catalytic thermal decomposition procedure described by Lubatti & Harrison[10] for the determination of methyl bromide. The fumigant is drawn in a stream of purified air through a train consisting of two furnaces and bubblers connected in series ; the bubblers contain a mixture of sodium hydroxide solution and hydrogen peroxide to convert the decomposition products into sodium bromide. The sodium bromide

* Part of a thesis approved for the degree of Ph.D., University of London

Table I

Analysis of the soil samples

	Ashurst Lodge	Black Fen	Bones Close	Method of analysis
	Percentage on dry weight			
Chemical analysis				
Calcium carbonate	0·00 0·00	0·05 0·03	0·47 0·45	Hutchinson & MacLennan[18]
Organic carbon	1·97 2·03 1·96	12·65 12·38 12·73	1·15 1·03 1·13	Walkley–Black titration[19]
Nitrogen	0·223 0·225	1·319 1·313	0·150 0·152	Kjeldahl[20]
Base-exchange capacity, milliequiv./100 g. of dry soil	11·3 11·4	36·0 33·3	14·6 14·2	Copper acetate method[22]
pH	6·00 6·00	4·62 4·62	7·93 7·92	Glass–calomel electrode[23]
Mechanical analysis				
Silt	16·5 16·6	37·7 37·0	25·4 26·0	Hydrometer[21]
Clay	5·0 5·2	3·8 3·8	26·4 25·0	Hydrometer[21]
Total sand	80·55 80·17	57·54 58·83	52·94 52·70	Sedimentation in water[20]
Fine sand	57·41 57·41	31·85 33·59	44·38 43·60	70-mesh sieve[20]

was determined by potentiometric titration with silver nitrate[12] after decomposition of the excess of peroxide by boiling.

The design of the fumigation units used in the present work is shown in Fig. 1. The mean volume of the twelve units used was 1270 ml. A correction for the volume occupied by the soil particles was made by measuring this volume by the manometric method of Jones.[13] The liquid fumigant was measured into the units by means of a micrometer syringe fitted with a 20-cm. steel needle. The needle was passed into the units *via* the straight-bore tap. The rate of evaporation of the fumigant was increased by dosing on to a strip of filter paper looped from the hooks at the lower end of the tube carrying the straight-bore tap. It was found by direct weighing that amounts of liquid ethylene dibromide of 43·2 mg. (100 divisions on the micrometer head) and 15·3 mg. (35 divisions) could be measured with coefficients of variation of 0·46 and 2·48% respectively. (Each coefficient was calculated from 12 weighings.) The calibration was found to be constant over the range 10–23°.

Units containing measured amounts of ethylene dibromide were connected to the decomposition apparatus and a stream of purified air was drawn through the system at the rate of 1 litre per minute for one hour. With amounts of ethylene dibromide of 49·7 mg., a mean recovery of 99·1% was obtained, with a coefficient of variation of 0·37%. With amounts of 15·3 mg. of fumigant, a mean recovery of 99·3%, with a coefficient of variation of 1·80%, was obtained.

The vapour-phase concentration of ethylene dibromide inside the units was determined by withdrawing samples of the vapour into evacuated 80-ml. sampling tubes (Fig. 2). The tubes were then connected to the thermal decomposition apparatus and

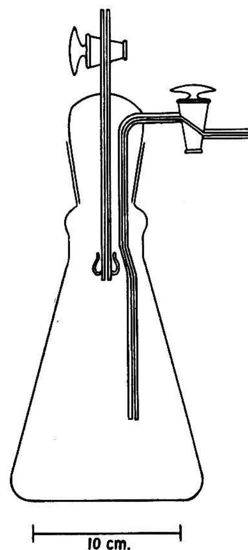

FIG. 1.—*Design of the fumigation units*

aerated at a flow rate of 250 ml./minute for 15 minutes. Under these conditions the recovery of fumigant from the sampling tubes was incomplete owing to the slow rate of desorption of traces sorbed by the walls of the tube. The last traces were recovered only by aerating for 1½–2 hours at room temperature or for 15 minutes with the tube immersed in boiling water. (An increase in the flow rate had little effect on the rate of recovery.) Neither of these procedures was considered suitable for routine use, and since the reproducibility (as measured by the coefficient of variation) was satisfactory with the original conditions (1·44% at a concentration of 39 mg./l., and 1·78% at a concentration of 12 mg./l.) a correction factor of 1·053 was applied to the results obtained under these conditions. The amount of fumigant sorbed by the walls of the fumigation units was negligible compared with the amounts sorbed by the soils.

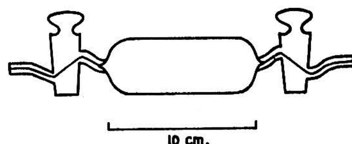

FIG. 2.—*Design of the sampling tubes*

Recovery of ethylene dibromide from fumigated soil

To determine the amount of fumigant irreversibly sorbed or decomposed in the soil it is necessary to recover and determine the unchanged fumigant. This recovery and determination was accomplished by a two-stage procedure.

After the samples of vapour had been withdrawn, the unit was connected to the thermal-decomposition apparatus and a stream of purified air was drawn through over the soil at a rate of 1 l./minute for one hour. At the end of this period, 99% of the ethylene dibromide in the vapour phase of the unit, together with that most readily desorbed from the soil, was recovered.

The residual fumigant was recovered by 'wet aeration' (cf. Higgins & Pollard[14] and Lubatti & Smith[5]). The soil was transferred, with washings, to a 2-l. bolt-necked flask. The washings were made up to one litre and the flask was closed with a bung carrying a reflux condenser and an entry tube reaching almost to the bottom of the flask. [The loss of fumigant by desorption during the transfer of the soil, which took 1¼ minutes, was determined, for the air-dried soils, by plotting the desorption curves (Fig. 3). These curves were obtained by prolonged aeration of 100 g. of the air-dried soils, which had been fumigated with 49·7 mg. of ethylene dibromide for two hours at 20°; the bubblers in which the decomposition products of the fumigant were absorbed were changed from time to time during the aeration. The rate of desorption after one hour was found to be 0·1–0·2 mg./minute. The loss on transfer of the soils is therefore a very small fraction of the original dose.] The suspension was heated on a glycerol–water bath boiling at 105° and the liberated fumigant removed in a current of air drawn through the suspension at the rate of 1 l./minute. The fumigant was determined as before.

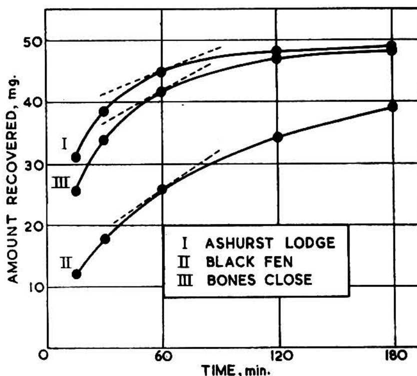

FIG. 3.—*Rate of desorption of ethylene dibromide from the air-dried soils*

The recovery of known amounts of ethylene dibromide added to one litre of water, and to suspensions of 100 g. of the air-dried soils in one litre of water, was measured during periods of up to three hours (Table II). Recoveries of 97–98% were obtained after one hour's aeration, and no significant increase in recovery was obtained by prolonging the aeration.

The sorption of ethylene dibromide by a sample of soil

A quantity of 100 g. of the sample was weighed into a fumigation unit and the unit placed in a constant-temperature room. (Unless otherwise stated all experiments were carried out at 20°.) A measured amount of fumigant was then added. After the required period of time had elapsed two samples of the vapour phase were withdrawn in succession into sampling tubes; the amount of ethylene dibromide in the tubes was determined as described above. The

Table II

Recovery of ethylene dibromide by 'wet aeration'

Dose, mg.	Period of aeration, min.	Amount recovered, mg.	Recovery, %
1 litre of water			
49·7	15	46·4	93·4
	30	48·2	97·0
	45	48·3	97·2
	60	48·4	97·4
	60	48·3	97·2
	180	49·3	99·2
15·3	60	14·9	97·4
	180	14·9	97·4
100 g. of Ashurst Lodge soil + 1 litre of water			
49·7	15	33·6	67·6
	30	48·1	96·8
	45	48·6	97·8
	60	48·7	98·0
	180	48·5	97·6
100 g. of Black Fen soil + 1 litre of water			
49·7	15	35·8	72·0
	30	48·4	97·4
	45	48·8	98·2
	60	48·9	98·4
	180	48·8	98·2
100 g. of Bones Close soil + 1 litre of water			
49·7	15	38·4	77·3
	30	48·1	96·8
	45	48·3	97·2
	60	48·4	97·4
	180	49·0	98·6

difference between the amount of fumigant found in the vapour phase and that originally added was taken as the total amount sorbed by the soil.

The unit and soil were then aerated dry for one hour, the soil was transferred to the wet-aeration apparatus, and the aeration was continued for a further period of one hour. The difference between the total amount of fumigant recovered in each of the above processes and the amount originally added was taken as the amount irreversibly sorbed or decomposed by the soil. Since different amounts of fumigant are recovered in each stage of the analysis, according to the type of soil and the experimental conditions, it is not possible to calculate an over-all experimental error for the method. It is possible, however, to calculate the maximum error in individual instances (see below).

Results

The rate of sorption of ethylene dibromide by soils

Experiments were carried out on each soil in the air-dried condition and at a moisture content corresponding approximately to the field capacity (p*F*, 2·7).[15] A series of six measurements extending over a period of one week were made on each sample, the units being dosed with 49·7 mg. of ethylene dibromide. When the period of exposure was 0·5 and 1 hour, the amount of fumigant in the vapour phase only was determined.

The initial concentration in each unit if the fumigant vaporized completely before sorption began would be approximately 40 mg./l. The results were corrected by simple proportion to give the amounts sorbed corresponding to a concentration of exactly 40 mg./l. This form of correction assumes a linear relationship between the initial (theoretical) concentration and the amount sorbed, but even if this assumption is not strictly true the error introduced is small. The calculated figures for the amounts of fumigant sorbed are only valid for the conditions of the experiment, but they serve to show the rate at which the fumigant is sorbed by the soils.

The curve obtained for the air-dried Bones Close soil is given in Fig. 4. Similar curves were obtained for each of the other soils. In each instance the initial rate of sorption was high, the greater part of the total sorption having taken place within the first 30 minutes. It is possible that the controlling factor during the early part of this period was the rate of vaporization of the fumigant. In a fumigation unit not containing soil, 49·7 mg. of liquid ethylene dibromide was completely vaporized in 15 to 20 minutes, as measured by the rate of contraction of a sensitive spring carrying a filter-paper strip dosed with fumigant. Little change in the amounts sorbed occurred after 24 hours, and, for the remainder of the investigation, equilibrium was supposed to have been established 24 hours after dosing.

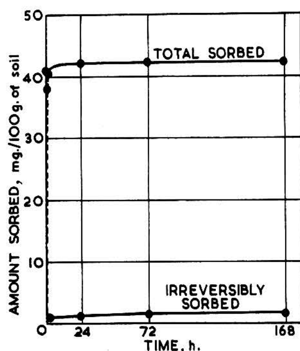

Fig. 4.—*Rate of sorption of ethylene dibromide by air-dried Bones Close soil*

The amounts sorbed varied with the type of soil and with the moisture content.

The proportions of fumigant not recovered were small but increased slightly with time. Table III shows the amounts of fumigant not recovered from the air-dried soils after one week compared with the calculated maximum experimental errors. The difference between the figures is in each instance small, and shows there can be very little, if any, irreversible sorption.

Table III

Soil	Amount of fumigant not recovered from air-dried soil after one week, %	Calculated experimental error, %
Ashurst Lodge	5·2	3·2 ± 0·5
Black Fen	5·8	2·8 ± 0·5
Bones Close	2·8	2·8 ± 0·5

Isotherms of the sorption of ethylene dibromide on soils

The sorption isotherms of ethylene dibromide were determined for each of the soil samples used in the previous experiment. The fumigations were carried out at 20° for 24 hours. The doses of fumigant were adjusted to give a range of equilibrium concentrations up to about 50 mg./l., about half the saturation concentration. The results, calculated as mg. sorbed per 100 g. of dry soil and plotted against the equilibrium concentrations, are shown in Fig. 5. (The moisture contents are calculated as a percentage of the oven-dry weight.) The curves, with the exception of the curve for the air-dried Bones Close soil, are linear over the greater part of the concentration range. The curve for the air-dried Bones Close soil is of Type III in the B.D.D.T. (Brunauer, Deming, Deming & Teller) classification,[16] as is a curve obtained by Stark[7] for chloropicrin on silt. It was noted that these soil samples had similar p*F* values, Bones Close 5·9 and silt 6·0; the other two air-dried soils had lower values, Ashurst Lodge 4·5, and Black Fen 4·3.

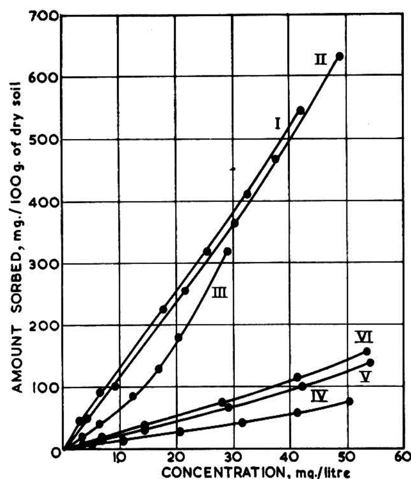

Fig. 5.—*Sorption isotherms of ethylene dibromide on the three soils*

Curve number	Soil	Moisture content, %
I	Black Fen	32·2
II	Black Fen	58·7
III	Bones Close	3·1
IV	Bones Close	15·9
V	Ashurst Lodge	4·4
VI	Ashurst Lodge	15·4

The recoveries of fumigant in these experiments, not shown in Fig. 5, also provided little evidence of irreversible sorption.

The effect of the moisture content of the soils on the amount of ethylene dibromide sorbed

A range of samples of different moisture content were prepared from each of the three soils by adding varying amounts of water from a spray gun to samples of the soils that had been dried in a current of air at 32° for 24 to 48 hours. In order to discover if the amount of fumigant sorbed depended upon whether the soil was wetting or drying, a range of samples of the Black Fen and Ashurst Lodge soils in the drying condition were prepared by drying in a current of air at 32° samples that had been soaked in water for 48 hours and drained at the pump, until the required loss in weight had occurred.

The fumigations were carried out in the usual manner, the doses being chosen to give equilibrium concentrations in the region of 10 mg./l. over as great a part of the range of moisture contents as possible. The results were calculated as the number of mg. of fumigant sorbed per 100 g. of dry soil from a constant concentration in air of 10 mg./l. Duplicate determinations were carried out, and the mean of the duplicates was used in plotting the results. When expressed in this manner all three soils gave similarly shaped curves. The curves are at first linear, and represent a sharp drop in the amount of fumigant sorbed for an initial small increase in moisture content. The rate of fall becomes less steep as the moisture content continues to increase, passes through a minimum, and then becomes linear again, the amount sorbed rising slightly with increasing moisture content. The curve obtained for the Black Fen soil is shown in Fig. 6. These curves bear some resemblance to the curve obtained by Lubatti & Smith[5] for the effect of moisture content on the amount of methyl bromide sorbed by a soil. Strict comparison with this curve is not, however, possible, since equilibrium between the methyl bromide and the soil could not be established.

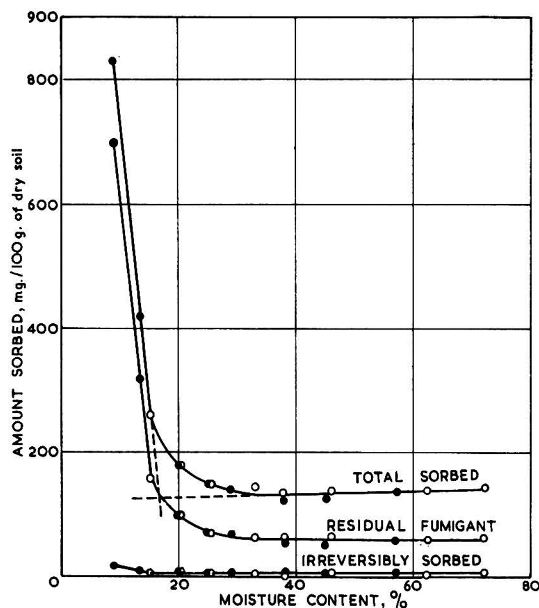

FIG. 6.—*Effect of moisture content of Black Fen soil on the amount of ethylene dibromide sorbed*

● Samples in drying condition
○ Samples in wetting condition

The points for both the wetting and the drying samples were found to lie on the same curve. No correlation was found between the moisture contents corresponding to the minimum amounts of fumigant sorbed (Fig. 6) and the p*F* values, but when taken on the drying branch of p*F*–moisture curves these moisture contents were observed to be in the region of p*F* 4·2,

the permanent wilting-point of the soils.[15] Since soils in this country are unlikely to dry below this point, particularly in autumn when fumigations are usually carried out, the second linear part of the moisture content–sorption curves can be taken to correspond with the field range of moisture contents. Over this part of the curves it was noticed that, whereas the total amount of fumigant sorbed increased slightly with increasing moisture content, the amount of residual fumigant recovered during the wet aeration process remained practically constant. This observation suggested that the increase in the total amount sorbed was caused by solution of the fumigant in the increasing amount of soil water. The solubility of ethylene dibromide, calculated from the slopes of the curves for the total sorption, was : Ashurst Lodge, 50 mg./100 ml. ; Black Fen, 56 mg./100 ml. ; and Bones Close, 38 mg./100 ml.

The solubility of ethylene dibromide in distilled water, in equilibrium with a vapour-phase concentration of 10 mg./l. at 20° was determined and found to be 43·1 mg./100 ml. The agreement between these figures is sufficiently close to support the suggestion that, over the field range of moisture content, the amount of fumigant held by the soil remains constant ; the total amount sorbed increases slightly with increasing moisture content owing to solution in the soil water.

The amounts of fumigant sorbed by soils of different types vary widely. Taken at corresponding moisture contents in the field range (p*F* 3, wetting) the order of increasing amounts sorbed is Bones Close, Ashurst Lodge, Black Fen, with the ratio of 1 : 2 : 10·8. This ratio agrees well with the ratio of increasing content of organic carbon of the soils, since the order of the soils is the same and the value of the carbon ratio is 1 : 1·8 : 11·8. The order of increasing clay content of the soils is Ashurst Lodge, Black Fen, Bones Close. It appears therefore that, over the field range of moisture content, the factor governing the amount of fumigant sorbed by a soil is its content of organic carbon.

Again, little evidence for irreversible sorption of the fumigant was found, since the slight rise observed at low moisture contents (see Fig. 6) was caused by the relatively large factor required to correct the results to an equilibrium concentration of 10 mg./l.

The effect of temperature on the amount of ethylene dibromide sorbed by soils

The amount of ethylene dibromide sorbed by the three soils was measured at two moisture contents over the range 10–25°. The fumigations were carried out in the usual manner, and the results were calculated as the amount of fumigant sorbed per 100 g. of dry soil from a constant concentration of 10 mg./l. The results obtained for the moist Ashurst Lodge sample are plotted in Fig. 7.

In each instance the curves were found to have pronounced negative slopes. This is as expected since sorption is an exothermic process. For the purposes of comparison the slope of each curve at 20° was calculated and expressed as a percentage of the amount sorbed at that temperature. The figures obtained are given in Table IV. In each instance the temperature coefficient of sorption for the drier sample is seen to be somewhat higher than that for the wetter.

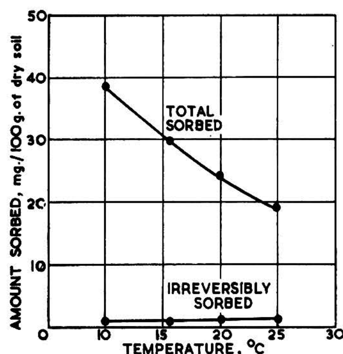

Fig. 7.—*Effect of temperature on the amount of ethylene dibromide sorbed by Ashurst Lodge soil of moisture content* 10·84%

The amounts of fumigant not recovered were again small and no systematic variation with temperature was observed.

Discussion

The establishment of an equilibrium between the ethylene dibromide in the vapour phase and that sorbed by the soil samples is in contrast with the results obtained by Lubatti & Smith[5] with methyl bromide. They found that sorption was most rapid during the first two hours, and that the amount sorbed afterwards increased linearly with time for periods up to one week ; this agreed with their observation of continued decomposition of the fumigant. Clegg[8] and Pauwels[9] have recorded progressive decomposition of carbon disulphide in soils but made no measurements of the amounts sorbed.

The near linearity of the isotherms obtained in five of the samples is again in contrast with the pronounced sigmoid isotherms obtained by Shipinov[4] with hydrogen cyanide, and by Stark[7]

Table IV

Soil	Moisture content, %	Temperature coefficient, % per degree at 20°
Ashurst Lodge	2·5	− 6·4
	10·8	− 4·9
Black Fen	40·6	− 5·1
	56·9	− 4·6
Bones Close	3·1	− 7·1
	16·0	− 4·9

with chloropricrin on dry soils. The higher p*F* of the sample of Bones Close with the lower moisture content, and the similarity of this curve to those obtained by Stark[7] at relative humidities of 50–90%, have already been mentioned.

The sharp change in direction of the moisture content–sorption curves as the moisture content increases from a low value suggests there are two different methods of retention of the fumigant at different parts of the range of moisture content. The initial sharp drop in the amount sorbed may be caused by a weakening of the forces binding the fumigant molecules as the surface of the soil particles becomes covered with an increasing number of layers of water molecules. The slight rise in the amount sorbed with increasing moisture content, in the second part of the curve, which has been shown to correspond to the solution of the fumigant in the soil water, suggests that, in this range of moisture content, the equilibrium between the fumigant in the vapour phase and in the soil may be made up of two equilibria; one between the fumigant in the vapour phase and that in solution, and the other between the fumigant in solution and that sorbed on the soil particles.

The change from sorption from the vapour phase to sorption from solution does not appear to occur at any critical p*F* value of the soils, but the first part of the curve requires further definition before the point of intersection of the two linear parts of the curve can be determined with accuracy. The correlation between the amounts of fumigant sorbed by the soils at moisture contents in the field range, and the content of organic carbon of the soils, is of practical importance. Stark[7] found a close correlation between the amounts of chloropicrin sorbed by a number of dry soils and their content of clay, but in this investigation wet clay was found to have little sorptive power for ethylene dibromide.

The marked effect of temperature on the amount of fumigant sorbed by the soils, of the order of 5% per degree at 20° C, may be a contributary cause of the result obtained by McClellan, Christie & Horn,[17] who found that, under otherwise similar conditions, ethylene dibromide was as efficient against *Heterodera marioni* in soil in one day at 98° F as in three days at 72° F.

The small amounts of ethylene dibromide found to be sorbed irreversibly during the present investigation do not rule out the possibility of decomposition of the fumigant in field soils. Clegg[8] and Pauwels[9] have found evidence that the decomposition of carbon disulphide is at least partly biological in origin. The power of a soil to decompose carbon disulphide decreased on storage, and in some instances repeated fumigation of a soil increased its power of decomposing this fumigant.

It is hoped to make a more detailed study of the decomposition of ethylene dibromide in soils, and to extend the measurements of the sorption of this fumigant to soils at low moisture contents, and to a wider variety of soil types.

Acknowledgments

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THE FIELD FUMIGATION OF GROUNDNUTS IN BULK

By L. A. W. HAYWARD

Sacks of decorticated groundnuts stored in two types of open-air stacks in Kano, Northern Nigeria, were fumigated with methyl bromide under gas-proof sheets to control *Trogoderma granarium* Everts. The distribution of the gas during fumigation was investigated.

Introduction

Storage of bagged decorticated groundnuts in Kano, Northern Nigeria, has been necessary on a large scale in recent years owing to the slow rate of transport by rail to port. The nuts are stored along the rail sidings in warehouses and in the open. It became customary, during the war years and after, to store nuts in the open in square-based pyramids of about 750 tons (9000 bags). These pyramids were made on specially constructed raised plinths, which were surrounded by a small trench as a protection against flooding, and were covered with groundnut husks or cinders, or both, to serve as dunnage and a termite deterrent. Overlapping tarpaulins were secured over these stows before the beginning of the wet season. A small number of pyramids were constructed on oblong rectangular bases, and consequently had a horizontal ridge apex instead of a point. These contained up to 2000 tons of groundnuts.

The storage problem of the 1950–51 crop was not so acute and, in general, the more permanent type of outside storage described above was not used. Storage in the open was on pyramid plinths, but flat-topped dumps were used, as at that time removal was possible before the rains were due to start. These dumps were oblong or square and contained up to 350 tons of groundnuts. As a result of prolonged storage, many dumps and pyramids became seriously infested with *Trogoderma granarium*,[1] and experiments were undertaken to determine whether they could be disinfested *in situ* by fumigation under gas-proof sheets.

Preliminary tests, with separate overlapping sheets placed on the pyramid and sealed together by a sprayed-plastic process, sometimes called 'cocooning', suggested that the method might be successful; more detailed trials were therefore undertaken, with pyramids specially built for the purpose, with gas-sampling tubes placed in position during the course of construction. A one-piece gas-proof sheet, large enough to cover a complete pyramid, was manufactured and used in the trials, the results of which are given in this paper.

Experimental

Pyramid.—In February, 1951, a square-based pyramid was made and gas-sampling capillary pipes were built into it during construction. The pyramid dimensions were : base, 50 ft. square ; height of vertical side, 7 ft., plus 37 ft. sloping to the apex ; volume approximately 29,000 cu. ft. ; weight prepared for fumigation, 730 tons.

It was arranged for the gas to be administered at the summit *via* two jets connected by a copper pipe to a gas cylinder on scales near the pyramid. Some minor restacking was carried out near the summit to provide a space to assist gas circulation.

Some initial difficulty was encountered in placing the one-piece cover over the pyramid. After this had been accomplished the edge of the cover was carefully sealed by an unbroken line of long narrow sand-bags. The gas-sampling capillary-lines and the copper pipe from the gas cylinder were led out under this seal.

Methyl bromide (150 lb.) was administered at the time of the usual early morning breeze. The dose was applied in 40 minutes without the assistance of a compressor, and gas samples were taken at intervals, with evacuated flasks containing monoethanolamine, over a period of 22 hours after the full dose had been given.

Analyses were carried out by means of a slightly modified method of Lewis.[2]

Flat-topped dump.—In early 1951 some 9000 tons of groundnuts in flat-topped dumps became lightly infested with *Trogoderma* and were successfully fumigated *in situ* by a technique developed in the United Kingdom. No particular difficulties were encountered.

One of the largest dumps fumigated was selected for examination of gas concentration and distribution. It was 60 ft. long, 50 ft. wide and 8 ft. high and contained 351 tons of groundnuts. Some surface restacking was carried out to make channels, about 4 ft. wide and 4 ft. deep, running the length and across the width of the stack. Copper gas-delivery pipes carrying eight atomizing jets in all were laid in these channels. Capillary gas-sampling lines had also been inserted into various internal and external sacks. Two-ply rubberized dinghy sheets 30 ft. × 40 ft. were placed over the dump. Their overlapping edges were rolled tightly together in pairs, then each pair was treated as a single sheet and lapped and tightly rolled to the adjoining pair. In this fashion the dump was covered with eight sheets. Small sand-bags were placed at intervals to prevent the sheets from becoming unrolled, and long narrow sand-bags were placed all round the base as for the pyramid. A dose of 70 lb. of methyl bromide was given in 20 minutes without the aid of a compressor. Gas samples were taken during a period of 20 hours after the full dose had been administered.

Results

Fig. 1 shows that the high initial concentration of gas inside external sacks fell off rapidly during the first few hours of fumigation. The concentration of methyl bromide in the most remote internal sack near the base gradually rose to rather a low maximum after 4–8 hours and then decreased. The minimum concentration–time product was at this point 81 mg. h. after 22 hours' fumigation.

The gas concentrations obtained in the dump fumigation were generally much higher and more uniform than in the pyramid experiment, and there was little difference between that of the free space and that inside an internally sited sack near the bottom layer (see Fig. 2). The minimum concentration–time product after 20 hours was 126 mg. h. Biological examination revealed no live insects after fumigation.

A halide-detector lamp showed that no escape of gas could be detected in either fumigation, and that when airing took place between two and three days after gassing much methyl bromide was still present.

Discussion

It appears that penetration of the gas into the internal regions of a large stack such as a pyramid is somewhat slow, and that much gas disappears in the process. As direct leakage did not appear to occur it is most probable that loss of gas was caused by diffusion into the very porous base.

Though loss of gas in this manner must occur in dump fumigation the shape of trenched dumps and mode of administering the gas mean that no sacks are very far from the circulation trench of high initial concentration, and receive a good dose before losses by diffusion occur.

Under conditions existing in Kano, gas distribution is assisted by flapping the covering of the stacks during the usual morning breeze between 9 and 11 a.m., and for this reason doses were given at about 9 a.m. when possible. Stirring of gases will also be caused by convection

due to the violent diurnal temperature changes to which objects in the open are subjected. During the day the temperature under the covering may reach 150° F, whereas at night it may fall to 60° or 70° F. Measurements with thermocouples showed that temperatures in the central portions of the pyramids and dumps were always fairly high and about 105° F.

The actual level of methyl bromide application in these experiments and in routine fumigations (namely 1 lb. of methyl bromide to 5 tons of groundnuts) is possibly about double the dose that might be expected to be adequate. This has ensured success in all fumigations and allowed for basal diffusion losses.

Pyramid storage has now become standard practice in Kano and there will be a serious storage problem for some years to come in view of two recent large crops and slow removal. The frequency of infestation by *Trogoderma* has recently increased, and the necessity for fumigation by methyl bromide continues.

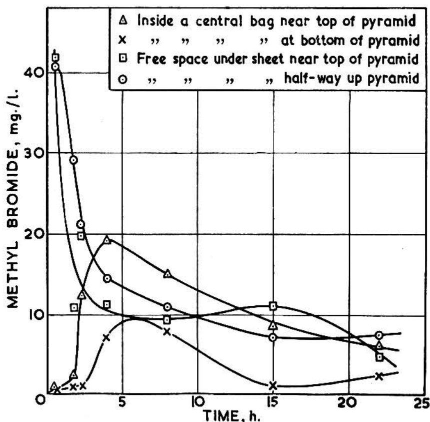

FIG. 1.—*Concentrations of methyl bromide in a pyramid of 730 tons*

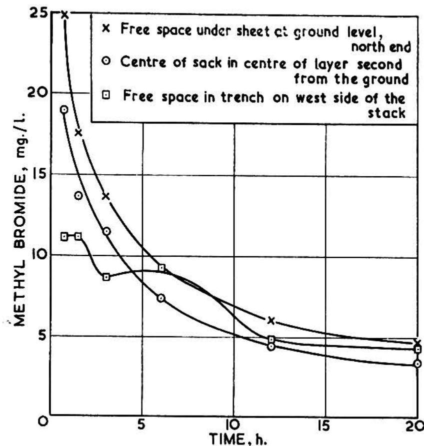

FIG. 2.—*Concentrations of methyl bromide in a rectangular dump of 351 tons*

The economizing in methyl bromide is now becoming a matter of some importance in view of the large quantities being used. Work is therefore being carried out to determine the minimum concentration–time value necessary to kill *Trogoderma* under conditions in Kano, and to ascertain whether the present level of dosage can be safely reduced. The effects on diffusion losses of constructing pyramid bases of tarmac, and also of covering conventional bases with sisalkraft paper, are to be investigated shortly.

Acknowledgments

Mr. L. Peverett of the London Fumigation Company assisted in introducing the current fumigation techniques. All materials and equipment were purchased by the Nigerian Groundnut Marketing Board through the London Fumigation Company.

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A RAPID METHOD FOR THE DETERMINATION OF NITROGEN, PHOSPHORUS AND POTASSIUM IN PLANT MATERIALS

By A. J. CAVELL

A rapid method for determining nitrogen, phosphorus and potassium has been developed : after the plant material is digested with sulphuric acid, nitrogen is estimated as ammonia, phosphorus as the yellow phosphovanadomolybdate and potassium with a flame photometer.

Agricultural laboratories concerned with the effect of applications to the soil of mixed fertilizers analyse large numbers of plant materials for nitrogen, phosphorus and potassium, and this paper gives an account of a simple rapid method, developed in this Laboratory, which requires the minimum of laboratory space and equipment.

Lindner[1] estimated nitrogen, phosphorus, potassium, calcium and magnesium after treating plant materials with sulphuric acid and hydrogen peroxide. Kelley, Hunter & Sterges[2] considered this procedure to be unsuitable for routine analysis and suggested a digestion with a salicylic acid/sulphuric acid mixture, followed, if necessary, by treatment with 10% perchloric acid.

A simple digestion with concentrated sulphuric acid in the presence of sodium sulphate and copper sulphate has been found to be a suitable method of treating plant material before the estimation of nitrogen, phosphorus and potassium.

The Kjeldahl[3] method for the estimation of nitrogen has been subjected to many investigations ; the experimental conditions described by Willits, Coe & Ogg,[4] which are applicable to plant materials, have been made the standard routine method in this Laboratory. However, for the purpose of this work it was necessary to replace potassium sulphate by sodium sulphate, a substitution which, according to Middleton & Stuckey,[5] should have but little effect on the boiling point of the digestion mixture. The replacement of mercuric oxide by copper sulphate will lead to the incomplete recovery of nitrogen only from certain refractory nitrogenous materials, and should introduce only small errors with normal agricultural products.

Colorimetric methods are usually more rapid than volumetric and gravimetric methods. Woods & Mellon[6] have shown that copper can interfere with the colorimetric estimation of phosphorus as molybdenum blue, but its presence, according to Kitson & Mellon,[7] causes less interference when phosphorus is determined colorimetrically as the yellow phosphovanadomolybdate. It has been found that the development of this yellow colour, first proposed by Misson,[8] takes place in the presence of sodium sulphate and sulphuric acid, and is a suitable and rapid means of determining phosphorus in an acid digest.

The presence of large amounts of sodium salts does not interfere with the determination of potassium with a flame photometer, and, after neutralization of the Kjeldahl digest, potassium can be rapidly determined with this instrument.

General experimental work

Nitrogen

Experimental work on the determination of nitrogen was not required, since the procedure adopted, subject to the limitations mentioned above, is in general use, and follows the general directions given in the Fertilisers and Feedingstuffs Regulations 1932.

Phosphorus

The yellow colour that develops when an excess of molybdate is added to an acidified mixture of vanadate and orthophosphate was used by Misson for the quantitative estimation of phosphorus in steels, and the range of materials to which his method has been applied has been extended by Willard & Center[9] to iron ores, by Koenig & Johnson[10] to biological materials, by Barton[11] to phosphate rock and by Hanson[12] to phosphate fertilizers. The colour was developed by Misson, by Murray & Ashley,[13] and by Hanson in the presence of nitric acid, by Barton in a mixture of hydrochloric and nitric acids, and by Koenig & Johnson and by Willard & Center in a mixture of perchloric and nitric acids. Earlier workers used separate solutions of ammonium molybdate and mixtures of nitric acid and ammonium vanadate of various acid concentrations. Barton, in addition, tried a mixed reagent containing nitric acid, which Hanson later modified. Kitson & Mellon indicated that it would not be necessary to

add the vanadate before the molybdate if the amount of ammonium molybdate were reduced, but they did not use a single mixed solution. The reproducibility of colours given by the two reagents used by them was found to be no better than that given by a single filtered aqueous mixture containing 1% of ammonium molybdate and 0·05% of ammonium vanadate. With standard phosphate solutions, in a final concentration of 3·5% of sodium sulphate and 0·75N-sulphuric acid, this single reagent (referred to later as the mixed reagent) gave reproducible results over a period of five weeks, and batch differences were negligible. The addition of nitric acid to the ammonium vanadate solution originated with Misson, but its presence does not appear to be necessary when dealing with phosphate solutions in a suitable concentration of sulphuric acid.

Effect of temperature on colour development.—Center & Willard reported that the transmittancies of ammonium phosphovanadomolybdate solutions were sensitive to temperature changes. In these experiments the mixed reagent and the phosphate solution were brought to 20° before mixing, and the transmittancies of the solutions were read after the colour had developed at 20° for 5 minutes. Subsequently, experiments indicated that variations caused by differences in temperature of 2° or 3° were negligible. Murray & Ashley reported that temperature differences in the range 20° to 30° had little effect.

Effect of variation of sulphuric acid concentration in 3·5% sodium sulphate solutions.—Variations from 0·5N to 1·0N in the acidity of solutions containing the mixed reagent and 3·5% of sodium sulphate had negligible effect on the transmittancy measurements made over the range 400–480 mμ. A final concentration of 0·75N was arbitrarily fixed as a suitable concentration of acid.

Effect of variations in the concentration of sodium sulphate.—Solutions (0·75N) of sulphuric acid containing 0 to 5% of sodium sulphate and the mixed reagent showed negligible differences in transmission over the range 400–480 mμ. When studying the effect of other ions a final sodium sulphate concentration of 3·5% was arbitrarily selected, but adjustment to a constant value is not normally necessary.

Transmittancy curves.—Fig. 1 shows transmittancy curves marked 0–5 obtained with six solutions containing 0, 1, 2, 3, 4 and 5 mg. of phosphorus pentoxide in 100 ml. of 7·0% sodium sulphate and 1·5N-sulphuric acid. A volume of 5 ml. of each of these solutions was diluted with an equal volume of the mixed reagent and the transmittancies were read at 20°. All transmittancies were compared with that of water as a standard. These curves show that the maximum absorption takes place at about 400 mμ, and absorption slowly decreases as the wavelength increases. During this work, therefore, measurements were taken at the wavelengths of maximum sensitivity (400 mμ) and wide range (480 mμ), and also at the intermediate wavelength of 440 mμ. At each of these wavelengths, log (transmittancy) plotted against concentration of phosphorus gives a straight line, indicating conformity to Beer's law (Fig. 2).

Effects of other ions.—The concentration of copper introduced by the catalyst into the final solution is about 25 p.p.m., and it was found that five times this amount had negligible effect on the spectrophotometric measurements. Kitson & Mellon reported that up to 100 p.p.m. of copper could be tolerated.

An investigation was made of the effects, on the intensities of the colours, of 0·4 mg. of iron, 4 mg. of aluminium, 4 mg. of manganese, 40 mg. of calcium, 20 mg. of magnesium, 20 mg. of potassium, when placed either individually or collectively into sets of seven 100-ml. graduated flasks, each flask containing 20 mg. of copper sulphate, 7 g. of sodium sulphate and 15 ml. of 10N-sulphuric acid, and each set containing 0, 1, 2, 3, 4, or 5 mg. of phosphorus pentoxide.

A volume of 5 ml. from each set of seven flasks containing the same amount of phosphate was treated with the mixed reagent; differences in transmission, at 400, 440 and 480 mμ, between the individual members of both these sets and pure solutions of equal phosphate concentrations were negligible.

The effects were also studied, on the intensities of the colours, of 2 mg. of iron, 20 mg. of aluminium, 20 mg. of manganese, 200 mg. of calcium, 100 mg. of magnesium, 100 mg. of potassium, and 200 mg. of silica, after digestion, individually and collectively, with 30 ml. of concentrated sulphuric acid, 0·1 g. of copper sulphate, 10 g. of sodium sulphate and 0·5 g. of sucrose, at six levels of phosphate concentration.

The six levels were obtained by adding 0, 5, 10, 15, 20 or 25 mg. of phosphorus pentoxide to a group of eight digestion flasks. After being digested, each solution was diluted to 250 ml., and 50 ml. of each was diluted to 100 ml. after adjustment of the final acidity to 1·5N and the final sodium sulphate concentration to 7%. Other 50-ml. portions from each

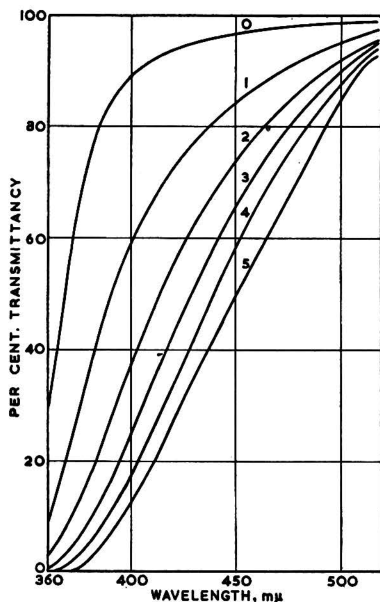

FIG. 1.—*Determination of phosphorus: variation of absorption with wavelength*

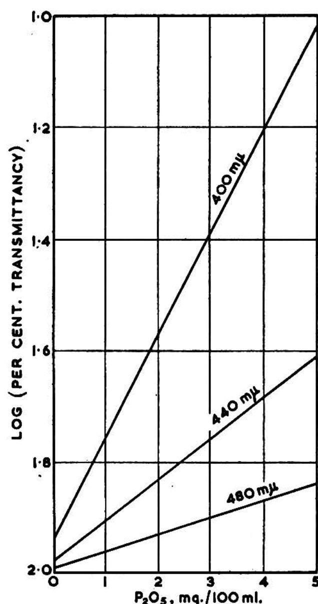

FIG. 2.—*Variation of transmittancy with concentration of phosphorus*

of the 250-ml. flasks were diluted to 100 ml. after the final acidity was adjusted to 1·5N, but without adjusting the sodium sulphate concentration. A volume of 5 ml. from both sets of eight flasks containing the same amount of phosphate was treated with the mixed reagent; differences in transmittancy, at 400, 440 and 480 mμ, between the individual members of both these sets and pure solutions of equal phosphate concentrations were negligible.

The presence of 0·1% of iron, 1% of aluminium, 1% of manganese, 10% of calcium, 5% of magnesium, 5% of potassium and 10% of silica in a plant material would not therefore interfere with the determination of phosphorus by this method.

Potassium

Cavell[15] showed that the determination of potassium in soil extracts in the presence of large amounts of sodium salts can be rapidly made with a flame photometer, and experiments were first directed towards neutralization of a suitable aliquot of the digested material with sodium hydroxide, and adjustment of the sodium sulphate concentration to a constant value. It was shown that the determination of potassium in the presence of up to at least 8% of sodium sulphate in neutral solutions could be made; sodium hydroxide of analytical-reagent purity contains a minimum of 100 p.p.m. of potassium and the original intention had to be modified. It was found later, however, that the acid could be neutralized with ammonium hydroxide, since variations in the amount of ammonium sulphate in the presence of slightly ammoniacal solutions of sodium sulphate did not affect the determination of potassium by the flame photometer.

Effect of other ions.—The effects, on the galvanometer deflections, of 2 mg. of iron, aluminium or manganese, 4 mg. of phosphorus pentoxide, 10 mg. of magnesium, 20 mg. of calcium or silica, when added, individually and collectively to five sets of eight flasks, each flask containing 1 g. of sodium sulphate, 10 mg. of copper sulphate and 3 ml. of concentrated sulphuric acid, and each set containing either 0, 1, 2, 3, or 4 mg. of potassium monoxide in each flask, were determined. Each solution was neutralized with ammonia and each solution of each set, after sedimentation had taken place if necessary, was sprayed into the flame photometer. All eight solutions at each of the five levels of potassium, when sprayed successively into the flame photometer, gave readings to within 2% of the deflection of the galvanometer caused by a solution containing only potassium, in corresponding concentration.

The presence in a plant material of 1% of iron, aluminium or manganese, 10% of calcium, 10% of silica, 5% of magnesium and 2% of phosphate, expressed as phosphorus pentoxide, would not therefore interfere with the determination of potassium. With an instrument error of up to 2%, results obtained by applying this method to plant materials can be expected to be within the required degree of accuracy usually considered desirable when analysing agricultural products.

Apparatus and reagents

Nitrogen.—A Markham[14] micro-distillation apparatus.

Phosphorus.—Transmittancy measurements were made with an SP 600 Unicam spectrophotometer.

Potassium.—Two flame photometers were used, the electronic instrument previously described by Cavell,[15] and a simpler instrument, also built in this Laboratory. The latter instrument had the same type of atomizing unit and burner as the former photometer but the R.C.A. 931A multiplier cell had been replaced by a Megatron infra-red barrier layer photocell. The flame is protected by a metal chimney, with an opening, about 2 in. square, through which light from the flame passes on to the cell.

Interposed between the flame and the photocell is the filter and a water cell, made from sheet Perspex and Perspex tubing joined with the aid of chloroform. The water cell, filter and photocell are mounted in a metal box, open at the top, which slips into two grooves on the chimney. The water issuing from the water cell passes through an outer jacket surrounding the sides of the box, and then goes to waste. Optical devices to increase sensitivity can be dispensed with in such an instrument as the water cell can be put 1 in. from the flame, with the filter and photocell immediately behind it, without danger of damage by heat.

Chemicals.—All chemicals were of at least A.R. quality. Sodium sulphate, A.R., but also low in phosphate, is obtainable from The British Drug Houses Ltd. and Hopkin and Williams Ltd.

Special reagent.—Ammonium molybdate/ammonium vanadate mixture. Dissolve 5 g. of ammonium molybdate and 0·25 g. of ammonium vanadate in warm water, cool, dilute to 500 ml. with water and filter. This mixed reagent, R, is stable for at least four weeks, and batch differences are negligible.

Methods

Digest 2 g. of dried plant material with 30 ml. of concentrated sulphuric acid, 10 g. of sodium sulphate, and 0·1 g. of copper sulphate until clear and then for 1 hour after. Cool, make up to 250 ml. and mix (solution A). It is usually convenient to set the product aside overnight at this stage so that the clear supernatant liquid can be pipetted off in the morning.

Determination of nitrogen.—Pipette 10 ml. of the solution A into a Markham micro-Kjeldahl distillation apparatus, add excess of sodium hydroxide and distil off and estimate the ammonia in the usual way. Results have been expressed as crude protein (N × 6·25).

Determination of phosphorus.—Pipette 50 ml. of supernatant liquor A into a 100-ml. graduated flask. Titrate 5 ml. of A with 2·5N sodium hydroxide with phenolphthalein as indicator (x ml.). Add ($10x - 60$)ml. of 2·5N sodium hydroxide to the 50 ml. of A in the 100-ml. graduated flask, cool and dilute to 100 ml. This solution, B, is now 1·5N with respect to sulphuric acid. After this solution is thoroughly mixed, centrifuge about 10 ml. of B in a 15-ml. centrifuge tube and pipette 5 ml. of the clear centrifuged liquid into a dry glass-stoppered tube; leave in a water bath at 20° for about 5 minutes and then add exactly 5 ml. of the mixed reagent R, also at 20°. Mix the solution by inverting the tube several times and then allow the tube to remain in the water bath at 20° for at least 5 minutes. Measure the light transmissions at 400, 440 and 480 mμ and read off the percentage of phosphorus pentoxide in the plant material from the calibration curve.

A standard graph may be obtained by plotting the transmittancy at these wavelengths of solutions made by mixing 5 ml. of R with 5 ml. of solutions containing up to 4 mg. of phosphorus pentoxide in 100 ml. of 2% sodium sulphate in 1·5N-sulphuric acid. This range is suitable for plant materials containing up to 1% of phosphorus pentoxide.

Determination of potassium.—Pipette 25 ml. of A into a 100-ml. graduated flask, add methyl red and make just alkaline with ammonium hydroxide. Cool, make up to 100 ml. with water, and mix well. Atomize the solution into the flame photometer and read off from the calibration curve the percentage of potassium (as K_2O) in the plant material.

The calibration curve may be obtained by atomizing standard solutions each containing, in 100 ml., 1 g. of sodium sulphate and 2 ml. of sulphuric acid, just neutralized with ammonium hydroxide, and from 0 to 6 mg. of potassium monoxide. It was found convenient to adjust the sensitivity of the instrument so that half the full-scale deflection of 100 divisions was given with the solution containing 4 mg. of potassium monoxide, corresponding to 2% of potassium monoxide in a plant material.

Results of analyses of various plant materials

Plant materials (15 kinds) were analysed for nitrogen, phosphorus, and potassium by means of the methods described above and by methods derived essentially from those described by Willits, Coe & Ogg, Richards & Godden,[16] and Green,[17] respectively. The potassium and phosphorus were determined after dry ashing at 550°. The results, set out in Table I, indicate satisfactory agreement between the various methods.

Table I

Plant material	Rapid method (N × 6·25), %	Rapid method P_2O_5, %	Rapid method K_2O, %	Standard methods (N × 6·25), %	Standard methods P_2O_5, %	Standard methods K_2O, %
Barley	12·6	0·95	0·75	12·7	0·97	0·64
Barley straw	3·7	0·14	0·60	3·8	0·19	0·56
Clover	16·2	0·56	2·08	16·6	0·57	2·12
Grass I	12·6	0·79	3·10	12·8	0·80	2·93
Grass II	23·5	1·06	2·48	23·7	1·05	2·25
Hay	10·4	0·58	1·78	10·8	0·59	1·78
Kale leaves	17·2	0·68	2·76	17·0	0·68	2·78
Kale stem	14·1	0·70	3·56	14·1	0·73	3·45
Lucerne	20·9	0·62	2·07	21·0	0·64	2·08
Oats	12·1	0·97	0·45	12·1	0·97	0·33
Pea meal	24·2	1·08	1·50	24·8	1·06	1·39
Potatoes	11·6	0·42	2·74	11·5	0·44	2·59
Swedes	19·0	0·67	3·56	19·3	0·66	3·51
Wheat	11·5	1·00	0·62	11·4	0·97	0·58
Wheat straw	2·3	0·12	1·00	2·4	0·15	1·02

Recovery of nitrogen, phosphorus and potassium added to a plant material

The recoveries of various amounts of nitrogen, phosphorus and potassium added to 2-g. quantities of barley straw are shown in Table II.

Table II

Nitrogen, as N				Phosphorus, as P_2O_5				Potassium, as K_2O			
Added, mg.	Found, mg.	Re-covered, mg.	Re-covery, %	Added, mg.	Found, mg.	Re-covered, mg.	Re-covery, %	Added, mg.	Found, mg.	Re-covered, mg.	Re-covery, %
0	11·8	—	—	0	2·8	—	—	0	12·0	—	—
0	11·8	—	—	0	2·8	—	—	0	12·0	—	—
5	16·6	4·8	96	5	8·0	5·2	104	17·1	30·0	18·0	105
5	16·7	4·9	98	10	12·8	10·0	100	20	32·6	20·6	103
5	16·4	4·6	92	15	17·4	14·6	97	14·1	26·4	14·4	102
5	16·8	5·0	100	15	18·0	15·2	101	10	22·0	10·0	100
10	21·6	9·8	98	5	7·7	4·9	98	10	22·0	10·0	100
10	21·7	9·9	99	10	12·8	10·0	100	34·2	46·4	34·4	101
15	26·6	14·8	99	5	7·7	4·9	98	10	21·6	9·6	96
15	26·0	14·2	95	5	8·0	5·2	104	17·1	30·0	18·0	105
15	26·3	14·5	97	15	18·0	15·2	101	30	41·6	19·6	99
15	26·3	14·5	97	15	18·0	15·2	101	51·3	63·6	51·6	101
Average recovery			97	Average recovery			100	Average recovery			101

Reproducibility of results

The results of twelve analyses on a sample of lucerne, analysed at intervals over a period of several weeks, are given in Table III, and indicate that agreement among replicate determinations is good, and that the coefficients of variation are satisfactory.

The agreement between the results obtained by the different methods, the excellent recovery of added nitrogen, phosphorus and potassium, and the good reproducibility of the results, together indicate that the proposed method is reliable.

Table III

	Replicate analyses				Mean	Standard deviation	Coefficient of variation	Standard error of the mean
(Nitrogen × 6·25), %	20·5	20·5	20·8	20·6	20·46	0·25	1·22	0·072
	20·6	20·5	20·9	20·1				
	20·4	20·2	20·2	20·2				
Phosphorus as P_2O_5, %	0·613	0·605	0·617	0·613	0·612	0·0064	1·05	0·00185
	0·619	0·619	0·621	0·614				
	0·605	0·605	0·605	0·605				
Potassium as K_2O, %	2·02	2·07	2·00	2·08	2·04	0·028	1·38	0·0081
	2·07	2·04	2·04	2·02				
	2·07	2·00	2·02	2·04				

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THE ORGANIC ACIDS OF GRASS EXTRACTS

By C. W. DAVIES and R. B. HUGHES

Ion-exchange chromatography indicates the presence of 12 organic acids in aqueous grass-extracts. Of these, acetic, lactic, succinic, malic, malonic and citric acids have been identified, though the lactic acid may have originated from catalytic action in the resin column. Some preliminary observations on the diurnal variations, and variations during growth, of the grass acids are reported.

The primary object of this work was to investigate some new procedures for studying the organic acid content of plant extracts. Most of the work previously reported in this field had been carried out by the potentiometric techniques of Pucher, Vickery & their collaborators.[1–4] This type of procedure can give consistent results as a routine method for closely similar samples, but we found it unsatisfactory for studying the complex mixtures of acids present in grass extracts.

Righellato & Davies[5] applied conductimetric methods to aqueous extracts of leather and tanning materials, and were able to determine the strong acid, the free and combined weak acid, and the neutral salt contents. These methods have been used and extended by other authors,[6] and seem well adapted to the investigation of plant extracts. Accordingly we gave considerable attention to their application to grass extracts. The results will not be reported here in detail, as they share some of the drawbacks of those from the potentiometric method. They distinguish clearly between amino-acids and the stronger carboxylic acids, and from a small sample of grass

it is readily possible to estimate the total quantity of each of these classes, as well as the mineral salts present, with an accuracy of approximately 5%. Greater accuracy seems unattainable, however, owing to the large number of constituents in the extracts and the overlapping of their pH ranges; in the early stages of titrating the acidified extract with alkali the conductivity is much affected by interaction of the stronger acids with the amino-acids, and, at the end of the titration, one-third of the free phosphoric acid remains unneutralized. As the work proceeded it became clear that attention should be concentrated on the individual separation and estimation of the acids present, and we therefore studied the chromatography of the extracts on ion-exchange resins.

Two strong anion exchangers were used, Dowex 1 and 2. After preliminary treatment, during which the amino-acids were removed by a cation exchanger, the aqueous grass-extract was passed on to the resin in the hydroxide form, and the adsorbed acids were displaced by hydrochloric acid. The separation was followed by paper chromatograms of the eluant, and 12 or 13 acids were detected, of which 7 have been positively identified. Some preliminary results have also been obtained for the diurnal variations, and variations during growth, of the organic acid content of grasses.

Methods

The grass samples, of perennial rye-grass, cocksfoot and timothy, were taken from plots of the pure strains, and were heat-dried and finely powdered. A heat-dried mixed herbage, used in some experiments, showed no marked difference in acid content.

Extraction.—The procedure finally adopted was as follows. A quantity of 30 g. of the sample was weighed, and gently boiled with 500 ml. of distilled water for 20 minutes. It was then filtered through a plug of cotton wool which was washed with 100 ml. of water and drained by solution. The residue and plug were then returned to the original beaker, 250 ml. of water was added, and the extraction repeated. The two extracts and washings gave just under 1 litre of dark greenish-brown solution containing much colloidal material. Its pH, measured with a glass electrode, was 5·6–5·9.

This extraction procedure gave reproducible results. When the residue from an extraction was put through the whole procedure a second time, the second extract, analysed by the method to be described, was found to contain phosphoric acid equal to 4% of that extracted by the standard procedure, but its organic acid content was less than 1% of that present in the first extract.

Purification.—The pH of the extract was adjusted to 7·5 with sodium hydroxide, 30 g. of sugar- or animal-charcoal was added, and the mixture shaken mechanically for 1 hour before filtering through a large Buchner funnel. The filtrate and washings were then reduced to a volume of 30 ml. by distillation under reduced pressure from a wide-necked flask at 20–25°. The concentrate contained a small quantity of precipitated material; after the liquid had been stored overnight in a refrigerator it could be readily filtered, leaving a clear and nearly colourless filtrate. This was passed through a system of two columns, the first (height 25 cm., diameter 1·8 cm.) containing Decolorite (Permutit Co. Ltd.) and the second (height 30 cm., diameter 1·5 cm.) containing Zeo-Karb 215 in the hydrogen form. The rate of flow was approximately 40 ml./hour. The Decolorite removed the residual colour; the Zeo-Karb adsorbed the amino-acids as a light-coloured band, and converted the organic-acid salts into the free acids. Control experiments showed that the treatment of the neutralized extract with charcoal and Decolorite did not lead to loss of organic acids; if animal charcoal was used there was an increase in the phosphate concentration, but this was normally of no importance. Blank experiments also indicated that there was no loss of acids on the Zeo-Karb column. The effluent from this treatment was clear and colourless, and after being combined with washings from the columns it was passed on to the Dowex columns.

Separation.—In the final arrangement three successive resin-columns were used. These consisted of resistance-glass tubes, fitted with taps at the lower end. The resin rested on a small plug of glass wool, and was always kept covered with liquid. A short tube passed through a rubber bung at the top of the glass column and was tapered to deliver small drops, which fell on a polythene disc or a hollow glass-sphere floating on the surface of the liquid. Column 1 was a short wide column, 6 cm. high and 3 cm. in diameter, containing 15 g. (40 milliequiv.) of air-dry Dowex 2 of 250–500 mesh. Column 2 was 10 cm. high and 1·2 cm. in diameter, and contained 7 g. of air-dry resin. Column 3 was 6 cm. in height and 0·5 cm. in diameter and contained Dowex 1.

Solutions were passed through the columns by gravity from a large separating funnel, and, to assist in adjusting the pressure head, a tube passed through the bung closing the top of the

funnel to a simple pressure device. This consisted of a long glass tube, closed at the bottom and partly filled with water; the two-holed rubber bung at its top carried the tube leading to the funnel, and a second long glass tube, to act as air inlet. The pressure was adjusted by varying the depth to which this tube was immersed in the water.

The fraction-collector was of the kind described by Phillips.[7] To avoid any danger of the sampling tubes' sticking it was found advisable to keep the fraction collector constantly agitated by a piece of rubber tubing, attached to an electric stirrer, which brushed against the framework at each revolution. With this precaution the fraction collector could be left unattended overnight, and the volumes of the fractions varied by not more than one drop.

The columns were first conditioned by two successive cycles with carbonate-free sodium hydroxide and hydrochloric acid, and were then regenerated with sodium hydroxide, followed by washing with distilled water until the effluent had a pH value of less than 8. It was essential that the sodium hydroxide and water should be entirely free from carbon dioxide (which would otherwise be liberated in the column during the acid displacement), and this was ensured by passing them through a preliminary column of a strong-base resin. The extract was then passed into column 1, where the acids were adsorbed in a light-coloured band at the top of the column. They were then displaced down the column by 0·1N-hydrochloric acid at a rate of flow of 2 cm./hour. When the front approached the bottom of column 2, column 3 was fitted in series with it, and the rate of flow was reduced to give the same boundary progression as in the previous column. The eluate from column 3 was collected in fractions of 1·0–1·5 ml. Considerable difficulty was caused by a tendency to 'gassing' in the third column, which was attributed to a catalysed decomposition at the advancing front. This difficulty could be avoided by rejecting the first 5 ml. emerging from the base of the second column. It is thought that no carboxylic acids were lost by this procedure.

Results

The general pattern of the results was the same for all the grass samples studied, and Fig. 1 illustrates a typical example, which for convenience of description is regarded as made up of the six fractions shown in the Figure. Fraction 1 contained the two acids shown, and transient spots corresponding to other acids were sometimes found in this region. The acid marked E, at the junction of Fractions 2 and 3, could not always be detected.

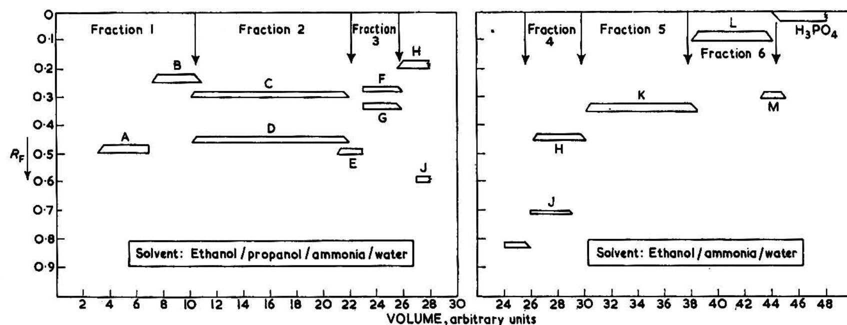

Fig. 1.—*Typical displacement chromatogram. R_F values for each fraction collected are plotted against the number of the fraction*

The quantities of acid in the various fractions were estimated by titration with sodium hydroxide, allowance being made for the drops withdrawn for paper analysis. A typical result, for a sample of heat-dried cocksfoot, is given in Table I. The total acid found is 0·321 g. equiv. per kilogram of dried grass.

Identification of acids

For the preliminary characterization of the various fractions some general arguments are available. In the first place the order of emergence of the acids from the column is characteristic, and, in general, monobasic acids will be expected to appear in the order of increasing dissociation constant,[8] followed by polybasic acids. The concentration of the eluate is also characteristic (provided the concentration of the displacing hydrochloric acid is kept constant), and in Table II

the acid concentrations of the various fractions are compared with those found for the fractions obtained from a synthetic mixture of lactic, succinic, malic and citric acids.

Table I

Fraction	Milliequiv. of acid/kg. of dried grass	Per cent. of total acids
1	24·9	7·8
2	55·7	17·3
3	18·9	5·9
4	38·6	12·0
5	100·6	31·3
6	82·7	25·7

These general arguments were supplemented by paper chromatograms with the butanol/water/formic acid solvent-system of Lugg & Overell,[9] Brown's ethanol/aqueous ammonia solvent,[10] the butanol/ammonia/water system used by Hiscox & Berridge,[11] and a new solvent, propanol/ethanol/water/ammonia (sp. gr. 0·880) (45 : 45 : 5 : 5), which was found to be suitable for the acid mixtures encountered. The R_F values are not very reproducible in ammoniacal solvents, and known acids were always run side by side with the test mixtures. Finally, specific tests were applied where possible, and led to the following conclusions:

Fraction 1.—Acid A is believed to be acetic acid. This supposition is based on its volatility, and because, when run side by side with acetic acid, it gives identical R_F values in ethanol/ammonia and in the butanol/water/ammonia system used by Hiscox & Berridge. Acid B has not been identified. The concentration of Fraction 1 implies that it is monobasic, and its position indicates that it has a higher dissociation constant or molecular weight than acetic acid.

Fraction 2.—A conductimetric titration of this fraction against sodium hydroxide produced a curve of the type expected for a weak monobasic acid, and analysis of the curve gave, for the dissociation constant, pK = 3·86. A potentiometric titration, to which the simple relationship :

$$pK = pH - \log \frac{[Salt]}{[Acid]}$$

was applied, yielded a constant value of pK = 3·86 ± 0·06 between 20% and 80% neutralization. This corresponds to the value for lactic acid, and the fraction gives the carbazole, guiacol and other tests for this acid. The higher R_F value of Fraction 2 also agrees with that of lactic acid in all the solvents tried. In addition, however, there is the second spot of lower R_F shown in Fig. 1, which is not given by commercial lactic acid that has been through the same treatment. Attempts to separate two acids from Fraction 2, by further treatment with Dowex in mixtures of dioxan and water, were unsuccessful, and the second acid, C, must be similar to lactic acid, both in dissociation constant and in affinity for the resin.

Fraction 3.—This small fraction contains two or three acids not yet identified. From their positions, and the concentration of this fraction, they appear to be monobasic acids of rather high dissociation constant or molecular weight, or both, and the potentiometric titration curve supports this.

Fraction 4.—This appears to contain two acids. Acid H, the one present in by far the greater amount, when run side by side with succinic acid in five solvents gives the same R_F values as that acid in each instance. Acid J, which has not been identified, appears to be a volatile monobasic acid of high dissociation constant. This is supported by the position of the spot, its absence in the Lugg & Overell chromatogram, the low concentration of Fraction 4, and its potentiometric titration curve.

Fraction 5.—This is a pure fraction, and acid K is malic acid. It gives identical R_F values in all solvents, and the melting point of its phenylhydrazine derivative (115·5°) is not depressed by admixture with malic acid.

Fraction 6.—Acid L is citric acid. This is confirmed by mixed-melting-point experiments with its phenylhydrazine derivative, and by the chromatographic results. Acid M, present in small amount, is believed to be malonic acid, with which the chromatographic results agree in both acid and basic solvents. Busch, Hurlbert & Potter,[12] using formic acid as eluant, have found that citric and malonic acids emerge together from a Dowex column.

Table II

Fraction	Concn., g. equiv./l.	Fraction	Concn., g. equiv./l.
1	0·102		
2	0·104	Lactic	0·102
3	0·106		
4	0·158	Succinic	0·192
5	0·197	Malic	0·191
6	0·274	Citric	0·264

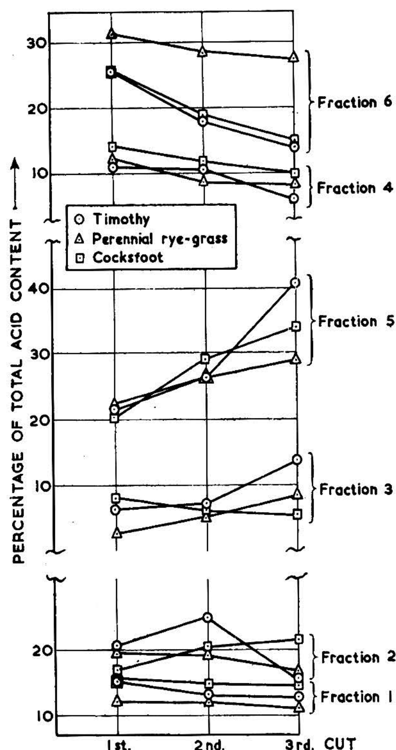

FIG. 2.—*Variations in acid content during growth*

Discussion

The various samples of grass examined all gave results closely similar to those of the example just discussed, although the relative proportions of the different acids varied within fairly wide limits. In no instance was oxalic, tartaric or *iso*citric acid detected.

Some observations were made of the changes in acid content during growth and development, by studying cuts from three pure strains of grass at periods corresponding approximately to the early, vigorous stage of growth, to the flowering stage, and to a stage intermediate between these two. The grasses were perennial rye-grass (5/5, 13/6, 25/6), timothy (28/4, 20/6, 14/7), and cocksfoot (2/5, 21/5, 20/6), the figures in parentheses giving the day and month on which each cut was made. The samples were dried in an electric oven in a current of hot air, and milled to a fine powder. For perennial rye-grass and timothy the total acid content fell off steadily, as might be expected, as the stem/leaf ratio increased, but for the cocksfoot sample there was a slight change in the opposite direction. The changes in the proportions of the separate fractions are shown in Fig. 2. The most marked effects are the increase in the percentage of the malic acid fraction (5) and the decrease in the percentage of citric acid (6). These effects roughly balance each other, so that malic and citric acid together account for about 50% of the total acid content at all stages of growth.

A preliminary study of the diurnal variation in the organic acid contents of two grasses, timothy and cocksfoot, was made by analysing cuts taken at 8 p.m. and 5.15 a.m. (on 10–11 June). The total acid content showed a small decrease overnight in the cocksfoot sample but a somewhat larger increase for the timothy. Both samples, however, gave a marked change in the malic/citric acid ratio, as is shown in Table III.

Table III

Milliequiv. of acid/kg. of dried grass

	Cocksfoot			Timothy		
	Fraction 5	Fraction 6	5 + 6	Fraction 5	Fraction 6	5 + 6
Evening	100·6	82·7	183·3	111·8	71·2	183
Morning	89·3	96·9	186·2	103·7	85·1	188·8

In reporting these preliminary results it should be emphasized that losses of volatile acids, and chemical changes, may occur during the heat-drying and extraction processes, and that aromatic acids may be lost by irreversible adsorption during the treatment of the extract. In particular, all the lactic acid found may result from secondary reactions on the Dowex column.[13] Attention is being paid to these uncertainties in further work now in progress.

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FUMIGATION OF AGRICULTURAL PRODUCTS. VII.*

Penetration and Sorption of Ethylene Oxide in Wheat Fumigated at Reduced Pressures †

By A. K. M. EL NAHAL

The fumigation of wheat in sacks with ethylene oxide is most efficient when the method of sustained-vacuum fumigation is used. Vacuum fumigation with simultaneous admission of air and fumigant is no more efficient than fumigation at atmospheric pressure over the same period. Ethylene oxide is much less strongly sorbed by wheat than is hydrogen cyanide, whichever method of application is used, but the moisture content of the wheat is even more important in determining the sorption of ethylene oxide than that of hydrogen cyanide. During fumigation of moist grain with ethylene oxide under sustained reduced-pressure, concentrations inside the sack consistently exceed those in the free space, and reach a maximum in the centre. The 'air-washing' procedures are as inefficient for ethylene oxide as for hydrogen cyanide in airing the grain after fumigation.

In the previous part (VI) of this series the sorption of hydrogen cyanide on wheat was found to be so heavy that, although penetration factors[1] give a measure of the amount of fumigant reaching the centre of a sack of wheat, the low values reached may suggest that all the three methods of fumigation with hydrogen cyanide give results too poor to need further consideration, in comparison with less strongly sorbed fumigants. Long practical experience, reinforced by the results described by El Nahal,[2] shows that hydrogen cyanide is an effective fumigant at the dosage levels selected when the three methods are compared on a biological as well as on a chemical basis. Nevertheless, the preliminary experiments carried out to determine the range of nominal dosages of ethylene oxide suitable for use in the main factorial experiments described here showed that the reduced levels of 6, 10 and 14 mg./l. could be used advantageously with ethylene oxide. In all other respects the experiments were designed and executed as described for hydrogen cyanide in Part VI.

Experimental

The fumigant, supplied in 60-lb. cylinders, contained an added 10% of carbon dioxide. In order to remove the carbon dioxide, which has been thought[3] to influence the toxicity of fumigants, the liquid ethylene oxide was siphoned from the cylinders into a stoppered glass-bottle and stored in a refrigerator until used. Only 0·75% of the carbon dioxide remains after this method of withdrawal,[4] and this small amount seems unlikely to exert any appreciable effect on the course of the experiments.

Lubatti's methods[5, 6] were used both for the determination of the concentrations of ethylene oxide reached at various sampling positions during the course of the fumigation and in the subsequent air-washing procedures, and for the estimation of the corresponding residues of ethylene oxide left in the wheat. Maximum chemical errors in these determinations were 0·7 and 0·8% at the highest gas concentrations and gas residues sampled, respectively, and 15 and 5% for the lowest levels.

* Part VI: *J. Sci. Fd Agric.*, 1953, **4**, 517
† Part of a thesis approved for the degree of Ph.D., University of London

Results and discussion

Analyses of variance were done on the concentration–time products attained, and on the residual-fumigant values, at the sampling points. The relative order of importance of the main factors (moisture content, dosage, method of fumigation and site of sampling) and of their interactions, agreed with the values found for hydrogen cyanide (Part VI). An exception was the greatly reduced concentration gradient in the wheat, which reflects the diminished sorption of ethylene oxide compared with hydrogen cyanide. The analyses of the residual fumigant in the wheat confirm this finding by showing a much smaller difference in residual fumigant between the central and outer sampling positions, when ethylene oxide is the fumigant.

Fig. 1 (*a*, *b* and *c*) shows the rate at which ethylene oxide penetrates the sack of wheat when applied by the three methods used. All the figures represent the fumigation of wheat of 13% moisture-content at a nominal dosage of 10 mg. per litre of ethylene oxide.

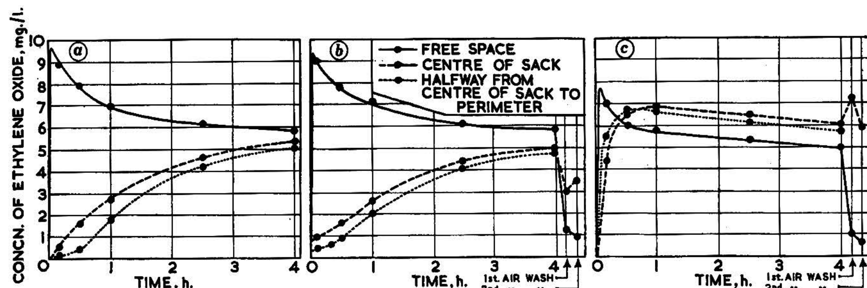

FIG. 1.—*Penetration of ethylene oxide into wheat fumigated by three different methods*

(*a*) Fumigation at atmospheric pressure
(*b*) Vacuum fumigation with simultaneous admission of air and fumigant
(*c*) Sustained-vacuum fumigation

Table I

Ethylene oxide concentration–time products, mg./h./l.

Moisture content, %	Dose, mg./l.	Method of fumigation	Free space: Fumigation period	Free space: Air washes	Free space: Total	Centre of sack: Fumigation period	Centre of sack: Air washes	Centre of sack: Total
9·0	6	Atmospheric	15·43	—	15·42	9·63	—	9·63
9·2	10		26·37	—	26·37	17·53	—	17·53
9·0	14		36·87	—	36·87	25·73	—	25·73
9·0	6	Vacuum, with simultaneous admission of air and fumigant	16·33	0·37	16·70	10·53	0·50	11·03
9·2	10		27·30	0·67	27·97	16·60	0·93	17·53
9·1	14		37·60	0·90	38·50	24·90	1·20	26·10
9·2	6	Sustained vacuum	14·40	0·37	14·77	14·17	1·13	15·30
9·1	10		24·13	0·63	24·76	23·77	1·63	25·40
9·2	14		36·10	0·93	37·03	35·53	2·63	38·16
12·8	6	Atmospheric	16·60	—	16·60	7·27	—	7·27
13·2	10		26·53	—	26·53	12·40	—	12·40
12·9	14		36·80	—	36·80	21·43	—	21·43
13·1	6	Vacuum, with simultaneous admission of air and fumigant	16·20	0·30	16·50	8·90	0·27	9·17
13·2	10		26·33	0·77	27·10	12·33	1·17	13·50
13·2	14		35·73	1·00	36·73	19·07	1·63	20·70
13·1	6	Sustained vacuum	12·60	0·37	12·97	15·03	1·37	16·40
13·1	10		22·37	0·63	23·00	24·77	2·17	26·94
13·0	14		31·03	1·00	32·03	34·83	2·93	37·76
16·8	6	Atmospheric	13·33	—	13·33	3·60	—	3·60
17·0	10		23·73	—	23·73	5·63	—	5·63
17·2	14		31·57	—	31·57	10·67	—	10·67
17·1	6	Vacuum, with simultaneous admission of air and fumigant	14·07	0·33	14·40	3·60	0·47	4·07
16·9	10		20·93	0·50	21·42	6·10	0·83	6·93
17·3	14		35·33	0·73	36·06	8·00	1·13	9·13
17·2	6	Sustained vacuum	10·97	0·27	11·24	10·87	0·87	11·74
17·1	10		18·07	0·40	18·47	17·87	1·43	19·30
17·0	14		25·37	0·67	26·04	25·77	2·03	27·80

The similarity between the results of the fumigation at atmospheric pressure and the vacuum fumigation with simultaneous admission of air and fumigant, together with the improved penetration under sustained vacuum, leaves little doubt that such results, supporting as they do the conclusions drawn from the hydrogen cyanide fumigation (Part VI) are of wide applicability (Tables I and II). The penetration factors[1] for the three experiments whose results are summarized in Fig. 1 are 31·0, 33·6 and 67·2%, respectively, for the three methods in the order just given. These figures are a great improvement on the corresponding penetration factors obtained with hydrogen cyanide. The rapid initial increase in the free-space concentrations was not sampled in these experiments, and has been drawn in the graphs in a tentative interpolation. The actual form of this early stage of the curve does not materially influence the total concentration–time product because of its short duration, and it has been more thoroughly investigated by other workers.[1]

Table II

Residual ethylene oxide in wheat, p.p.m.

Moisture content of wheat, %	Dose, mg./l.	Method of fumigation	Residual ethylene oxide, p.p.m.	
			Top of sack	Centre of sack
9·0	6	Atmospheric	12·3	9·5
9·2	10	Atmospheric	18·2	17·0
9·0	14	Atmospheric	24·7	22·0
9·0	6	Vacuum, with simultaneous admission of air and fumigant	16·2	13·8
9·2	10	Vacuum, with simultaneous admission of air and fumigant	21·6	18·7
9·1	14	Vacuum, with simultaneous admission of air and fumigant	27·0	25·0
9·2	6	Sustained vacuum	14·7	17·0
9·1	10	Sustained vacuum	18·4	20·9
9·2	14	Sustained vacuum	25·8	30·3
12·8	6	Atmospheric	14·6	15·1
13·2	10	Atmospheric	19·7	19·2
12·9	14	Atmospheric	28·8	31·6
13·1	6	Vacuum, with simultaneous admission of air and fumigant	10·2	14·1
13·2	10	Vacuum, with simultaneous admission of air and fumigant	15·7	21·8
13·2	14	Vacuum, with simultaneous admission of air and fumigant	24·7	31·7
13·1	6	Sustained vacuum	12·3	20·2
13·1	10	Sustained vacuum	21·3	33·0
13·0	14	Sustained vacuum	24·6	38·1
16·8	6	Atmospheric	23·8	18·9
17·0	10	Atmospheric	35·9	29·2
17·2	14	Atmospheric	48·8	47·2
17·1	6	Vacuum, with simultaneous admission of air and fumigant	22·3	19·6
16·9	10	Vacuum, with simultaneous admission of air and fumigant	30·4	29·8
17·3	14	Vacuum, with simultaneous admission of air and fumigant	45·0	40·1
17·2	6	Sustained vacuum	19·1	27·0
17·1	10	Sustained vacuum	31·2	46·0
17·0	14	Sustained vacuum	40·7	67·5

One feature of Fig. 1 (*c*) calls for comment. The concentration, under sustained reduced-pressure, builds up in the grain to a level that exceeds the free-space concentration by an amount considerably in excess of the possible errors of determination. Moreover, this phenomenon is systematic in that it has been observed for the same conditions of fumigation with wheat of 17% moisture-content, and also in that it is more pronounced in the centre of the sack than in the sampling position midway between the centre and perimeter of the sack. This enhanced concentration is of sufficient magnitude to ensure that residues of ethylene oxide from the centre of the sack (33 p.p.m.) are notably higher than at the outer sampling point (21 p.p.m.). Leakage of air into the chamber on the scale necessary to produce this build-up of concentration in the grain can be ruled out. The pressure-recording manometers were checked before and at the end of the exposure to reduced pressures.

Turtle[7] found a similar effect during fumigation of 1-cwt. sacks of maize with a 1 : 2 mixture of ethylene oxide and carbon dioxide at atmospheric pressure. In this example the carbon

dioxide concentration inside the sacks rose to a value in excess of the free-space concentration, in a manner strongly reminiscent of Fig. 1 (*c*). The ethylene oxide concentrations in the sack did not exceed the free-space concentrations. As Turtle indicates, the carbon dioxide concentration inside the sack has been enriched, relative to that of the ethylene oxide, by 35 : 1, yet the two gases possess approximately the same rates of diffusion.

Some differences between the behaviour of the gases are attributable to sorption, but the enhanced concentration of carbon dioxide inside the sack, like that of ethylene oxide found during the experiments reported in this paper, requires another explanation.

One peculiarity of the phenomenon is its apparent restriction to fumigations of fairly damp wheat (moisture content greater than 10%). This restriction suggests that water, either sorbed or as vapour, plays some part in determining the enhancement of the intergranular concentration of fumigant. When the experiments, to be described in Part VIII, are analysed, with methyl bromide as the fumigant, only the drier wheat illustrates this effect.

There is evidence that evaporation of water from the seed-coat during the vacuum fumigations may account for some desorption of fumigant from the outside layers of those grains that lie towards the perimeter of the sack, but the quantity of fumigant that can be transferred in this way to the centre of the sack seems insufficient to account for the phenomenon observed.

There are a number of unexplained 'skin effects', such as that found by Turtle,[7] which have been observed when fumigants are allowed to diffuse into a packed stored product,[8] and this may prove to be a further example.

The air-washing procedure, as described in Part VI, seems to afford an inefficient removal of fumigant from the free space, and, applied at the end of the fumigations under sustained vacuum, barely compensates for the enhanced concentration in the wheat after the restoration of atmospheric pressure, even after two cycles have been completed; see Fig. 1 (*c*). The rise of the intergranular concentration after the second air-washing cycle following vacuum fumigation, with simultaneous admission of air and fumigant, resembles that which occurs in the first cycle after the sustained-vacuum method.

In the work described here, the samples are taken from the air-washing cycle at reduced pressure, the cycle being considered to end at this stage, whereas Brown & Heuser[1] end their cycle on a restoration of atmospheric pressure. The discussion of the residual fumigant found after hydrogen cyanide fumigations (Part VI) applies, with one important modification, to ethylene oxide fumigations. The moisture content of the wheat influences the sorption much more strongly with ethylene oxide than with hydrogen cyanide, despite the generally lower sorption of ethylene oxide on wheat.[9] Table II shows how these residues, for wheat of the three moisture contents employed, vary according to the method of application. For a given increase in the moisture content, sorption not only increases but does so more rapidly at the higher moisture content of the grain. Further, a covariance analysis, in which the concentration–time products are used as a concomitant variate, discloses that the changes in the concentration–time products associated with differences of moisture content are insufficient to account for the variation of the residual fumigant. Sorption on damp wheat, in fact, is so heavy that further fumigant moves into the sack to replace that sorbed, and is itself sorbed, to an extent that more than accounts for the initial interstitial dosage. The same covariance analysis failed to disclose any differential sorption associated with differences between sampling sites or doses of fumigant, apart from those accounted for by concentration–time product alterations. In particular, no such difference associated with reduced pressure was detectable, although when hydrogen cyanide was used the sorption did increase disproportionately in the sustained-vacuum method.

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CONTENTS

PAGE

Unsolved problems in the preservation of food : the influence of cultural conditions on the quality and preservation of fruits and vegetables 161
By R. G. Tomkins

Analytical studies on the carbohydrates of grasses and clovers. IV.—Further developments in the methods of estimation of mono-, di- and oligo-saccharides and fructosan 167
By Clare B. Wylam

The fluorine and chromium contents of treated aluminium surfaces 173
By R. Holt

The properties of the algal chemicals. II.—Some derivatives of laminarin 176
By W. A. P. Black and E. T. Dewar

Soil fumigation. I.—The sorption of ethylene dibromide by soils 184
By Peter Wade

The field fumigation of groundnuts in bulk 192
By L. A. W. Hayward

A rapid method for the determination of nitrogen, phosphorus and potassium in plant materials 195
By A. J. Cavell

The organic acids of grass extracts 200
By C. W. Davies and R. B. Hughes

Fumigation of agricultural products. VII.—Penetration and sorption of ethylene oxide in wheat fumigated at reduced pressures 205
By A. K. M. El Nahal

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