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FOOD AND THE FUTURE

PRESENT-DAY FACTORS LIMITING FOOD PRODUCTION*

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MR. LE GROS CLARK'S paper[1] has shown how far the food production of the world falls short of the essential needs of the present population if it is to exist at a reasonable standard of health, and he has further shown how the solution of this problem is made doubly difficult by the rapid increase of population that is taking place. The function of this paper is to discuss the factors that are limiting food production from the land at the present time; future papers will be devoted to methods of overcoming these limitations. Naturally in places it is hardly possible to discuss the factors limiting food production without indicating the means that can be used to neutralize them, but any discussion on this latter aspect is incidental to the main theme of this paper.

The basic needs of plants and animals

Land plants have a number of essential requirements if they are to make good growth: they need an adequate depth of soil, an adequate water supply, an adequate number of days when there are no serious frosts, and an adequate food supply. Further, most plants need a soil that is adequately aerated. Thus there are no immediate prospects of growing large amounts of vegetation in deserts, unless water is brought in from elsewhere, or in Antarctica or in the northern tundra belt, or on rock outcrops or the bare rocky sides of mountains.

Not all land plants can be used as human food. The human body cannot directly use celluloses or lignins for its nutrition, so that the primary sources of vegetable food are plant organs that store starches, sugars, oils and proteins in fairly high concentration, e.g. the seeds of the cereals and the roots or tubers of various plants, such as potatoes, yams and cassava, the seeds of many types of pea and bean, and the oil from the seeds or fruits of plants such as the olive (and now cotton and oil palms). Leaves, which always contain a fairly high proportion of cellulose, can at most form only a minor part of the food supply.

However, many human beings derive much of their food from certain animals which feed, or can be fed, primarily on plant foods. Some animals, such as the hen and the pig, have very similar food requirements to the human being, as they also are unable to digest celluloses or lignins. But others, and in particular the ruminants, differ in the important respect that they have evolved a mechanism for utilizing some of the energy contained in cellulose though not in lignin; hence they can be fed entirely, or almost entirely, on the leaves of plants—which in practice means the leaves of some grasses and some leguminous plants.

The natural vegetation on much of the earth's surface was forest before man exerted any appreciable influence on it. But forest trees provide very little food either for man or for any of the animals on which he feeds. Hence forested areas at the present time make almost no contribution to the feeding of the human race; indeed large tracts of land on which human food is grown are kept out of forest only by the labours of the farmers and peasants who are tilling that land.

Human beings, then, can use only a restricted number of crops for food production, but the list of plants actually utilized is still more restricted because only a few plants either naturally yield, or have been selected to yield, a reasonable amount of food per acre. Nor is this the full story of limitations, for human beings have strong food preferences and will tend to grow a crop that they like to eat, even if it means a certain loss of food per acre, rather than one they do not like which gives a higher yield. Thus wheat is the cereal preferred to all others for bread, so it is grown in areas more suited to oats or barley, though in the past people living in these

* Read before the Food Group (Nutrition Panel) on 10 December, 1952

areas had to put up with barley or oatmeal. Again, in the sub-tropics and tropics maize is preferred to the sorghums, although it is a more exacting cereal to grow, particularly in its greater sensitiveness to drought. The most sought-after tropical cereal for human consumption is rice, particularly paddy or wet rice, but it typically grows in well-watered valley-bottoms or flat alluvial lands with high rainfall, i.e. in conditions in which no other cereal will grow. There is also an upland rice grown in drier conditions which usually gives lower and more erratic yields, and it is probable that this is grown in areas where it does not give as good a yield as sorghums or millets, but this loss of yield, and therefore of food supply, is accepted as the price of the increased palatability of the diet.

Factors limiting food production in the United Kingdom

Anyone travelling in England during the growing season cannot but help being impressed by the great variation in the growth of crops on different fields or farms. Unfortunately no systematic work seems to have been done on the causes of these large variations, though there are now enough isolated lines of evidence available for a partial discussion to be possible. But, at the outset, a distinction will be made between the hill lands of the north and west on the one hand and the lower lands of the south and east on the other, for the problems are so distinct that a general discussion covering both would hardly be profitable.

Most of the land in lowland England is used for agriculture, and through most of the southern and eastern areas the climate is sufficiently similar for it to have no very great effects on agricultural production. Hence the main causes of variation in crop production between different farms are differences in inherent soil fertility and in the skill and ability of the farmers themselves. Table I gives an example of differences in crop yields on four classes of soils, all occurring in a restricted area of East Anglia. The data have been taken from the results of routine farm surveys made by the agricultural economists at Cambridge [1a] for the years 1946 to 1950 and the areas selected are: the silts in the Holland Division of Lincolnshire, the fens in the Isle of Ely, the clays of West Cambridgeshire and Huntingdonshire, and the chalks of South Cambridgeshire. But it must be borne in mind when examining this Table that the data have been derived from farms in these districts; so, although these are the dominant soil types, they will not be the only soils occurring on each farm.

Table I

Average crop and milk yields on various soil types in East Anglia, 1946–50

	Wheat, cwt./acre	Barley, cwt./acre	Sugar-beet, tons/acre	Potatoes, tons/acre	Milk yield, gal./cow
Silts	30·6	24·4	13·2	9·4	575
Fens	26·6	21·7	12·6	8·8	630
Clays	18·6	18·1	8·3	5·8	570
Chalks	19·7	18·6	8·4	4·9	550

Table I shows quite clearly that the crop yields are systematically highest on the silts, somewhat lower on the fens, and appreciably lower on the clay and chalk soils. These differences are almost certainly due to inherent differences in soil fertility, for they can hardly be due to climate, which is very similar over this small area, or to differences in the average skill of the farmers between the different areas. It was to demonstrate this point that the last column, average milk yield per cow, was inserted, as this is much more dependent on farming skill than on soil fertility. Table I shows that it is almost the same on the silts, clays and chalks; the higher figure for the fens may be solely a reflection of the higher water-tables there, which allow grass to grow longer in the summer, giving both more hay in winter and better grazing in summer.

It is more difficult to bring forward figures showing the effects on production of the varying skills of the farmers themselves, as one can never be certain one has eliminated the full effects of soil variation. However, Table II, taken from a study of the causes of variation of productivity on a number of farms in the South Midlands and South of England made by the agricultural economists of Reading University,[2] illustrates the kind of data available. The statisticians computed a yield index for each farm which takes account of the yields of both crops and stock, and put the index at 100 for the average yield of each of the commodities produced.

Table II shows that low yields run parallel with low expenditure on manures, labour etc., and although average rents fall somewhat with yield, the fall is not pronounced. Hence on the face of it low yields are due not so much to inherent low soil-fertility as to low expenditure, and this could be either because these farmers were very short of money,

Table II

Farm expenditure in the South Midlands and south of England at different levels of farm yields, 1946–49

Yield index	Per cent. of farms	Milk yields per cow, gal.	Expenditure in £ per 100 acres on			
			Rent and rates	Manures	Labour	Total
Under 85	29	490	136	57	592	1163
85–99	25	610	146	69	661	1293
100–114	24	670	142	85	705	1401
115 and over	22	750	158	115	820	1695

or because they had not the ability to use more money profitably. The Table indicates that the latter must be, in part at least, to blame, for it is very unlikely that shortage of working capital could be solely responsible for the great variation in the average milk yield per cow, for this is dependent, to a large extent, on good management and skill. Independent evidence for this conclusion can be found in some results obtained by Plant[3] in a survey he made into the causes of low yields on a number of farms in Herefordshire in 1945. He found that 70% of all low-yielding fields were short of phosphate, and 70–80% of all low-yielding wheat fields were short of nitrogen. Here is a case where a very small extra expenditure of money, at a time when money was fairly readily available on the farm, could have increased yields appreciably, but it was not spent because of lack of skill or knowledge.

It is very difficult to estimate how far yields in the country as a whole could be increased if all farmers used as much fertilizers as those having the highest yields ; for if it is the less skilful farmer, and not merely the farmer with less money, who uses less than the average amount, and gets less than the average yield, he would increase his yields appreciably only if the fertilizers were as effective on badly farmed as on well farmed land. A good farmer taking over poorly farmed and half-starved land can often get striking increases in yield by the proper use of fertilizers, but it does not follow the bad farmer would get these, for the fertilizers sometimes benefit the weeds in the crop more than the crop itself. But if one assumes that responses as a whole will be as high on poorly farmed as on well-farmed land, yields of cereals and grasses could be increased by 10–20% with a very modest expenditure on fertilizers. Yates & Boyd[4] have estimated that, for the United Kingdom as a whole, 0·25 cwt. per acre of nitrogen, given to cereals as an ammonium or nitrate fertilizer, will increase the yield of starch equivalent by about 3 cwt. per acre. Other data indicate that the crude protein in a cereal or grass crop can be increased by 1–2 cwt. for each 1 cwt. of nitrogen used, and if the management is good both of these increases can be obtained simultaneously on grassland even when rates as high as 2 cwt. per acre of nitrogen are used. However, increases such as these need a skill in farming that is much above the average.

One can thus summarize the causes that bring out the variability of farm yields found in a restricted district. Low yields are correlated with low expenditure on fertilizers as well as low general farm-expenditure, and they are also undoubtedly due in part to lack of skill on the part of the farmer. But it is not yet possible to estimate how far the present farmers on the lower yielding lands could put up their yields if they were lent the money to buy additional fertilizers, for if the low yields were due more to bad farming than to lack of capital, the fertilizers might be less effective on these badly farmed areas than on neighbouring well-farmed ones.

The upland areas of England and Wales

So far this discussion on the factors limiting food production has been confined to the lowland areas of England, but there are very large areas of hill lands that are producing only a fraction of the food per acre produced by the lowland areas. Anyone who has walked over Dartmoor, or on the Welsh hills or the Pennines, will have seen large areas of semi-derelict land, and have noticed that the upland pastures usually grow more rushes, bracken and unpalatable grasses, and a good deal less of the nutritious grasses and clovers, than do typical good lowland pastures.

The principal factor limiting production, or at least making high production difficult, is the climate, which is characterized by high rainfall and low rates of transpiration. This means that adequate drainage is essential for any kind of productive agriculture. Further, the climate limits the types of crop that can be grown, for neither seed-time nor harvest are likely to be dry. This rules out cereal cropping and makes even haymaking a difficult and time-consuming process which produces a fodder of only low feeding-value. Present-day agricultural techniques can help here, for haymaking can be replaced by silage-making, which needs far shorter periods of relatively dry weather. However, silage has the disadvantage that it possesses a much higher

water content than hay, so that it is much heavier per unit weight of dry matter ; hence moving silage in wet weather is likely to damage farm-tracks much more severely than moving hay, since between three and four times as much weight has to be shifted.

The other agronomic factors limiting production are a great lack of plant nutrients in the soil, and the natural vegetation. Fertilizers and lime can be brought into the uplands and the poor natural vegetation can be replaced by a more productive one if the land is drained, ploughed and re-seeded with higher-yielding strains of grasses and clovers, or even with green crops such as rape and kale. Now these crops are all fodder-crops for sheep or cattle, so that actual products of these lands must either be meat, or else young stock for fattening or breeding on the lowland farms. However, these crops can be converted profitably into animal products only if they are grazed either by cattle or sheep, or possibly by cattle alone, but the improved pastures cannot be maintained by sheep alone ; yet the normal animal in these areas is the sheep. Replacing sheep by cattle, however, introduces many new problems, for cattle are not as hardy as sheep, so they need much more attention in winter ; in fact they usually need to be given fodder during winter, whereas sheep, if used at a low stocking per acre, can be left to find their own. Hence the introduction of cattle will almost certainly mean introducing more men into these areas ; these men must know how to handle cattle as well as sheep, and how to manage the improved pastures, which will soon revert to their initial unproductive condition unless they are expertly managed.

The most intractable problem of increasing productivity in the hills can now be appreciated. It is to get more men with new farming skills into these areas, and to alter the skills of the men, largely shepherds, who are already there. Yet these are the very areas where men, and particularly the more enterprising and intelligent men, are unwilling to live. This unwillingness applies with still greater force to the women, for life in these areas is still isolated and primitive, and these men and women are no longer content to live under the conditions that their fathers and mothers before them had to endure.

There is no easy solution to this human problem. It is almost certain that no return of men to these hills can be expected until the amenities of these areas have been vastly improved, by the building of all-weather roads, of houses fitted with running water and electric light, by bus services to take their children to school and the men and women themselves to market towns for shopping and entertainment. Although these would all be very costly, they could be provided relatively quickly if it ever became the policy to do so ; but it might also require a complete change in outlook, almost a new faith in the future of the hills, to persuade the more intelligent men to undertake the difficult task of introducing new methods of farming into these marginal areas rather than remain content to earn a competence more easily in the lowlands.

Factors limiting food production in the world as a whole

The factors limiting food production can be divided into three distinct groups. First there are the inherent factors of the site : lack of soil or of water, lack of plant nutrients and too short a growing season for useful plant-growth. Then there are factors that harm the growing crop, such as plant and insect diseases and pests. Finally there are the human factors, which themselves fall into several groups. First there are those concerned with lack of skill, and these can usually be put right by education. Then there are those due to lack of desire to produce more food. This may be due to an inherent dislike of work and an acceptance of a low material standard of living involving little work in preference to a high material standard of living involving harder work ; or it may be due to debility caused by disease, which can only be cured when the prevalent diseases are controlled ; or it may be due to discouragement caused by excessive demands of landlords, money-lenders or tax collectors, which can be alleviated only by political action. Social and religious customs can also act as a brake on production. Thus so long as a man must buy his wife for a fixed number of cattle, no matter in what condition they are so long as they are living, there is no possibility of improving the quality or productiveness of these animals. Nor if religion is held to forbid the slaughter of cattle, so that many are left to roam the common lands, can there be any possibility of improving the quality of the communal pastures. Altering these customs and taboos must in the nature of things be a very slow process, if one wants to rely on the methods of democracy rather than those of the police state. Finally, if too many people have to feed themselves from too little land, farming practices are adopted that are likely to cause serious erosion and loss of yield, so that bad farming-practice hastens the arrival of the day when the community must suffer catastrophe or disperse. The limitations imposed by too high a density of population will probably be found to be the most intractable of all these sociological problems.

Limitations of production due to disease come primarily within the province of the scientist

but also within that of the local administrative officers. The scientist's problem is to recognize the symptoms of the various diseases, discover their causes and devise methods for reducing the damage they cause to crops. But after all these scientific problems have been solved, the methods of control developed must be put into practice. These methods may only involve changing the variety of seed used, or some minor alteration in crop management, in which case simple demonstrations are normally adequate to get at least a reasonable proportion of the farmers to adopt them. But the methods often require co-operation between different people, and can be used effectively only if backed by administrative action, such as the requirement that only seed certified free from the disease can be used, or that certain methods of husbandry must be followed to prevent the disease being carried over from one crop to the next. Such administrative acts can be effective only if there is an honest and enthusiastic band of inspectors and local administrators willing to try to make the methods work. We are accustomed in this country to assume that such men will always be forthcoming in adequate numbers, but it is an assumption that unfortunately is not yet true for many parts of the world. These administrative problems are still more difficult when the methods require co-operation between different nations, particularly if some are much richer than others, as can be seen in the difficulties facing the international locust-control organization. However, it seems reasonable to assume that lack of scientific knowledge will be the principal factor limiting the effective control of crop diseases and pests for a considerable time to come.

Site factors limiting yield

The principal site-factors limiting yield are, as already stated, too little soil, too short a growing season, too little water, or a lack of plant nutrients. But before the importance of these is discussed, it must be realized that we cannot always recognize what factors are limiting crop yields in any particular instance; probably the best example here is paddy rice.

Rice is second only to wheat as the preferred cereal for human consumption, and it forms the basis of the food supply of nearly half the world's population. The common types of paddy are grown in water, so their yields cannot be limited by lack of water. Their cultivations are confined to tropical and subtropical regions, so cold can hardly affect them seriously. Merely adding fertilizers to the soil often does not have much effect, and simple nutrient deficiencies do not therefore seem important. Yet the yields of paddy vary enormously between countries, as is illustrated in Table III.[5]

Table III

Total acreages and average yields of paddy rice in 1948–50

Country	Acreage, millions of acres	Av. yield (unhusked rice), cwt. per acre
Africa	67	10
Asia	212	13
India	74	8·8
China	45	20
Japan	7·3	32
Italy	0·34	37

The variation of yield between the different countries is most striking, and no good explanation can yet be given of their cause. Here, then, lack of scientific knowledge is the principal factor limiting yields. It takes little imagination to realize the beneficent political consequences that would follow if India could quadruple her average yield of rice in the next decade, though her average yield would still be below that of the Italian or Spanish peasant. Naturally a certain amount of research on methods of increasing the yields of rice in Asia is going on, but it is nowhere near commensurate with the magnitude of the problem or the great probability of finding practical methods to bring about spectacular improvements. The methods will almost certainly involve introducing a great many changes into the methods of cultivation; merely changing the variety of seed or applying a fertilizer is unlikely to be sufficient. Thus Ramiah and his co-workers[6] at Cuttack in India have already shown that by using better varieties of rice, better water-management, and a simple manuring-programme, yields on native areas can be doubled in certain parts of India, and under some favourable conditions they can be increased to over 50 cwt. per acre.

Limitations of crop yield due to cold

No crops can grow if the weather is too cold, and in particular all crop growth ceases in periods of frost. Now there are extensive areas in Russia and North America, and much smaller

ones in Europe, where the principal factor limiting crop growth is the length of the frost-free season in summer. In these northern regions living conditions are hard for man and beast, so hard that it is not usually economically worth while trying to keep animals, although the Eskimo and the Lapps keep or hunt herds of reindeer which feed on the tundra mosses. The crops grown on the edge of these regions are the cereals—wheat, barley and oats; and the problem of importance is to select or breed varieties that need the minimum number of days to come to maturity after being sown. The spring wheats furnish an excellent example of what has been achieved. In this country a spring wheat like Atle is sown in February or March and harvested about six months later. But at the turn of this century the Canadian wheat-breeders had introduced wheats that needed only four months to come to harvest, and now they have wheats that can be harvested only 100 days after sowing. Each week that can be taken off the growing period of a wheat means that another large area of land can be brought into cultivation; and at the present time, it is believed, the principal factors limiting wheat production in the Canadian prairies are lack of men and lack of markets, rather than lack of suitable land.

Limitations of yield due to lack of water

Lack of water is probably the most important factor limiting crop yields in the world today. Crops need water to keep their leaves cool, for if they had no mechanism for dissipating the sun's energy they absorb except that of black-body radiation, their leaves would get too hot for their vital processes to proceed properly. Hence the cooler the weather, and the shorter the period the crop takes to come to maturity, the smaller the amount of water it needs.

The problems of wheat-growing are typical of those encountered in growing crops under restricted rainfall, as wheat is the typical crop of the non-equatorial semi-deserts, such as the steppes of Russia, the north of the Sahara, the Great Plains of the United States and the Pampas of the Argentine, and the desert fringe in Australia. The characteristic of these regions is uncertain rainfall, good one year and deficient the next, so wheat yields are extremely variable, fluctuating from complete failures to yields comparable with the average in the United Kingdom. As an example of the control that climate exerts, one can compare the wheat yields in Australia and New Zealand, where the farmers are of the same level of intelligence and (presumably) skill, but the Australian grows his wheat in dry areas and the New Zealander in moist areas. In Australia there are about 11–15 million acres of wheat, yielding 8–9 cwt. per acre, about the average for the world as a whole. In New Zealand there are only 120–150 thousand acres yielding 20–23 cwt. per acre, about the same as in the United Kingdom.

Lack of rain is not the only factor limiting wheat yields in the semi-desert. Fungus diseases such as rusts, insect pests such as locusts and other hopping insects, hot dry winds and hail can all take a large toll of yield even if there is adequate water. The first method of combating these is to breed wheat for frost-resistance so that it can be sown in the autumn and come to maturity early in the summer; thus it uses less water and also tends to escape the rusts and insects, which are most prevalent in the hotter, drier part of the summer.

Another factor that may limit yields in these regions is loss of soil structure, with consequent increased liability to soil erosion. In this country we would deprecate growing the same crop on the land every year, so the problem of whether continued cropping of these semi-arid regions to wheat is going to remain feasible or not is important. In the first place wheat and the other small grains are the only crops of commercial value that will grow there, as all the usual alternative crops need too much water. It is possible that the structure can be maintained by allowing the land to fall down, or be seeded down, to natural prairie and to be left like this for several years without disturbance or grazing, but the problem still appears to be unimportant. The real problem of the dust bowl of America in the thirties was not loss of structure, or continued cropping, as has so often been stated, but lack of rain for several years running; no system of agriculture could prevent very serious soil blowing under conditions like that.

Use of irrigation to overcome lack of rain

Irrigation is the practice of taking water either from rivers or wells and applying it to the land to make good a water-deficiency in the soil that is due to lack of rain. Even in humid temperate countries like England, yields are limited by lack of rain, and this even in years like 1951 which give the impression of being wet, though in which May and early June are dry. Penman[7] at Rothamsted has just begun an irrigation experiment on a sandy-loam soil at Woburn in Bedfordshire, and in 1951 he found that by applying 2–3 inches of water as irrigation during the summer, to supplement the natural rainfall, yields of grass, barley, sugar-beet and early potatoes were all very definitely increased.

The amount of irrigation that can be practised, even in England, is limited much more by the amount of water available than by the amount of irrigable land. Thus in the valley of the Nile and in the Punjab, for instance, there are large tracts of irrigable land unirrigated solely for this reason. The area of land irrigated could be increased if the waters normally flowing in the rivers could be held back when the crops were not needing it and released later when they are. Suitable sites for reservoirs on the principal rivers flowing through the desert must be provided by the topography of the land—they cannot be made by engineers—and to a large extent the amount of water stored in such reservoirs is determined more by the site than by any limitations on dam size imposed on the engineers because of the inadequacy of their building materials. Thus the Nile, which almost ceases to flow into the sea at the height of the irrigation season, pours large volumes of water into it at other periods of the year, because not enough works at the head waters of its rivers have yet been constructed; and at one of these at least, Lake Tana in Abyssinia, the problems are political rather than technical.

Once one begins to irrigate land in desert and semi-desert areas, adequate amounts of water must be used per acre, otherwise the land soon becomes sterile. For irrigation water always contains salts dissolved in it, and most of the salts put on the land when it is irrigated remain in the soil after the crop has used the water. Hence irrigated land is always liable to accumulate soluble salts in it, and if the salt concentration in the soil rises too high, so also does the osmotic pressure of the water in the soil, and plants cease to be able to use it. It is therefore essential to use some of the irrigation water for dissolving these salts and washing them down beyond the root range of the crop, either into drains or into a deep ground-water. Now in any area where the density of population is high and subsistence farming is being practised, i.e. where the primary function of the irrigation scheme is to feed and clothe the cultivators and their families, the administrators are always tempted to let the maximum number of people have some water, by letting the water be spread just thick enough for crop growth but not thickly enough for flushing out the salts. This does not matter for a few years, but crop yields then begin to fall and land begins to go out of cultivation, and it can be reclaimed only by the use of large quantities of water, a scarce and literally vital commodity. This dilemma is already confronting those responsible for allocating water in some of the Punjab schemes.

Limitation of production due to lack of nutrients

In many areas of the world, crop production is possible only if there is an adequate supply of fertilizer, because the soil is virtually lacking in some essential plant-nutrient. Large areas of Canada, Australia and South Africa have soils so deficient in phosphate that crop production is entirely dependent on the supply of superphosphates, a fertilizer which still requires sulphuric acid for its manufacture. Even in England during the war, extensive areas of land on the chalk could be brought into cultivation only by using potassic fertilizers, and many old pastures over the whole country could grow arable crops only if given superphosphate.

There are, however, very large areas in the tropics where the soils seem to lack almost all plant foods. They occur in areas where the land surface has been subjected to high temperatures and rainfall for many millennia, and probably for millions of years, and where the only soil-constituents that have been able to resist this intense leaching are quartz, kaolinite and iron oxide, all of which are devoid of plant food. Admittedly under natural conditions they are covered with tropical forests, but all the plant food is in the trees themselves or in the top few inches of soil; and when these have gone, so also have the plant nutrients.

Limitation of production due to soil erosion

Soil erosion can be due to two quite different processes: wind may blow the soil away and rain-water may wash it away. In either case the land which has lost the soil will have lost productivity, but so also will the land on which the soil is re-deposited, for it is never deposited in the form in which it was removed. Serious wind erosion is confined to the semi-arid regions of the world, and then only to soils containing large numbers of particles in the range 0·5–0·1 mm. In general the soils that blow most, such as soils in the dust bowl of America, are loess soils, which were blown there at an earlier period.

Water erosion is due to rain falling faster on the soil than it can soak into the soil, so some of it runs off the surface if it is on a slope, however gentle. Now rain-water can only enter a soil at an appreciable rate through pores at least 60μ in size, so unless there are a large number of pores exceeding this size in the soil surface itself, heavy rain will have to run off the land, and in doing so is liable to carry away much soil with it. This has two disadvantages. In semi-arid regions, or in regions with dry summers, water is already sufficiently scarce to limit crop growth, and the more fertile top-soil is being removed. Hence erosion increases the effective

aridity of the climate and the poverty of the soil. This increasing poverty may not be very serious if the subsoil itself is fertile and amenable to cultivation, but it can be disastrous if it is poor in plant nutrients and difficult to cultivate.

The basis of all methods of preventing erosion is to keep the maximum amount of the soil covered either with a crop or with a vegetable mulch which can take the momentum and dissipate the energy in the falling raindrops ; for soil crumbs, when wet, are usually so soft mechanically that fast-falling raindrops can disrupt them, allowing their constituent particles to block all the coarse pores down which water can enter the soil quickly. Hence in areas liable to heavy storms, the proportion of the land that can be bare at any one time must be very much restricted. This can be achieved in two ways. Methods of cultivation can be developed in which the soil surface and the plant residues on the soil surface are undisturbed but the soil below the surface is cultivated and made into a seed-bed. This involves discarding the mould-board plough with which we are so familiar and developing entirely new types of machine. Alternatively, and usually in addition, some of the land must be left in grass or allowed to revert to forest for periods of a few years, so that the soil particles can once again become aggregated together so firmly that they will not break down easily when they are wetted or when rain falls on them. This will allow the necessary wide pores to have a fairly long life.

This latter requirement of keeping a proportion of the land under grass or forest to allow it to recover its natural tilth raises very difficult problems in areas where subsistence farming is being practised and the population is increasing. Not only will it be difficult to prevent the increasing population shortening the interval for soil recuperation, but also they will tend to break up for human food crops every available piece of land, even that on steeply sloping hills, where surface erosion occurs most easily. The great advantage of a reasonable density of population is that they can afford to use some of their land for feeding livestock, hence they can keep all easily erodable land in pasture and rotate arable with grass on the rest ; and the great disadvantage of a rapidly increasing population is that it becomes very difficult to enforce good conservation practices, even if these begin by increasing the yield per acre.

Factors limiting animal production

Animal husbandry can be practised under two quite separate conditions : in areas where only animal food crops can be grown, and in areas suitable for human food crops. The first type of husbandry is practised by the Asiatic and African nomads, the ranchers in America, the Argentine and Australia, and the mountain and hill shepherds and graziers, who, however, have often to rely on lower land to carry their stock through the winter. The fundamental factor limiting production in the desert and semi-desert areas is obviously rainfall, and the direct factor is usually overgrazing, owing to graziers trying to keep too many stock in the area, particularly during the more arid years. A dictator could unquestionably increase the production from many of the ranges by limiting stock-numbers to the carrying capacity of the land, by allowing stock access to each grazing area only at a particular season each year (thus allowing all palatable and nutritious grasses and herbs to make reasonable growth before being eaten by the stock), and by using water-spreading devices such as very low contour banks or furrows to spread any water running down hillsides or valleys over the maximum area of the land. The normal difficulties of enforcing these policies on the nomad are that their population numbers are getting much too high for them to live off their flocks in their own grazing areas, and on the rancher are lack of adequate experimental and demonstration work to show him exactly how he ought to manage his range, and the economic benefits he would get by proper management. It would also often mean that several ranchers would have to put a co-operative scheme into practice in each river-catchment area.

A further limiting factor, already pointed out earlier, is that nomads tend to measure their wealth by the head of stock they own, and not by the amount of human food—milk, milk products or meat—that it produces. Until they can be persuaded to slaughter all the poorer animals and breed from the better and keep them properly fed, so long will grass be converted to creatures more resembling bags of bones than carriers of good meat.

The principal factor limiting animal production on tillable land is lack of food. The land can be used to produce either human food—with animals consuming the by-products, such as straw of a cereal crop or sugar-beet leaves and pulp from a sugar-beet crop—or animal food—grasses, roots or kale. The more abundant the human food supply or the higher the standard of living, the greater the proportion of the land that can be used for animal production. But the scarcer the food supply, the lower is the proportion of land that can be spared for animal production, since it takes 4–12 acres of animal crop to produce the same amount of human food as 1 acre of human food crop.

Even if food is adequate, other factors can still limit animal production. Of these, disease is the most serious. There are large areas in Equatorial Africa where no cattle can be kept because they would be infected by various trypanosomes carried by tsetse fly. Again even in England, disease takes a very heavy toll of animals. Thus it is reckoned that the average cow has less than three lactations, yet there are a number of cows in normal herds in this country that have ten or more, and this is due primarily to the incidence of disease of one kind or another.

Climate seems to play a very minor role in limiting animal production. Human beings can breed and grow up in almost all parts of the world, and although no one animal is as adaptable as man, some breed of domesticated ruminant can be found adapted to every part where there is food. At the present time all the productive breeds of animals have been built up by over two centuries of careful selective breeding from local stocks by a large number of farmers in the temperate regions, and it is quite true that these breeds are much less productive when transferred to the tropics. But there is no reason to assume that one could not build up, in a few decades, breeds that are much more productive than these temperate ones by starting with the relatively unproductive local breeds adapted to the tropics by using the best present-day breeding techniques, if the need were really urgent.

Conclusions

1. In countries with well-developed agriculture, farmed by educated farmers, production is limited by lack of farming skill as well as by poor soils. It is probably also limited to some extent by lack of capital.

2. In many of the hill lands of this country, production is limited by a lack of suitable men and women willing to live the rather hard and isolated life that would be their lot at the present time.

3. In the world at large, factors limiting food production can be divided into those due to site, those due to disease, and those due to the characteristics of the local societies living on the areas.

4. Lack of soil, too short a growing season, and lack of water and poverty of soil are all important factors limiting production. The plant breeder is helping to reduce the importance of a short growing-season, irrigation can sometimes make good the lack of water, and manuring and a suitable system of crop rotations can alleviate soil poverty.

5. Animal production is limited mainly by lack of land available for growing animal food, but disease, particularly insect-borne and mite-borne diseases, can be very important.

6. The outlook of the farmer himself is also important. Probably the most important property of the farm community is its density per acre. Too high a density of a subsistence-farming population almost always involves bad farming-practices, leading to erosion of the soil or salt efflorescences in irrigated regions, which in turn lead to reduced yields per acre. Another important limiting factor, particularly in the sub-tropics and tropics, is the great prevalence of debilitating diseases such as malaria and intestinal worms, which sap the desire and the ability to do much work in a day.

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THE COMPOSITION OF BRACKEN: SOME MAJOR- AND TRACE-ELEMENT CONSTITUENTS

By JAMES G. HUNTER

Periodic samples of bracken taken during the growing season have shown that the concentration of major elements (except calcium and sodium) decreases with age in fronds, and remains relatively constant in rhizomes, whereas trace-element concentration varies widely. Soil type has little effect on the mineral composition of fronds. Examination of three rhizome types revealed no wide differences in the content of major elements; pinnules generally contained higher concentrations of major and trace elements than fronds. Comparison of results with those published elsewhere for moorland plants grown under similar conditions indicates that bracken contains rather more potassium and less molybdenum.

Introduction

Previous investigations on the composition of the bracken plant (*Pteris aquilina* Linn.) have been carried out principally to determine the usefulness of the frond as a source of potash, the suitability of the frond and rhizome as food, and the nature of the toxin apparently present in the frond.

Berry[1] and Berry *et al.*,[2] investigating the value of bracken ash as a fertilizer, recorded the potash content of fronds from a number of areas, samples being taken from some at intervals during the growing season; high concentrations of potash were found in the young fronds, but older plants gave larger yields per acre. Hendrick[3, 4, 5] published proximate-analysis figures for bracken rhizomes sampled several times during a year, and in one paper[5] gives figures for fronds also. Smith & Fenton[6] reported on the potash content in the ash of fronds throughout several seasons, commenting on the high concentration of potassium in young fronds, and recording the changes in the nitrogen, phosphorus and potassium contents of rhizomes (unconnected with the frond samples) sampled periodically between April and September. Ferguson & Armitage[7] and Moon & Pal[8] determined the composition of bracken 'stem' and 'leaf' (presumably the petiole plus rachis and pinnae) at several stages of growth, giving many figures for major-element and organic constituents. Shearer[9] investigated the poisonous properties of bracken, recording the nitrogen, phosphorus, potassium, calcium and sodium contents of fronds sampled six times between May and October.

Schwabbe[10] approached the subject in another way by studying the phosphorus and potassium requirements of bracken grown in sand culture, and describing the symptoms produced when these nutrients were deficient.

The trace-element content of bracken has received little attention. Simpson[11] has described a lime-induced chlorosis of bracken growing on a calciferous sand, and Hunter[12] has described a chlorosis due to manganese deficiency (the manganese content of the fronds being given); Moon & Pal[8] give figures for chlorine in 'leaf' and 'stem' samples.

The present paper provides further information on the composition of bracken. It records the concentrations of some major and trace elements in fronds and rhizomes sampled at various stages of growth, the uptake of these elements by the fronds, their distribution between pinnules and entire fronds, and between rhizome types, and the effect of soil type on the composition of fronds. The author has already briefly reported on the concentration of major elements in fronds and on the rate of uptake of these elements.[13]

Change in composition of fronds and rhizomes with growth

Two series of bracken samples were used in examining the changes in composition which occur in fronds and rhizomes with growth. The samples were obtained from Ballochraggan (Perthshire), the bracken experimental area of the West of Scotland Agricultural College. The first series of samples, consisting of fronds only, were taken from an area of uniform bracken (elevation 500 ft.) during 1942, at intervals from May to October. Frond and rhizome samples from another area (elevation 600 ft.) formed the second series, the sampling being between January and December 1944. The bracken was more vigorous in the second area.

The soil was similar in the two areas, a typical profile description being:

> Very dark brown sandy loam, mull surface horizon, 0–4 in.
> Dark red-brown stony loam, B horizon, 4–15 in.
> Loose disintegrating rock (Old Red Sandstone conglomerate).

Analyses of typical surface horizons gave the following results:

pH		4·3
Loss on ignition		17%
P_2O_5	soluble in 2·5% acetic acid	< 1 mg./100 g. of soil
K_2O		20
CaO		14
MgO		4

Fronds and rhizomes were sampled and prepared for analysis as follows: Between 100 and 200 fronds were selected at random, cut off about 2 in. above ground level, and the lower part of the petiole was wiped with a clean damp cloth. Rhizomes were obtained by selecting three plots (each about 2 sq. yd.) at random at each time of sampling, removing and discarding the fronds, and digging up (and including as one sample) all the rhizomes below the cleared plots; these were scrubbed in cold running water, broken or bruised pieces were cut out and discarded, and surface moisture was removed with a dry cloth.

The data determined for both series of samples are recorded in Tables I and II. It will be noted that sampling dates differed in the two series, the first sampling of the second series corresponding to the second sampling of the first. Moreover, the sampling intervals were not evenly spaced and this has been indicated by reporting the number of days between the sampling date and the date on which the shoot had expanded but the pinnae had not unfolded.

Fronds

Examination of the data in Table I shows that in both series the weight of the fronds became greatest about 80 days after the initial stage, the fronds of the second series being heavier than those of the first; after 80 days the weight decreased almost as rapidly as it had increased. The final stage of this decrease was probably related to the drying-out of the fronds (as shown by the large increase in percentage dry matter), but the initial decrease was not associated with a corresponding increase in percentage dry matter and appears to have been related to translocation of material from fronds to rhizomes, being accompanied by an increase in percentage dry matter in the rhizomes. The change in percentage dry matter in the fronds therefore seems to occur in three stages: (i) a period of rapid increase due to assimilation and to maturing of the fronds, (ii) a period during which the percentage dry matter is relatively constant, when assimilation is proceeding in the mature fronds and material is being translocated to the rhizomes, and (iii) a period at the end of the season when the senescent fronds are losing water and the percentage dry matter is increasing rapidly.

The percentages of ash, nitrogen, phosphorus, potassium and magnesium in the dry matter of the fronds decrease as the age of the fronds increases. The decrease was rapid in each case for about 50 days after the initial stage; thereafter, the ash content remained relatively constant, the nitrogen and potassium contents continued to decrease, though less rapidly, and the phosphorus and magnesium contents decreased less rapidly, eventually becoming relatively constant. The magnesium contents in the second series were considerably lower than in the first, and the potassium contents (except in the young fronds) were higher.

Calcium in the dry matter behaved very differently from the elements already described, the percentage increasing gradually until the frond became senescent (120 days). The sodium results were erratic but they also increased for about 100 days, and thereafter remained relatively constant. Both calcium and sodium contents were consistently lower in the second series than in the first.

The trace-element contents reported in Table II are subject to analytical errors of $\pm$ 10% or more;[14] generalization is therefore more difficult from these results than from the major-element figures. The results in both series agree quite well, though the iron, manganese, titanium, barium, strontium and chromium concentrations are uniformly higher in the second series, possibly indicating greater soil contamination in that series.[14]

The results show, however, that the cobalt, chromium, vanadium and silver concentrations remained relatively uniform throughout the sampling periods, and that the molybdenum, iron, zinc, and lead concentrations, though also relatively uniform, were slightly higher in the young and in the old fronds. The nickel and tin concentrations decreased rapidly for 50 days from the initial stage and then remained relatively constant, whereas the manganese and strontium concentrations increased gradually with the age of the frond. The results for the remaining three elements—copper, titanium and barium—are less easy to interpret: as the fronds became older, the copper concentrations decreased in the first series and remained relatively constant in the second; titanium and barium concentrations remained relatively constant in the first series, though they increased in the second.

Table 1

Composition of bracken sampled periodically: major-element constituents and other data

Predominating condition of fronds	Date sampled	Age *	Wt. of 100 fronds, kg.	Dry matter, %	% in dry matter						
					Ash	N	P	K	Ca	Mg	Na
1st series (fronds)											
Pinnae not unfolded	15 May	0	0·56	9·2	11·20	4·31	0·64	4·65	0·15	0·26	0·06
1 pair of pinnae unfolded	25 May	10	1·34	13·6	8·52	3·32	0·50	3·72	0·16	0·21	0·06
2 pairs of pinnae unfolded	10 June	26	3·05	19·0	7·74	2·74	0·35	2·88	0·20	0·18	0·13
7 pairs of pinnae unfolded	2 July	48	3·45	33·8	5·60	2·25	0·23	2·23	0·25	0·17	0·10
Fully expanded (many tips brown)	3 Aug.	80	6·00	32·8	5·20	1·87	0·14	1·64	0·37	0·17	0·20
Many pinnae brown	11 Sept.	119	5·50	34·0	4·93	1·47	0·10	1·34	0·51	0·16	0·19
Almost completely brown	5 Oct.	143	3·70	45·1	4·54	0·60	0·10	0·71	0·50	0·15	0·10
2nd series (fronds)											
1 pair of pinnae unfolded	22 May	0	2·55	14·1	8·40	3·31	0·41	3·64	0·11	0·19	—
2 pairs of pinnae unfolded	19 June	29	4·35	20·0	6·96	2·63	0·13	2·81	0·15	0·15	0·08
7 pairs of pinnae unfolded	11 July	51	6·56	26·3	6·23	2·37	0·19	2·57	0·19	0·14	0·08
Fully expanded	25 July	65	7·68	28·9	5·95	1·94	0·15	2·48	0·28	0·11	0·07
Fully expanded (many tips brown)	8 Aug.	79	8·55	26·0	6·45	2·12	0·12	2·69	0·29	0·11	0·14
1st pair of pinnae brown	29 Aug.	100	6·52	30·7	6·29	2·02	0·10	2·15	0·35	0·10	0·15
Almost completely brown	27 Sept.	129	4·74	32·9	6·80	1·40	0·09	2·00	0·41	0·09	0·14
Completely brown	28 Oct.	160	3·86	35·2	5·79	1·01	0·10	1·25	0·40	0·09	0·13
2nd series (rhizomes)											
Unexpanded	18 Jan.	− 124		26·8	6·60	1·35	0·073	1·33	0·16	0·15	0·24
Unexpanded	17 Apr.	− 34		24·9	6·53	1·14	0·062	1·24	0·19	0·21	0·20
1 pair of pinnae unfolded	22 May	0		28·3	6·69	1·36	0·064	1·40	0·28	0·29	0·22
2 pairs of pinnae unfolded	19 June	29		22·7	7·95	1·14	0·054	1·24	0·37	0·36	0·25
7 pairs of pinnae unfolded	11 July	51		19·8	7·03	0·90	0·045	1·05	0·35	0·34	0·24
Fully expanded	25 July	65		20·6	7·23	1·09	0·053	1·29	0·26	0·28	0·28
Fully expanded (many tips brown)	8 Aug.	79		20·8	5·71	1·05	0·051	1·24	0·18	0·18	0·26
1st pair of pinnae brown	29 Aug.	100		24·5	6·17	1·07	0·060	1·30	0·17	0·20	0·24
Almost completely brown	27 Sept.	129		25·1	5·85	0·96	0·056	1·08	0·18	0·21	0·25
Completely brown	28 Oct.	160		27·1	6·39	0·81	0·063	1·05	0·15	0·18	0·23
Completely brown	27 Dec.	220		27·8	6·23	1·12	0·058	1·20	0·18	0·24	0·25

* Number of days since shoot expanded but pinnae not unfolded

10 June	0·53	20 *	0·22	146	83·6	51	7·7	2·3	20·5	6·1	27	5·9	3·4 *	0·25	0·1						
2 July	0·31	4·0	0·14	64	122	37	5·7	1·5	11·0	3·9	20	5·5	0·4	0·15	0·1						
3 Aug.	0·24	0·63	0·13	116	122	47	3·3	0·8	7·5	6·6	36	10	0·4	0·30	0·8						
11 Sept.	0·28	1·43	0·15	121	216	61	9·8	1·3	8·4	6·9	34	12	0·6	0·49	0·2						
5 Oct.	0·29	1·57	0·25	188	209	83	10·7	1·8	6·6	9·9	37	15	0·6	0·63	0·2						
2nd series (fronds)																					
22 May	—	—	—	—	101	—	—	—	—	—	—	—	—	—	—						
19 June	0·30	11 *	0·13	336	150	67	8·8	3·1	13·0	19	22	6·8	1·7	0·38	0·08						
11 July	0·22	3·06	0·04	213	207	45	5·1	1·7	11·0	11	35	8·2	1·1	0·26	0·06						
25 July	0·29	3·60	0·16	257	229	46	5·3	1·7	8·5	15	38	10	0·9	0·61	0·10						
8 Aug.	0·24	1·34	0·15	167	319	39	4·8	0·9	7·5	22	60	15	0·7	0·47	0·05						
29 Aug.	0·38	2·16	0·16	208	350	73	4·2	1·4	8·6	34	68	22	0·7	0·53	0·10						
27 Sept.	0·37	2·24	0·22	327	474	72	10·3	1·5	11·0	34	86	23	0·7	0·47	0·17						
28 Oct.	0·65	2·64	0·37	530	370	110	9·2	2·0	10·0	66	99	20	1·5	1·75	0·23						
2nd series (rhizomes)																					
17 Apr.	0·80	4·60	0·43	790	132	49	2·9	2·8	10·0	111	124	12	2·0	2·20	0·17						
11 July	1·07	6·40	0·46	838	121	64	6·6	3·9	10·5	97	154	17	1·7	2·10	0·31						

* Unexplained abnormal value

Table III

Major-element and trace-element uptake by 100 fronds (expressed as percentage of greatest amount determined)

Date sampled	Dry matter	N	P	K	Ca	Mg	Na	Co	Ni	Mo	Fe	Mn	Zn	Pb	Sn	Cu	Ti	Ba	Sr	Cr	V	Ag
1st series																						
15 May	3	6	1	7	1	4	1	4	9	10	2	1	4	1	12	8	5	2	1	5	2	3
25 May	9	16	33	21	3	11	3	15	36	10	9	4	14	7	38	19	14	5	2	20	14	19
10 June	30	43	74	52	12	31	19	60	100	31	27	12	21	24	44	76	21	22	13	*	14	16
2 July	59	71	97	81	31	59	29	69	40	38	24	35	31	36	58	82	28	33	25	42	16	32
3 Aug.	100	100	100	100	76	100	100	90	11	62	73	59	68	35	52	94	79	100	80	71	56	*
11 Sept.	95	75	68	78	100	89	90	100	23	67	72	100	82	100	81	100	79	90	87	100	88	100
5 Oct.	85	27	67	37	88	75	80	92	23	100	100	86	100	97	100	70	100	87	100	89	100	89
2nd series																						
22 May	16	25	29	22	6	28	—	—	—	—	—	5	—	—	—	—	—	—	—	—	—	—
19 June	39	49	59	41	19	53	23	33	100	24	46	18	40	48	72	60	20	14	13	70	16	25
11 July	78	87	97	74	47	98	44	49	52	16	58	48	53	55	80	100	24	44	32	92	21	36
25 July	99	91	100	93	89	99	59	83	28	89	89	69	79	73	100	99	43	63	51	100	64	79

The percentage uptake figures are given in Table III. The actual amounts absorbed are illustrated by the results given below for the August sampling in the first series, calculated as weights of elements in 100 fronds.

N	37		Co	0·5		Ti	13		Fe	228	
P	2·8		Ni	1·3		Ba	71		Mn	240	mg.
K	32		Mo	0·3		Sr	20				
Ca	7·3	g.	Zn	93	mg.	Cr	0·8	mg.			
Mg	3·4		Pb	6·5		V	0·6				
Na	3·9		Sn	1·6		Ag	1·6				
			Cu	15							

As would be expected, the elements fall into two well-defined groups, namely, major and trace; iron and manganese were in greater amounts than the other trace elements. The minuteness of the amounts of the trace elements (especially the essential element molybdenum) in the fronds is noteworthy.

The results in Table III show that the amount of dry matter increased steadily for 80 days after the initial stage and then decreased, probably because of translocation of constituents to the rhizomes. It is possible that towards the end of the season parts of the fronds, which were then brittle, might have been lost before or during sampling, but this is unlikely to have seriously affected the results; some loss of dry-matter components may also have been due to leaching from the frond during rain.

The uptake of nitrogen, potassium, magnesium and sodium likewise increased for about 80 days and then decreased, again presumably because of translocation to the rhizomes; considerable amounts of nitrogen and potassium were apparently returned in this way. The rate of phosphorus uptake was greater than that of the other elements, 75% of the total phosphorus being absorbed in less than 30 days; a high percentage of the total amount of phosphorus in plants is often absorbed at an early stage.[15] Calcium, on the other hand, accumulated in the frond for 100–120 days, remaining relatively stable thereafter; calcium is generally recognized as being relatively immobile in the tissues.[16]

The trace-element results for the first series are satisfactory in that general trends are apparent; the results for the second series are more erratic, though generally confirming those for the first. The analytical errors and those from contamination, which were mentioned previously, must be taken into consideration when interpreting results. The amounts of manganese, copper and barium taken up by the fronds are seen to have increased rapidly at the beginning of the season, and to have remained constant or decreased slightly towards the end; the amounts of the other trace elements increased more gradually and were greatest when the fronds were old. The uptake of trace elements is thus very different from that of major elements, in that there is no evidence of return of significant proportions to the rhizomes.

Rhizomes

The major-element composition of the rhizomes (Table I) was relatively constant throughout the sampling period. The difficulty in obtaining uniformity in samples was great because of the limited number of sampling areas used each time, and because of the various proportions of the several rhizome types which can occur.[17]

Certain trends, however, are apparent, though they are mainly ill-defined. The reduced dry-matter content between 19 June and 8 August, when the fronds had expanded and the main movement of carbohydrate and other substances from fronds to rhizomes had possibly not been completed, may be significant; Millard & Stubbs[18] found that starch reserves in the rhizomes were low from about the beginning of June to the middle of July, and this is in fair agreement with the results obtained here. Also, a decrease in phosphorus content in the June–August period, and increases in calcium and magnesium contents in the May–July period are indicated.

The trace-element contents of two rhizome samples were determined and the results are recorded in Table II. The very high iron and titanium concentrations may be significant, but it is much more likely that they were due to soil contamination, in which case the other results are suspect. For this reason, trace elements were not determined on the other rhizome samples.

It is clear from the results that translocation of one or more elements from the ageing fronds to the rhizomes was not substantiated by corresponding increases in the rhizomes. This could be explained if the increase in total dry-matter constituents of the rhizomes had been sufficient, or more than sufficient, to counteract any increase in percentage of an individual element. The fall in phosphorus concentration and the rise in calcium and magnesium concentrations in the rhizomes about the June period may have been due to a difference in rate of

movement, phosphorus being relatively rapid in movement when compared with carbohydrate and other substances being translocated, and calcium and magnesium relatively slow.

The results in Table I show that the rhizomes contained lower concentrations of nitrogen, phosphorus and potassium than the mature fronds, about the same concentration of calcium, and higher concentrations of magnesium and sodium.

Composition of rhizome types

The rhizomes whose analysis is described in the previous section were composite samples taken irrespective of the types distinguished by Braid,[17] and the possibility of sampling error due to composition differences between these types has already been mentioned. Rhizomes were therefore taken in October from two areas of bracken on the Countesswells soil association[19] in Aberdeenshire. The bracken from which Sample 1 was taken was well developed, and rhizomes were found to a depth of several feet ; Sample 2 was from much poorer bracken growing at a greater altitude and in a more exposed position, and the rhizomes were confined to a thin layer of soil and rock-detritus mixture. When sampled and cleaned, the rhizomes were separated into the three types described by Braid (Leaders, A, Secondaries, B, and Tertiaries, C), weighed and analysed ; the results are recorded in Table IV. These show that differences occurred in the contents of the three rhizome types, the Leaders usually containing the highest concentration and the Tertiaries the lowest. The differences, however, were not very great (except perhaps in the calcium and magnesium contents of Sample 2), and it is unlikely that any significant error resulted from the small variation in proportion of types which probably occurred in the periodic samples.

Effect of soil type on frond composition

To determine the effect of soil type on frond composition, a bracken area was chosen in each of eight distinct soil associations[19] in Aberdeenshire. In the Tarves association, two additional areas were chosen within 100 yards of the first ; the bracken in one of these was growing extremely vigorously as compared with the normal for the region, whereas the other yielded a sample of typical woodland bracken, all other samples being from open country.

About the end of August, fronds which were almost fully expanded, or newly so, were sampled from these areas as previously described. Analysis was carried out as before, but, in addition, the pinnules from fronds forming part of each sample were separated and analysed. The soil profile in each area was also examined and a sample of soil was taken from the main rhizome zone, the A1 horizon in all except one case (Countesswells association), where the zone was the A1 + A2 horizons.

The soils were analysed, and the results of the analysis and other data are reported in Table V. It will be seen that the parent material was usually boulder clay formed from rocks of acidic to ultra-basic types. Most of the soil pH values were less than 4·5, the lowest being 3·6, but the pH of one sample was 5·4 and of another 6·2 ; a wide range of soil pH was therefore considered. The phosphate content of the soils was invariably low, the lime and magnesia usually low and the potash moderate to high. The amounts of trace elements extracted by acetic acid did not differ greatly from soil to soil, and with one exception were within the ranges of results given, for all the elements except iron and silver, by Mitchell ;[20] the exception was the nickel content of the Leslie association, which was abnormally high, but similar and higher values have been found by Mitchell[21] and Hunter & Vergnano[22] in soils of serpentine origin.

The composition of the fronds and pinnules, and other data, are given in Tables VI and VII. Samples showed considerable differences in vigour, as estimated by height and weight of fronds, and in pinnules/frond ratio, but no consistent relationship was apparent between these measurements.

Fronds

The values for the major-element percentages in the dry matter of frond samples were as follows : nitrogen, 1·31–1·96 ; phosphorus, 0·09–0·22 ; potassium, 1·02–2·95 ; calcium, 0·15–0·32 ; magnesium, 0·11–0·27 ; sodium, 0·07–0·24. These ranges are remarkably small considering the wide variety of soil types examined.

The ranges of trace-element concentrations (p.p.m. in dry matter) found in the fronds were : cobalt, 0·06–1·54 ; nickel, 0·60–10·7 ; molybdenum, less than 0·05–0·19 ; iron, 38–670 ; manganese, 32–484 ; zinc, 21–73 ; lead, 2·1–4·8 ; tin, 0·7–5·1 ; copper, 7·0–16·8 ; titanium, 2·3–22·0 ; barium, 14–50 ; strontium, 6–25 ; chromium, 0·2–1·0 ; vanadium, less than 0·05–0·54 ; silver, less than 0·05–0·20. Except for lead and copper these ranges are greater

Composition of rhizome types

	% by wt. of total rhizome system	Dry matter, %	% in dry matter					
			Ash	N	P	K	Ca	Mg	Na
Sample 1									
Leaders	61	28·5	4·59	1·04	0·035	1·33	0·30	0·28	0·34
Secondaries ..	25	35·6	4·19	1·03	0·037	1·25	0·26	0·21	0·21
Tertiaries	14	34·8	3·92	0·94	0·034	0·94	0·19	0·16	0·20
Sample 2									
Leaders	51	27·6	6·59	0·91	0·037	1·47	0·55	0·20	0·17
Secondaries ..	38	29·7	4·43	0·86	0·038	1·12	0·14	0·09	0·24
Tertiaries	11	34·8	2·92	0·88	0·028	0·81	0·08	0·04	0·11

Table V

Composition of soil samples from various soil types

Soil association	Parent material *	pH	Loss on ignition, %	Soluble in 2·5% acetic acid														
				mg./100 g. soil				P.p.m. in soil										
				P_2O_5	K_2O	CaO	MgO	Co	Ni	Mo	Fe	Zn	Pb	Sn	Ti	Cr	V	A
Corby	Acid-igneous and metamorphic	4·3	11	< 1	15	27	22	0·13	0·78	0·03	44	11	1·4	< 0·4	0·7	0·2	0·09	<
Countesswells	Granitic	4·0	9	1	8	23	15	0·05	0·28	0·02	133	10	1·0	< 0·4	1·4	0·1	0·1	<
Strathfinella	Arenaceous Old Red Sandstone	4·1	8	2	12	23	13	0·09	0·26	< 0·02	121	8	2·0	< 0·4	0·7	0·2	0·08	<
Strichen	Quartz schist	4·4	8	2	11	27	15	0·44	0·65	< 0·02	17	13	2·0	< 0·4	0·4	0·3	0·08	<
Foudland	Argillaceous schist	4·0	11	< 1	7	27	17	0·49	2·36	0·03	123	8	3·5	< 0·4	1·1	0·3	0·08	<
Tarves (normal bracken)	Acid and basic igneous	4·3	9	< 1	15	30	12	0·35	0·65	< 0·02	45	10	1·2	< 0·4	0·7	0·3	0·09	<
Tarves (woodland bracken)		5·4	8	< 1	15	126	43	0·52	0·49	< 0·02	15	12	0·5	< 0·4	0·4	0·2	0·07	<
Tarves (vigorous bracken)		3·6	12	1	22	56	15	0·09	0·45	< 0·02	44	12	1·2	< 0·4	0·8	0·1	0·13	<
Insch	Basic igneous	4·5	21	1	7	86	10	0·68	0·60	0·11	53	13	< 2·0	< 0·4	1·4	0·6	0·69	<

Corby	2·5	8·23	0·66	Pinnules	27·6	2·28	0·17	2·44	0·37	0·19	0·16
Countesswells	4·5	9·43	0·65	Frond	30·9	1·31	0·12	1·20	0·28	0·11	0·22
				Pinnules	30·2	1·56	0·16	1·36	0·33	0·15	0·07
Strathfinella	3	4·45	0·43	Frond	19·6	1·96	0·22	2·95	0·19	0·15	0·13
				Pinnules	23·7	3·20	0·39	3·09	0·29	0·22	0·13
Strichen	3	5·63	0·60	Frond	30·8	1·61	0·20	1·71	0·21	0·14	0·12
				Pinnules	30·9	2·61	0·30	1·91	0·26	0·20	0·07
Foudland	3·5	4·81	0·60	Frond	32·8	1·85	0·12	1·02	0·20	0·19	0·24
				Pinnules	33·4	2·58	0·17	0·93	0·25	0·28	0·24
Tarves (normal bracken)	3·5	5·42	0·42	Frond	22·3	1·61	0·12	1·83	0·21	0·14	0·07
				Pinnules	35·3	2·27	0·14	1·88	0·24	0·16	0·05
Tarves (woodland bracken)	2·5	4·08	0·57	Frond	28·6	1·85	0·09	2·12	0·32	0·18	0·08
				Pinnules	30·4	2·52	0·13	2·45	0·34	0·26	0·07
Tarves (vigorous bracken)	5·5	10·77	0·48	Frond	28·2	1·79	0·10	1·95	0·20	0·16	0·07
				Pinnules	31·3	2·48	0·16	2·05	0·29	0·20	0·06
Insch	4·5	7·95	0·60	Frond	35·1	1·79	0·16	1·45	0·29	0·16	0·13
				Pinnules	37·3	2·24	0·19	1·54	0·34	0·20	0·13
Leslie	3	5·01	0·43	Frond	32·1	1·49	0·15	1·70	0·15	0·27	0·13
				Pinnules	34·2	2·18	0·21	1·75	0·17	0·40	0·08

Table VII

Composition of bracken fronds from various soil types : trace-element constituents (p.p.m. in dry matter)

Soil association	Part analysed	Co	Ni	Mo	Fe	Mn	Zn	Pb	Sn	Cu	Ti	Ba	Sr	Cr	V	Ag
Corby	Frond	0·89	4·74	0·14	310	224	68	4·3	1·8	9·7	22	34	25	0·7	0·54	0·20
	Pinnules	1·08	4·83	0·17	346	225	65	4·5	1·5	11·3	34	25	23	0·9	0·61	0·15
Countesswells	Frond	0·17	1·16	0·05	84	484	34	3·6	1·5	8·1	3·4	31	13	0·3	0·09	0·12
	Pinnules	0·20	0·64	0·10	96	556	39	3·8	2·4	8·2	5·2	24	11	0·4	0·16	0·11
Strathfinella	Frond	0·48	3·00	0·19	670	326	73	4·8	5·1	16·8	4·0	33	14	1·0	0·25	< 0·05
	Pinnules	0·37	1·88	0·18	238	534	79	—	0·5	17·1	6·3	29	18	0·9	0·20	< 0·05
Strichen	Frond	0·06	0·60	0·18	38	154	21	3·7	0·7	12·7	6·7	50	10	0·4	0·13	< 0·05
	Pinnules	0·08	1·23	0·14	44	212	29	3·4	0·4	11·3	5·6	69	12	0·5	0·12	< 0·05
Foudland	Frond	0·72	4·42	0·07	226	409	35	4·3	1·2	10·2	3·3	27	11	0·2	< 0·05	0·11
	Pinnules	0·82	4·66	0·05	174	497	40	3·2	0·7	10·0	4·1	28	11	0·3	< 0·05	0·16
Tarves (normal bracken) ..	Frond	—	—	< 0·05	97	240	38	2·4	0·8	7·5	3·4	24	10	0·4	0·07	0·06
	Pinnules	0·24	4·48	< 0·05	144	283	48	3·7	0·7	9·2	4·6	20	10	0·4	0·12	0·09
Tarves (woodland bracken) ..	Frond	0·34	1·68	0·17	135	139	35	2·1	2·2	8·9	9·8	24	14	0·4	0·18	0·11
	Pinnules	0·43	2·48	0·22	238	338	48	3·7	1·3	10·5	13	29	14	0·6	0·27	0·12

than those found for the major elements; for example, a difference more than tenfold occurs in the cobalt, nickel, iron, manganese and vanadium ranges.

There is some degree of correlation between the major-element results and the soil analyses in Table V. The two largest concentrations of phosphorus and calcium in the fronds correspond to the two highest in the soils; there is also good correlation between the potassium figures in the plants and in the corresponding soils, and the high magnesium content of the Leslie soil is reflected in the plants from that association.

The trace-element results in Table VII, however, show little correlation with the soil analyses, though the exceptionally high nickel content of the Leslie soil is also found in the plants growing on it. This lack of correlation is not surprising; Mitchell[14] has emphasized the empirical nature of acetic acid extraction of soil, though the value of the technique has been proved in many instances. The composition of plants is the result of many factors, including soil composition, soil pH and environment; in the samples compared in this section, the number of variables was too great to allow satisfactory correlation of results.

Pinnules

The analytical results for the pinnule samples (Tables VI and VII) differ, though not markedly, from those for entire fronds. Without exception, the contents of nitrogen, phosphorus, calcium and magnesium are greater in the pinnules, whereas the concentrations of potassium, though similar in both, are also, with one exception, greater in the pinnules. The sodium contents of the fronds and pinnules are similar.

The trace-element results give no evidence of any consistent differences. In most samples, cobalt, nickel and manganese are found in greater concentrations in the pinnules than in the entire fronds. The concentrations of zinc, copper, titanium, chromium and silver in the pinnules are similar to, but usually greater than, those in the fronds. The results for molybdenum, lead, barium, strontium and vanadium are similar in pinnules and fronds and vary irregularly, but the iron concentrations vary considerably between the pinnules and fronds, but again irregularly. Finally, the tin results are similar for pinnules and fronds, but usually greater for fronds.

The composition of these samples gives no indication of a relationship between the vigour of the plants and their nutrient status; it is probable that the controlling factor in these cases was water supply, or some other feature of the environment. Similarly, the differences between the three Tarves samples cannot be explained nutritionally from the analyses. The difference in vigour between the two series of periodic samples is also inexplicable nutritionally, though the higher concentrations of nitrogen and potassium in the second series might have been significant.

Discussion

The results obtained here for frond samples are in good agreement with those published by other authors. Exceptions are the relatively low potassium content reported by Shearer[9] for a May sampling, and the sodium results reported by Ferguson & Armitage[7] and Shearer,[9] where the concentrations decrease with age of the frond. In the case of rhizomes, the results for nitrogen are comparable with those reported by Hendrick[3, 4, 5] and by Smith & Fenton;[6] potassium results are also similar to those given by Smith & Fenton, but their phosphate figures are about ten times greater.

The ranges of concentration of elements reported here are not excessive. Bracken, like most other plants, will absorb some elements to excess if sufficient amounts are present in an available form in the soil, and consequently considerably greater values are possible than are reported here. Similarly, where soils are deficient in a specific element, low values will occur in the plant, and if the element is essential, deficiency symptoms may be produced; evidence of this is given by Hunter,[12] who found that chlorotic fronds sampled at the beginning of August had a manganese content of 11 p.p.m. in the dry matter (which is lower than the lowest value in Table VII) and were presumed to be suffering from manganese deficiency.

It is of interest to compare the bracken data given here with the composition figures published for other plants, particularly moorland species and hill pastures which, like bracken, grow mainly in uncultivated soils. Of particular interest is the potassium content of bracken fronds, which is frequently regarded as unusually high, though Ferguson & Armitage[7] have stated that bracken may contain less potassium than ordinary pasture grass. An attempt to evaluate the status of bracken in these terms is made below.

Thomas *et al.*[23] determined the composition of heather sampled periodically between June and October, the edible portions being analysed and results given for phosphorus, potassium,

calcium, magnesium, sodium, cobalt, iron, manganese and copper. In general the percentage values reported are similar to those for the bracken fronds. The calcium concentrations were rather higher in heather than in bracken, but the potassium contents were lower, being less than 0·7% in the dry matter. The periodic changes in heather were less pronounced than in bracken (probably because of the type of sample selected) and only changes in potassium, sodium and manganese could be discerned; the potassium and manganese changes were similar to those noted in bracken, but the concentrations of sodium decreased slightly as the season advanced, whereas in bracken it increases.

The changes in composition that occurred throughout the growth of several species of moorland plants are described by Thomas & Trinder,[24] the data given being for the elements mentioned above. The data vary from plant to plant but the ranges covered are on the whole similar to those given here for bracken. A comparison of the composition of mature (but not senescent) plants shows that in the moorland plants phosphorus, magnesium, iron and copper contents were similar to those of bracken. The potassium and sodium contents are usually somewhat greater in bracken, and for calcium the bracken figure is similar to the intermediate values for the moorland plants. Apart from a high concentration in one species (*Vaccinium myrtillus*), the manganese contents in the moorland plants were also comparable to those in bracken. The cobalt content of bracken, however, is usually considerably higher, but the cobalt status of the moorland species may have been a characteristic, not of the plants, but of the particular soil on which they were grown. Though change in composition with age did not occur consistently in the moorland plants, it is clear that trends were often similar to those noted in bracken; the changes in magnesium and sodium contents differed from those in bracken, magnesium being exceptional in that the initial decrease was often followed by an increase towards the end of the season, and sodium tending to decrease with age.

Thomas & Thompson[25] give the composition of various grasses and herbs grown on long-term manurial plots sampled in June. The results for the no-treatment plot are suitable for comparison with the bracken data, particularly as the soil pH was low (4·8). Of the seven species described four contained more phosphorus, calcium and sodium than fully-expanded bracken fronds, one had more potassium (none had less) and one had markedly less magnesium. The cobalt, iron, manganese and copper contents were all comparable with those for bracken.

Mitchell[20, 26] gives the contents of some trace elements in pastures from north-east Scotland, and in cocksfoot, rye-grass and red clover from a granitic soil. Figures are given for all the trace elements found in bracken except silver, and the data are usually in close agreement. Comparison shows that the cobalt value for the Insch fronds and the nickel value for the Leslie are considerably higher, iron values sometimes higher, and the molybdenum and tin contents considerably lower.

Mitchell[27] has also reported the trace-element composition of several moorland plants sampled periodically over three seasons. Concentrations varied with the plant species, but on the whole the ranges of values given do not differ widely from those for bracken, though the molybdenum content of bracken is lower and the iron and tin contents are rather higher. In the moorland plants, changes in composition with age were usually similar to those taking place in bracken, though often less pronounced.

Nitrogen concentrations are not dealt with in the papers mentioned above, but the nitrogen content of bracken compares well with that of good grass, and Moon & Pal[8] have shown the digestibility of the crude protein of fairly young bracken to be reasonably satisfactory.

These comparisons do not indicate any serious abnormality in concentrations of the bracken constituents studied. The potassium concentration in the fully expanded fronds is rather higher than is found in the moorland plants (including heather), but is similar to that in the grasses and herbs, and it is probable, as suggested by Ferguson & Armitage[7] that ordinary pasture grass may contain higher concentrations. In the young fronds the potassium concentration was considerably higher than in the mature fronds; it is higher than any of the values given by Thomas *et al.*[28] for eight grasses grown on fertile soil and sampled periodically, but not higher than in some herbs and legumes reported in the same paper. There is abundant evidence (e.g. Berry *et al.*;[2] Beeson[29]) that other plants, especially if grown on soil which has received potassic fertilizer, can have potassium concentrations as high as or higher than that of the young fronds, but it may be that young bracken contains higher concentrations than other plants growing on similar soils under similar conditions.

The concentrations of the other major elements in the bracken are probably not exceptional for plants grown on similar soils, and the concentrations of the trace elements iron, manganese and copper are not markedly different from those in the moorland plants. The cobalt content, however, is definitely greater than in the moorland plants described by Thomas & Trinder[24]

and Thomas *et al.*[23] (though not greater than in those described by Mitchell[27] or in pastures), and the molybdenum concentration is relatively low.

Summary

1. The concentrations of nitrogen, phosphorus, potassium, calcium, magnesium, sodium, cobalt, nickel, molybdenum, iron, manganese, zinc, lead, tin, copper, titanium, barium, strontium, chromium, vanadium and silver have been determined in bracken fronds and rhizomes.

2. The changes in concentration which occurred with growth have been described. Nitrogen, phosphorus, potassium and magnesium contents in the fronds decreased with age of frond, and calcium and sodium contents increased; considerable differences were found in the changes in concentration of trace elements. The major-element composition of the rhizome was relatively constant throughout the growing season; some of the trace-element results are probably of little value because of soil contamination.

3. The percentage uptake figures for the above elements in the frond have been recorded. The uptake of nitrogen, phosphorus, potassium, magnesium and sodium increased for about 80 days and then decreased (possibly because of translocation to the rhizome), the uptake of phosphorus being more rapid, and that of calcium, slower; the amounts of manganese, copper and barium in the frond increased rapidly at the beginning of the season, but those of other trace elements increased gradually through the season.

4. The major-element contents of three rhizome types have been determined. No large differences were found.

5. The major-element and trace-element contents of fully expanded fronds from ten areas (including eight distinct soil types) and of soil from the rooting zones, have been determined. The ranges of concentration found for each element were not wide.

6. The major-element and trace-element contents of pinnules have been determined. Differences were not great but the elements were usually in higher concentration in the pinnules.

7. Results obtained have been compared with those published by other authors for bracken and other plants. It was concluded that though the major-element and trace-element composition of bracken is not exceptional, rather more potassium and less molybdenum are present than in other plants grown under similar conditions.

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SOUTH AFRICAN FISH PRODUCTS. XXXIII.*—The Rock Lobster : the Composition of the Hepatopancreas Oil

By S. P. LIGTHELM, L. NOVELLIE, H. M. SCHWARTZ and M. M. VON HOLDT

The main sites of fat deposition in the rock lobster are the hepatopancreas and the gonads. Data on the yield and characteristics of the oils from these organs are given. The composition of the saponifiable and unsaponifiable fractions of the hepatopancreas oil was determined. The component acids (weights per cent.) are : saturated acids C_{14} 1·1, C_{16} 10·3, C_{18} 8·1, C_{20} 4·2, C_{22} 0·5 ; unsaturated acids C_{14} 2·4 (— 2·0 H), C_{16} 7·3 (— 2·1 H), C_{18} 14·3 (— 2·9 H), C_{20} 28·4 (— 6·5 H), C_{22} 19·0 (— 8·3 H), C_{24} 2·6 (— 2·1 H) and C_{26} 1·8 (— 2 H). Cholesterol and α-glyceryl ethers are the main components of the unsaponifiable fraction.

An examination of the lipids of the rock lobster, *Jasus lalandii* M. Edw., was undertaken as part of an investigation of potential products from the waste of the rock-lobster fishery in South Africa and South West Africa (cf. Part XXXII of this series).

Data on the distribution of the lipids between the various parts of the body are given in Table I. These figures together with those published by Karnovsky, Rapson & Black (1946) show that the lipids are located mainly in the gonads and the hepatopancreas, the hepatopancreas being the main fat depot. The variations encountered in the oil content of these organs and in the characteristics of the oils extracted from them are shown in Table II. The oils are highly coloured, the hepatopancreas oils being greenish brown and the gonad oils deep red. They do not contain vitamin A (negative Carr–Price test). The gonad oils contain a high proportion of phospholipids, the hepatopancreas oils appreciably less ; thus the figures for the phosphorus contents of the oils in Table I correspond to 35% phospholipid (as lecithin) in the gonad oils and 1% in the nepatopancreas oils. The high phospholipid content of the gonad oils may be expected to cause trouble owing to emulsion formation during extraction of the oil by the alkali digestion process, which is generally used in South Africa for the processing of fish livers and viscera, and, in fact, troublesome emulsions were frequently encountered in the laboratory during extraction of the gonads by this method. Hence the later extractions were made by desiccating the tissue with anhydrous sodium sulphate and extracting with ether in a Soxhlet apparatus (see Table II).

The potential yield of hepatopancreas and gonad oil from rock-lobster waste is approximately 150 tons per annum. It is doubtful, however, whether this represents an economic

Table I

Distribution of lipids in the rock lobster

Organ	Wt. as % of wt. of animal	Oil in organ, %	I.V.	Characteristics of oil		
				Sap. value	Unsap. matter, %	Phosphorus, %
Tail	31·0	—	—	—	—	—
Cephalothorax minus viscera ..	62·2	0·5	—	—	19·3	—
Hepatopancreas	4·5	15·0	158·9	177·4	5·8	0·05
Stomach and intestine	1·3	2·8	125·4	176·7	8·6	1·27
Gonads	2·0	10·4	146·5	182·2	6·7	1·35

Table II

Yield and characteristics of hepatopancreas and gonad oils

Date	Hepatopancreas oils			Gonad oils		
	Yield, %	I.V.	Unsap. matter, %	Yield, %	I.V.	Unsap. matter, %
1945	21·4	—	5·3	6·5	—	8·5
11.12.47	7·4	168·4	5·0	4·8	165·2	4·6
20.1.48	15·4	171·1	8·3	7·2	186·3	4·3
19.2.48	19·1	157·4	3·1	—	—	—
27.2.48	14·0	167·2	5·0	11·2*	166·3	6·5
24.11.48	13·0*	161·9	6·8	8·0*	163·8	9·6
5.11.50	15·0*	158·9	6·7	10·4*	146·5	6·7

* Tissue desiccated with anhydrous sodium sulphate and oils extracted with ether
All other oils extracted by alkali digestion (Rapson, Schwartz & van Rensburg, 1943)

* Part XXXII : *J. Sci. Fd Agric.* 1951, **2**, 571 ; Part XXXI : *J. Sci. Fd Agric.* 1950, **1**, 248 ; Part XXX : *J. Sci. Fd Agric.* 1950, **1**, 182

source of oil in view of the low value of the oils and the difficulties likely to be encountered in their extraction.

Component acids of the hepatopancreas oil

Since very little is known about the composition of the oils from the larger marine invertebrates (Hilditch, 1947), it was felt that it would be of interest to make a study of the component acids of a sample of the hepatopancreas oil. This was obtained in 13·0% yield by extraction of the desiccated tissue (anhydrous sodium sulphate) with ether and had the following characteristics:

I.V.	161·9
Sap. value	172·9
Unsap. matter, %	6·8
Phosphatides (as lecithin), %	8·5

The oil (400 g.) was saponified, the fatty acids freed from unsaponifiable matter and separated into three fractions by crystallization from acetone (10 ml./g.) at — 60°, followed by two recrystallizations of the insoluble acids from ether at — 40° (Table III). Each fraction was converted to the methyl esters and fractionally distilled. The component acids of each fraction were determined as described by Hilditch (1947). The composition of the three fractions and of the saponifiable fraction as a whole is given in Table IV.

Table III

Low-temperature crystallization of acids from hepatopancreas oil

	Fraction	Weight, g.	Total acids, %	I.V.
A	Insoluble in ether at — 40°	49·8	16·1	8·0
B	Insoluble in acetone at — 60°	91·7	28·6	100·7
C	Soluble in acetone at — 60°	185·4	55·3	267·3

Table IV

Component acids of hepatopancreas oil

Component acids: increments, % wt.	A	B	C	Total
Saturated acids:				
C_{14}	0·5	0·5	0·1	1·1
C_{16}	7·4	2·9	Trace	10·3
C_{18}	5·0	3·1	—	8·1
C_{20}	1·0	3·2	—	4·2
C_{22}	0·5	—	—	0·5
Unsaturated acids:				
C_{14}	—	0·2	2·2	2·4
C_{16}	0·1	1·9	5·3	7·3
C_{18}	0·2	4·1	10·0	14·3
C_{20}	0·3	8·3	19·8	28·4
C_{22}	1·0	2·3	15·7	19·0
C_{24}	—	0·4	2·2	2·6
C_{26}	—	1·8	—	1·8
Mean unsaturation of homologous groups of unsaturated esters:				
C_{14}	—	— 2·0 H	— 2·0 H	— 2·0 H
C_{16}	— 2·0 H	— 2·0 H	— 2·1 H	— 2·1 H
C_{18}	— 2·0 H	— 2·5 H	— 3·1 H	— 2·9 H
C_{20}	— 2·0 H	— 4·0 H	— 7·6 H	— 6·5 H
C_{22}	— 2·0 H	— 6·0 H	— 9·0 H	— 8·3 H
C_{24}	—	— 3·0 H	— 2·0 H(?)	— 2·1 H
C_{26}	—	— 2·0 H(?)	—	— 2·0 H(?)

The only previous component-acid analyses of oils from marine crustaceans appear to be those of Lovern (1935) for the copepod *Calanus finmarchius* and Klem (1935) for the prawn (*Leander serratis*). For *C. finmarchius* the component acids were reported as: saturated acids (weights per cent.) C_{14} 8, C_{16} 11, C_{18} 1; unsaturated acids C_{14} 1 (— 2 H), C_{16} 12 (— 2·4 H), C_{18} 17 (— 5·1 H), C_{20} 25 (— 7·8 H) and C_{22} 25 (— 8·1 H). For *L. serratis* they were: saturated acids C_{14} 1·5, C_{16} 9·5, C_{18} 2, C_{20} traces; unsaturated acids C_{14} 0·5 (— 2 H), C_{16} 13 (— 2 H), C_{18} 32 (— 3·3 H), C_{20} 34 (— 6 H) and C_{22} 7 (— 10 H). Compared with the other two oils, the characteristic features of the rock-lobster oil appear to be its higher content of the higher-molecular-weight saturated acids, stearic acid and arachidic acid, together with the presence

of small amounts of C_{24} and C_{26} unsaturated acids. All three oils show the characteristic features which Lovern (1932) has shown distinguish fish oils of marine origin from fresh-water types.

The unsaponifiable matter of the hepatopancreas oil

The unsaponifiable fraction of the oil was examined in some detail. Squalene, saturated hydrocarbons, sterols and α-glyceryl ethers were determined by the methods described in Part XXVII of this series (Karnovsky, Rapson, Schwartz, Black & van Rensburg, 1948). The unsaponifiable fraction was chromatographed on alumina as described by Black & Schwartz (see Part XXXI). Evidence was obtained of the presence of fatty alcohols in the fraction eluted with ethyl ether. The fraction was assumed to contain only fatty alcohols and sterols, and the amount of the fatty alcohols was estimated by difference from the weight and sterol content of the fraction. The fraction eluted with ethanol–acetic acid (9 : 1) was found to contain, in addition to α-glyceryl ethers and a trace of sterols, 25% of material of unknown composition. The approximate composition of the unsaponifiable fraction is given in Table V.

Table V

Composition of the unsaponifiable fraction

	%
Sterols (as cholesterol)	41·1
α-Glyceryl ethers (as selachyl alcohol)	38·7
Squalene	0·5
Saturated hydrocarbons	1·1
Fatty alcohols	7·0
Unidentified component(s)	11·7

Chromatography of the unsaponifiable matter on alumina was repeated on a larger scale in an attempt to obtain the various constituents sufficiently pure to permit their characterization. Approximately 6 g. of unsaponifiable matter dissolved in *n*-pentane was applied to a column 18 mm. in diameter containing 60 g. of alumina. The chromatogram was developed successively with *n*-pentane, *n*-pentane containing increasing proportions of ethyl ether, ether, ether–ethanol mixtures and finally absolute ethanol, or 90% aqueous ethanol, which was found to be more effective in the elution of α-glyceryl ethers than absolute ethanol. It was not possible to achieve a clean separation of the various groups of components present in the unsaponifiable matter. This was apparently due to the fact that the column was overloaded with the sterol component, which therefore overlapped the fatty-alcohol band on the one side and the glyceryl ether band on the other. By stopping the development when the tall sterol peak appeared in the eluate–volume curve, and taking the residual material off the column and applying it to another column, better separation was obtained and it was possible to collect fractions containing mainly sterols, fatty alcohols or glyceryl ethers for further examination. Although the glyceryl ethers tended to be eluted before the unknown component or components with ethanol, it was not possible to obtain the unknown components free from substantial amounts of glyceryl ether, and their identification was therefore not attempted.

Sterols.—Fractions melting at 144–145° (uncorr.) and having $[\alpha]_D^{20}$ (chloroform) — 25° to — 30° were combined and recrystallized from ethanol to give cholesterol, melting point 147·5–148·5°, this melting point being undepressed on admixture with an authentic sample. The acetate was also prepared and it melted at 115·5°, the melting point being undepressed by the addition of cholesteryl acetate. Cholesterol is thus the principal sterol present. Small amounts of 7-dehydrosterols have also been shown to be present in rock-lobster hepatopancreas oil (Karnovsky, 1946). The 7-dehydrosterol content was, however, only 2% of the sterol fraction, i.e. approximately 1% of the unsaponifiable matter. The rock lobster is thus of little interest as a source of provitamins D, in contrast with many marine molluscs, the lipids of which are relatively rich in 7-dehydrosterols.

The α-glyceryl ethers.—Fractions which were shown by periodic oxidation to contain over 96% of α-glyceryl ethers (as batyl alcohol) were combined and recrystallized first from *iso*heptane and then from acetone, to give a solid melting at 64°. The phenylurethane was prepared and melted at 95–96°. These figures and also the C and H values indicate the presence of a mixture of batyl and chimyl alcohols.

Fatty alcohol fraction.—Separation of the fatty alcohols from the sterols was difficult. By using two columns, however, it was possible to obtain fractions rich in fatty alcohols. These were freed from sterols by formation of their urea complexes. The crude fatty alcohols so obtained melted at 37°, but it was not possible to characterize them owing to the small amount of material available.

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STUDIES ON COMMERCIAL OCTAMETHYLPYROPHOSPHORAMIDE (SCHRADAN). V.*—Insecticidal Comparisons of the Two Main Constituents

By L. A. LICKERISH

Solutions of pure octamethylpyrophosphoramide, $(Me_2N)_4P_2O_3$, and of a mixture of this insecticide with its higher homologue $(Me_2N)_5P_3O_5$, were sprayed on plants and the insecticidal effects were compared. Three species of aphids were used on chrysanthemum plants, and two species on Windsor bean seedlings.

The different insect species showed not only different levels of susceptibility to the insecticides and different relationship between dosage and response, but also different relative effects between the two insecticide samples. In particular, the mixture containing $(Me_2N)_5P_3O_5$ was found more toxic or less toxic than pure $(Me_2N)_4P_2O_3$, according to the insects used for testing.

In all cases, the insecticidal activity of the mixture was greater than would be expected from its content of $(Me_2N)_4P_2O_3$ alone; it is concluded that the higher homologue is itself an insecticide of the same order of toxicity as $(Me_2N)_4P_2O_3$, but rather more or less toxic than this according to the insects used for testing.

The insecticide Pestox III can be regarded as a mixture of two insecticides, $R_5P_3O_5$ and $R_4P_2O_3$ (R = Me_2N—) (Parts I and II). This paper describes experiments to compare the effectiveness of the various constituents as systemic insecticides. As explained in Part I it has not proved possible to isolate the second major constituent, triphosphoric acid penta(dimethylamide), ($R_5P_3O_5$, where R denotes the dimethylamino group) in a state of purity. Experiments were therefore carried out with the enriched mixtures obtained as described in Part I in comparison with pure pyrophosphoric acid tetra(dimethylamide), ($R_4P_2O_3$, octamethylpyrophosphoramide or Schradan).

Plants and insects

I. Cuttings of *Chrysanthemum indicum*, a hardy, November-flowering, bronze variety, were rooted in sand. The plants were then put into John Innes potting compost in 1½-in. diameter thumb pots. Only cuttings 3 to 4 cm. in height were used for the experiment.

The plants, after spraying with insecticide solutions, were infested simultaneously with the aphids: (*a*) *Aulacorthum circumflexum* (Buckton), the mottled arum aphid, (*b*) *Coloradoa rufomaculata* (Wilson), the green chrysanthemum aphid and (*c*) *Macrosiphoniella sanborni* (Gillette), the brown chrysanthemum aphid.

II. Seedlings of Windsor beans, *Vicia faba*, were grown in John Innes potting compost in 3-in. diameter pots. The seedlings used were 8 days old, with 2 true leaves expanded.

* Part IV: *J. Sci. Fd Agric.* 1952, **3,** 69; Part III: *J. Sci. Fd Agric.* 1952, **3,** 60; Part II: *J. Sci. Fd Agric.* 1951, **2,** 310; Part I: *J. Sci. Fd Agric.* 1951, **2,** 303.

The plants, after spraying with insecticide solutions, were infested simultaneously with the aphids: (*d*) *Aphis fabae* Scopoli; the black bean aphid and (*e*) *Megoura viciae* (Buckton), the vetch aphid.

The species (*a*), (*d*) and (*e*) were reared on seedlings of *Vicia faba* (Windsor bean) in controlled conditions in the laboratory at a temperature of 20°. Species (*b*) and (*c*) feed only on *Chrysanthemum indicum* and cannot be satisfactorily reared in the laboratory; they were reared in the greenhouse in a temperature range of 10° to 18°.

Chemicals

(1) OMPA, pure pyrophosphoric acid tetra(dimethylamide); (2) 'Mixture', 23·4% pyrophosphoric acid tetra(dimethylamide) + 76·6% triphosphoric acid penta(dimethylamide). (See Part I for method of analysis.)

In the text which follows, these samples are referred to as OMPA and Mixture respectively. For convenience in comparison of the results, the OMPA and the Mixture were both assessed as though they were distinct, pure (100%) materials.

Method

The plants were sprayed with dilute solutions of the insecticides in an apparatus specially designed for spraying small potted plants in the laboratory. The apparatus consists essentially of a compressed air atomizer, a turn-table on which the plant is rotated during spraying, and a transparent cover to exclude draughts. This apparatus will be described elsewhere.

Each plant was covered after spraying with an insect-proof cage, consisting of a transparent cellulose acetate cylinder closed at one end with bolting silk. One function of the cage is to exclude draughts from the surface of the plant, while allowing free ventilation. The maintenance of a constant atmosphere around the plants is very important when dealing with volatile insecticides (see Part III), since 'the amount of insecticide which enters the plant depends on a competition between the rate of absorption and the rate of loss by evaporation'. It should be pointed out here that, although octamethylpyrophosphoramide is sufficiently volatile to show significant losses by evaporation from sprayed surfaces, it is not sufficiently volatile to kill insects by fumigation at room temperatures. David & Gardiner (1951) showed that OMPA does not kill aphids by fumigation.

Twenty-four hours after spraying, insects were put on the plants, two species on each plant. The percentage mortality of the insects was determined 24 hours later and again 48 hours later. The chrysanthemums were then reinfested (i.e. 3 days after spraying) with *Coloradoa rufomaculata*, and further counts made.

The plants were kept throughout the experiment in a chamber with forced ventilation, controlled at a temperature of 22°, under constant illumination from 'blended' bulbs. These bulbs are of the incandescent type with a fluorescent coating on the glass; they give a radiation rather like direct sunlight, including a large infra-red component. The plants were stored in this chamber for six days before spraying, to bring the foliage into a standard condition for spraying.

Results

The results are summarized in Tables I, II and III.

Analysis of results

Examination of the results in Tables I, II and III shows the following: (1) The level of susceptibility of the insects to the insecticides is different for each species; (2) the rate of increase of percentage mortality with concentration is different for each species; (3) the relative effect of the two insecticides is different for the different species. In particular, although *Aphis fabae* shows that OMPA is more toxic than the Mixture, *Megoura viciae*, confined on the same plants at the same time, shows that the Mixture is more toxic than OMPA. (4) The relative effect of the two insecticides is different at different concentrations. For instance, the first column of results in Table I shows that the Mixture is more toxic than OMPA at 0·08 and 0·06%, but is less toxic at higher and at lower concentrations.

The aim of these experiments was to compare the insecticidal action of the mixture rich in $R_5P_3O_5$ with that of pure $R_4P_2O_3$, therefore we must derive from the results some quantitative comparisons between the two insecticide samples. The quantitative relation between percentage mortalities given in Tables I, II and III must be transformed before they can be used for quantitative analysis.

***

Table I

Mortalities of three species of aphids on cuttings of Chrysanthemum indicum *sprayed with insecticide solutions, infested one day after spraying. Temperature, 22°. For* Coloradoa rufomaculata *each figure was determined from 100 to 200 insects on four plants treated alike. For the other species, each figure is determined from 50 to 100 insects on two plants*

Insecticide	Concn., % of active ingredient	Percentage mortality of *Coloradoa rufomaculata*		Percentage mortality of *Macrosiphoniella sanborni*		Percentage mortality of *Aulacorthum circumflexum*	
		After 24 hr.	After 48 hr.	After 24 hr.	After 48 hr.	After 24 hr.	After 48 hr.
OMPA	0·12	93·4	100·0	72·0	96·1	65·6	98·5
	0·10	94·0	100·0	88·2	100·0	61·8	97·1
	0·08	89·8	100·0	92·2	100·0	58·4	100·0
	0·06	87·4	100·0	41·2	90·2	70·2	90·0
	0·04	84·3	99·2	26·0	86·0	34·2	84·3
	0·02	66·7	100·0	6·3	43·0	30·4	71·7
	0·01	55·6	97·0	—	—	24·3	67·5
	0·005	25·0	75·2	—	—	23·8	61·9
Mixture ..	0·12	90·4	100·0	40·8	93·9	45·4	87·9
	0·10	88·9	100·0	28·1	82·5	54·4	97·2
	0·08	90·0	100·0	20·6	79·5	55·1	90·0
	0·06	95·2	100·0	30·5	61·1	47·0	94·1
	0·04	82·6	100·0	19·6	53·0	41·7	91·7
	0·02	50·9	95·5	10·3	33·8	31·4	65·8
	0·01	22·6	62·1	—	—	13·3	53·4
Water	—	3·3	7·5	14·3	20·4	2·9	4·0

Table II

Mortality of aphids on cuttings of Chrysanthemum indicum *sprayed with insecticide solutions, infested three days after spraying. Temperature, 22°. Each figure was determined from 150 to 200 insects on four plants treated alike*

Insecticide	Concn., % of active ingredient	Percentage mortality of *Coloradoa rufomaculata*	
		After 19½ hr.	After 24 hr.
OMPA	0·12	83·0	97·0
	0·10	95·0	96·5
	0·08	77·1	88·2
	0·06	70·0	86·0
	0·04	59·8	81·1
	0·02	50·0	61·2
	0·01	30·8	42·6
	0·005	20·4	25·5
Mixture	0·12	80·9	93·9
	0·10	78·2	90·9
	0·08	75·8	95·1
	0·06	60·9	75·8
	0·04	56·0	68·2
	0·02	40·0	48·0
	0·01	27·7	37·1
Water	—	5·9	6·5

The usual practice in dealing with dosage–mortality relations is to transform the dosages to logarithms and the percentage mortalities to probability integrals (e.g. see Finney, 1947). This often gives a relation between the two quantities which is linear, or nearly so, and therefore is easily manipulated. We have used a simplified version of this method. The results are plotted on graph paper scaled for dosage in logarithms and for percentage mortality in probability integrals. To the results for OMPA a straight line is drawn to give the nearest fit to the points. Then for each result with the Mixture we can read off from this line a concentration of OMPA that would give the same percentage mortality. The ratio of the concentration of OMPA which would give a certain percentage mortality to the concentration of the Mixture which was found to give that percentage mortality is an estimate of the toxicity of the Mixture

Table III

Mortalities of two species of aphids on seedlings of Windsor beans sprayed with insecticide solutions, infested one day after spraying. Temperature, 22°. Each figure is determined from 200 to 500 insects on four plants treated alike

Insecticide	Concn., % of active ingredient	Percentage mortality of *Aphis fabae*			Percentage mortality of *Megoura viciae*		
		After 24 hr.	After 48 hr.	After 70 hr.	After 24 hr.	After 48 hr.	After 70 hr.
OMPA	0·10	27·0	42·8	59·5	82·5	100·0	100·0
	0·08	11·6	33·0	40·0	51·5	90·1	98·5
	0·06	5·1	23·0	35·3	40·5	84·5	92·3
	0·05	11·6	24·3	42·2	53·5	93·5	98·5
	0·04	5·9	15·5	28·2	36·8	88·1	94·8
	0·03	3·3	14·9	28·0	41·0	84·5	89·5
	0·02	1·2	6·5	11·3	17·3	54·3	71·9
	0·01	2·9	7·8	12·7	3·5	13·0	19·0
Mixture ..	0·12	7·5	15·7	37·7	96·5	100·0	100·0
	0·10	8·1	27·9	57·5	93·5	100·0	100·0
	0·08	5·5	11·0	30·0	91·5	100·0	100·0
	0·06	4·2	9·0	23·9	82·5	99·5	100·0
	0·04	8·9	18·4	30·3	78·5	88·5	90·0
	0·02	5·3	9·6	16·3	36·1	62·2	72·7
Water	—	5·2	9·7	13·1	1·6	1·9	6·9

Table IV

Estimates of the toxicity of the Mixture (regarded as a pure substance with 100% active ingredient) expressed as a percentage of that of OMPA

Insect species	Days after spraying	Exposure of insects on treated plants, hr.	Concn. of Mixture, % of active ingredient						
			0·12	0·10	0·08	0·06	0·04	0·02	0·01
Coloradoa rufomaculata ..	1	24	63	65	89	220	105	51	34
,, ,, ..	3	19½	82	84	90	58	70	72	78
		24	95	85	164	58	63	64	83
Macrosiphoniella sanborni	1	24	44	43	45	74	87	137	—
		48	52	41	48	45	59	86	—
Aulacorthum circumflexum	1	24	37	69	89	93	103	100	50
		48	35	98	72	117	129	84	76
Aphis fabae	1	48	30	62	32	37	100	—	—
		70	44	108	49	48	98	96	—
Megoura viciae	1	24	—	>100	>100	>100	400	220	—
		48	—	>100	>100	>100	100	115	—
		70	—	>100	>100	>100	78	110	—

relative to that of OMPA. This ratio multiplied by 100 gives the toxicity of the Mixture as a percentage of that of OMPA. Percentages determined in this way are given in Table IV.

When the results were treated in this way, OMPA gave good straight lines with *Coloradoa rufomaculata* and *Aphis fabae*, though the scatter was rather greater with *Aphis fabae*. OMPA with *Macrosiphoniella sanborni* gave irregular results at the higher concentrations, and so the percentage toxicities for this insect in Table IV are not very reliable at the higher concentrations. With *Aulacorthum circumflexum* the transformed dosage–mortality line had a pronounced curvature. This was rectified by using log(concentration + 0·01) for the dosage scale (for this method of rectifying relations, see Gaddum, 1945).

One fact stands out clearly from Table IV : in no case is the toxicity of the mixture as low as 23·4%, which is its actual content of $R_4P_2O_3$. Therefore $R_5P_3O_5$ must be an insecticide.

The fact that the toxicity of the mixture is generally less than that of OMPA is partly explained because $R_5P_3O_5$ is absorbed into the plant more slowly than $R_4P_2O_3$, probably at about a quarter the rate (see Part III). This is also the reason for the differences between the

effects of the two samples being less when plants are tested 3 days after spraying, than when they are tested only 1 day after spraying. Probably the differences between the samples would have been greater if the plants had been treated by watering at the roots instead of spraying. In Part III it was shown that in dahlias treated at the roots with a 39 : 57 mixture of $R_4P_2O_3$ and $R_5P_3O_5$, the concentration of $R_4P_2O_3$ was more than 5 times as great as of $R_5P_3O_5$.

Conclusions

Pure octamethylpyrophosphoramide, $(Me_2N)_4P_2O_3$, was compared with a mixture containing 23·4% of this insecticide with 76·6% of its higher homologue, $(Me_2N)_5P_3O_5$, by measuring the percentage mortalities of insects confined on plants that had been sprayed with solutions of the 2 samples. Five species of insects were used, and each species showed not only a different level of susceptibility to the insecticides, but also a different relationship between dosage and mortality for each insecticide, and a different relative effect between the 2 samples. In particular, the mixture containing $R_5P_3O_5$ was shown to be more toxic or less toxic than $R_4P_2O_3$, according to the insect used for testing.

In no case was the toxicity of the mixture rich in $R_5P_3O_5$ as low as 23·4% of $R_4P_2O_3$ which was the actual content of $R_4P_2O_3$ in the mixture.

It is concluded that $R_5P_3O_5$ is itself an insecticide of the same order of toxicity as $R_4P_2O_3$, but rather more or less toxic than this, according to the insects used for testing.

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THE AMINO-ACID COMPOSITION OF EXTRACTED GRASS-PROTEIN. I.—The Basic Acids

By R. WAITE, A. FENSOM and S. LOVETT

Protein has been extracted from four species of grass at stages of growth varying from young and leafy to old and woody. The lysine, arginine and histidine contents of the extracted protein have been determined and considerable uniformity has been found between all samples, especially those of a single species.

Many analyses of leaf protein of various plants, including grasses, have been made in the past, principally by Chibnall[1], Tristram[2] and Lugg & Weller.[3, 4] Although none of these authors were able to extract more than half of the total protein from the leaf, it seemed reasonable to conclude that the protein samples they analysed were truly representative of the whole. Their collected results, as reviewed by Lugg,[5] showed that there was no outstanding difference between the proteins of different species of grass and that no significant change in amino-acid composition occurred at different stages of growth, although there was some indication that in senescent leaves the distribution of sulphur between methionine, cystine (or cysteine, or both) might be affected.

In the past, however (e.g. Morris, Wright & Fowler[6]), there has been a tendency to attribute differences in the apparent value for milk production of grass grown at different seasons of the year to differences in the quality of its protein. In a long series of experiments on the growth, management and utilization of grass now in progress at the Hannah Institute, an excellent opportunity was afforded of obtaining grasses at different stages of growth and at different times of the year grown under closely controlled conditions, and as one part of the work it was decided to re-investigate the amino-acid content of the protein prepared from some of these

grasses using recently introduced methods of analysis. Albanese[7] had previously used electrodialysis to separate protein hydrolysates into the three amino-acid fractions, basic, neutral and acidic, and paper chromatography can now be employed to separate these fractions into the individual amino-acids. These methods have been applied in the present work to investigate the amino-acid content of samples of protein prepared from grasses cut at widely differing stages of growth, and also at the same stage of growth in spring and in autumn. The present account gives the results for lysine, arginine and histidine.

Experimental

Grasses used, and manurial treatment

Perennial rye-grass (*Lolium perenne* strains S23 and S101), meadow fescue (*Festuca pratensis* strain S53), timothy (*Phleum pratense* strain S48) and cocksfoot (*Dactylis glomerata* strain S143) were grown in small plots (36 sq. yd.) on land previously used as a short-term rye-grass ley. Two series of plots were grown, the first being sown in 1947 and used in 1948, the second being sown in 1950 and used in 1951 and 1952. The manurial treatment of the two series was the same. In February of each year superphosphate (18% P_2O_5) and potassium chloride (60% K_2O) were applied at the rate of 2 cwt./acre each, followed by Nitro-Chalk in April at 2 cwt./acre. One half of each plot was allowed to grow without check until September, when the grass was cut for protein extraction. The other half was cut when it was about 8 in. high, usually in late April or early May, and again each time this height was reached; Nitro-Chalk was applied at 3 cwt./acre after each cut. Protein was extracted from the first (spring) cut, the third (summer) cut and the fifth or sixth (autumn) cuts, all of which were in the long leafy stage of growth.

Extraction of protein

The borate buffer (pH 9·2) extraction method of Lugg & Weller[3] was used with little change. The grasses for extraction were cut about 9 a.m. and except for those which had been allowed to grow to maturity, they were used immediately. The mature grasses were dry and woody, about 30–40 in. in height and difficult to mince. Preliminary soaking in the borate buffer overnight softened the stems sufficiently to allow them to be minced. After cytolysis with ethanol–ether it was found that filtration through loosely-packed paper pulp gave a clearer liquor and purer protein after precipitation than passage through a Sharples centrifuge ($g \times 14{,}500$), although the centrifuge method was employed in the early extractions. The purified protein powder was allowed to come to moisture equilibrium in the air before moisture, ash and nitrogen contents were determined. Nitrogen was estimated by the Kjeldahl method of Hiller, Plazin & Van Slyke,[8] using a mercury catalyst which was inactivated by formation of a zinc amalgam during the distillation.

Protein hydrolysis

Two methods of hydrolysis were investigated: (*a*) refluxing with 20% hydrochloric acid for 24 hours,[7] and (*b*) heating in a sealed tube with 6N-hydrochloric acid at 105° for 48 hours. The ratio of acid to protein in the first method was 30 : 1 (v/w) and in the latter 20 : 1 (v/w). After hydrolysis the liquor was centrifuged to settle the humin and the clear solution was decanted. The humin was twice washed with distilled water and centrifuged after each washing; the washings were added to the hydrolysate and the whole solution taken to dryness *in vacuo* over phosphorus pentoxide and solid sodium hydroxide. The dry residue was redissolved in water to give a solution containing about 0·1 g. of original protein in 10 ml., of which 1 ml. was used for the determination of nitrogen by the micro-Kjeldahl method in order to estimate nitrogen loss in the humin.

Electrodialysis

The amino-acid analysis was to be confined in the first instance to the basic group, lysine, arginine and histidine and hence preliminary separation of the protein hydrolysate could easily be effected in the three-compartment cell of Albanese.[7] The cell was made of Perspex and incorporated the modifications of Macpherson.[9] The redissolved hydrolysate (9 ml.) was placed in the centre compartment and electrodialysed at 250 v. d.c. through a parchment membrane between the cathode and centre compartments, and through formolized gelatin supported on cloth between the centre and anode compartments. The progress of the separation was checked by one-dimensional chromatography, and it was found that after the initial dialysis (45 minutes), the cathode solution (referred to subsequently as the catholyte) required three further recyclings (10–15 minutes each) before complete separation was obtained. The final catholyte was partially evaporated and made up to 25 ml.

Chromatography

The catholyte was prepared for chromatography by the evaporation of 3 ml. to dryness in conically-ended 10-ml. centrifuge tubes, by blowing a current of warm air on the surface of the liquid. The solid residue was dissolved in 0·5 ml. of 0·001N-hydrochloric acid and the tube was stoppered. The equivalent of about 200 μg. of the original protein was contained in 10 μl. of this solution. Lysine, arginine and histidine were estimated on one-dimensional chromatograms from the final catholyte by a slight modification of the spot-area method of Fisher, Parsons & Morrison.[10]

Using 22 in. × 18 in. No. 4 Whatman paper, four standard solutions containing 5, 10, 15 and 20 μg./10 μl. of lysine and arginine and 2, 4, 6 and 8 μg./10 μl. of histidine were spotted in duplicate using a 10-μl. automatic pipette similar to that described by Meinhard & Hall.[11] The catholyte solution to be analysed was replicated six times, being placed between successive standards. To allow for any irregularities in the paper, the duplicates of standard spots were placed as widely apart as possible on the paper. Each spot was dried immediately over a 100-W. lamp to minimize its initial size. Four sheets, each carrying one replicated unknown and the same four standards, were run at the same time in a solvent mixture containing 2 : 4 : 6-collidine, 2 : 4/2 : 5-lutidine and water, in which the pH had been reduced to 8·5 with 2N-hydrochloric acid. These three-component mixtures are sensitive to temperature, and good separation of all three acids was obtained with a mixture in the proportions 1 : 2 : 2 only when the temperature did not exceed 10°. For more general use up to 20°, a mixture in the proportions 2 : 5 : 4 was suitable. With this mixture, however, lysine and arginine are only completely separated after 40–48 hours, by which time the histidine has run off the paper and must be estimated on a separate chromatogram run only for 8 hours. If this last course has to be taken, the standards should still include lysine and arginine, otherwise the value for histidine in the unknown will be too high. As for all accurate chromatography, the developing tank should be gas-tight, and the atmosphere inside saturated with respect to the solvent.

The sheets were dried in a forced-draught oven at 80°, sprayed with a solution of 0·2% ninhydrin in ethanol, and allowed to dry in the dark for 24 hours without heating. This method of locating the amino-acids, recommended by Patton & Chism[12] gave deeper-coloured spots, particularly with histidine, than with ninhydrin in butanol and made easier the next stage of cutting out the coloured area.

The weight of the paper occupied by each spot (20–75 mg.) was determined and the average weight of the eight replicates of each standard and of the six replicates of each unknown was calculated. Standard curves for each acid were obtained by plotting the average weight of spot (instead of its area as suggested by Fisher *et al.*[10]), against the logarithm of the concentration of amino-acid. Interpolating the mean weight of the unknown then gave its concentration. Although the slope of the standard curves varied only slightly from day to day, only those standard values obtained during a single run were used to assess the corresponding unknowns. With concentrations of amino-acids greater than 3–4 μg./μl. the areas of the spots became diffuse and the standard curves ceased to be linear.

Check of the whole method using purified casein from cows' milk

Casein was precipitated from cows' milk, washed, dialysed for three days against running tap-water and for one day against distilled water, then dried and ground. The dry material contained 15·73% nitrogen and was hydrolysed in a sealed tube for 48 hours in 6N-hydrochloric acid at 105°. The hydrolysate was electrodialysed and chromatographed as described above, and gave the following basic amino-acid analysis: lysine 6·9% ± 0·2%, arginine 3·7% ± 0·2%, histidine 1·7% ± 0·08%. Recent values by chemical gravimetric analysis for casein are: lysine 6·0–7·9%, arginine 3·7–4·2%, histidine 1·7–3·0%.[13–17] The figures obtained by the present method are therefore comparable to those obtained gravimetrically.

Results

Protein extraction

As would be expected, the protein from young leafy grass of high moisture content was more easily extracted, in greater quantity and of greater purity than from any older grasses. Thus, when the moisture content was about 87%, roughly 23–28% of the protein was extracted and contained 15–15·8% nitrogen. When the moisture content was 45–50%, as in the senescent autumn grass, the weight of protein extracted was only 15% and its nitrogen content had fallen to 8–12%. In all the extracted proteins except those from the senescent autumn grasses the ash content was 0·2–1% of the dry matter, but in two samples, the cocksfoot and fescue protein from the old grass, the ash had risen to 11% of the dry matter. Chibnall, Miller, Hall & Westall,[18]

using their ether–water method of extraction on young leafy material, record yields of extracted protein of 20–30% of the theoretical value, with nitrogen contents of 13·2–15·0% and ash contents of about 1%. Lugg & Weller[3] obtained higher yields, 30–50% of the protein, with nitrogen contents of 14·2–14·9% and ash contents of 1·4–2·8%.

Protein hydrolysis

In the hydrolysis of protein extracted from grasses and other plants it has been generally recognized that some loss of amino-acids may occur. The small amount of polysaccharide or pectin material found by Chibnall & Miller[19] and Lugg[20] to be present in most ' pure ' extracted proteins is thought to combine during hydrolysis with amino-acids in the formation of humin, but on the evidence of extensive analyses, Lugg & Weller[4] suggest that the destruction, with the exception of trytophan and tyrosine, is probably small. Armstrong[21] has more recently discussed this problem and adds the sulphur-containing acids to those seriously affected during acid hydrolysis. From the present work it appears equally important to ensure that the conditions of hydrolysis are sufficient to cause complete breakdown of the protein, particularly of the more impure proteins extracted from older herbage.

At the start of these experiments the proteins were hydrolysed according to the method of Albanese,[7] (i.e. refluxed for 24 hours with 20% hydrochloric acid), but after analysis of the basic amino-acids considerable differences were found between the young and old grasses. Although this could conceivably have been the result of the stage of growth of the grass, it was noticed that the lowest values invariably came from the protein of lowest nitrogen content. In view of this, the hydrolyses were repeated in sealed glass tubes at 105° for 48 hours in 6N-hydrochloric acid (lower concentrations than 6N-hydrochloric acid resulted in incomplete hydrolysis). Table I gives values for cocksfoot which are representative of the results for all four grasses and show the effect of this change in hydrolysis conditions. It can be seen that the sealed-tube method was considerably more effective in promoting breakdown of the more impure protein. Apart from the differences in time and temperature, the sealed tube allows much more vigorous shaking, early intimate mixture of protein and acid and greater exclusion of air. The method finally adopted was therefore to hydrolyse 0·1 g. of finely ground protein in 2 ml. of 6N-hydrochloric acid in a sealed tube for 48 hours at 105°. Under these conditions loss of nitrogen to humin amounted to 5–10%.

Table I

The effect of the conditions of hydrolysis on the basic amino-acid composition of extracted grass-protein

Conditions	Young-grass protein (14·2% N)			Old-grass protein (7·9% N)		
	Lysine	Arginine	Histidine	Lysine	Arginine	Histidine
	(% of dry protein of 16% N)					
Reflux with 20% HCl for 24 hr.	3·6	6·1	1·2	1·9	4·7	0·6
Sealed tube with 6N-HCl at 105° for 48 hr.	4·7	6·8	1·3	6·3	7·6	1·3

The basic amino-acids of the extracted grass protein

Table II records the values obtained for the proteins extracted from the different grasses cut at the various stages of growth. The values for the amino-acids have been calculated to a common basis as a percentage of each acid in moisture-free, ash-free protein containing 16% nitrogen. The young spring and autumn grasses were very leafy, the young summer grasses were slightly more stemmy but had not headed (with the exception of the rye-grass) and the old autumn grasses were dry, woody and with few, brown leaves. It will be seen that the purity of the protein samples, as judged by their nitrogen contents, varied considerably. This was chiefly the effect of the stage of growth of the grass. As grass matures the cells become more lignified, and during protein extraction the impurities which are precipitated with the protein or adsorbed on it during acidification are present in increased proportion. Successive purification in ethanol, citric acid and ether removes less from these proteins than from those extracted from younger grasses. Similar difficulties were encountered by Chibnall *et al.*[18] when dealing with grasses cut at a mature stage. The use of the Sharples centrifuge for clarification after cytolysis instead of passage of the liquor through paper pulp may also have contributed slightly to the impurity of the proteins from the older grasses, although passage of the filtered liquor through activated alumina did not result in a purer protein.

The accuracy of the amino-acid estimation was $\pm$ 4% of the values given in Table II. Variation in the purity of the extracted proteins and hence in the humin formation during

Table II

The basic amino-acid content of extracted grass-protein for four species of grass at different stages of growth, and cut in spring, summer and autumn

Grass		Date cut	N in dry protein, %	Lysine (% of dry matter, calc. to 16% N protein)	Arginine	Histidine
Rye-grass	Young spring	27.4.48	14·5	5·3	6·6	1·6
	,, ,,	24.4.52	15·4	6·4	7·8	1·8
	Young autumn	5.9.50	14·5	5·5	7·2	1·5
	,, ,,	29.8.51	14·9	6·0	8·2	1·5
	Headed summer	27.6.51	15·5	6·3	7·9	1·1
	Old autumn	23.8.48	11·4	6·2	7·8	1·5
	Mean			5·9	7·5	1·5
Fescue	Young spring	5.5.48	14·9	6·9	7·6	1·5
	,, ,,	21.4.48	15·8	6·0	6·1	1·5
	Young autumn	1.9.50	14·0	6·4	8·0	1·4
	,, ,,	26.9.51	15·2	6·4	6·6	1·6
	Young summer	30.7.51	13·4	6·0	6·6	1·3
	Old autumn	6.9.48	8·0	5·8	7·1	1·2
	Mean			6·3	7·0	1·3
Timothy	Young spring	21.5.48	12·6	6·5	7·5	1·5
	,, ,,	21.4.52	15·8	5·9	6·8	1·7
	Young autumn	7.9.50	13·8	5·0	6·2	1·3
	,, ,,	18.9.51	15·0	6·1	6·9	1·3
	Young summer	9.7.51	13·4	5·5	7·5	1·6
	Old autumn	18.8.48	13·3	5·3	6·5	1·7
	Mean			5·7	6·9	1·5
Cocksfoot	Young spring	3.5.48	14·2	4·7	6·8	1·3
	,, ,,	28.4.52	14·8	4·9	7·7	1·7
	Young autumn	11.9.51	13·4	4·9	8·6	1·5
	,, ,,	24.8.51	15·0	5·3	7·5	1·2
	Young summer	16 7.51	13·7	5·0	8·1	1·2
	Old autumn	26.8.48	7·9	6·3	7·6	1·3
	Mean			5·2	7·7	1·4

hydrolysis probably accounts for the major differences between nominally similar samples. In any one species of grass the chief feature of these results is the great similarity of the values, despite the considerable differences in stage of growth. Even between the four species the differences are not great, although the cocksfoot appears to have a slightly lower lysine content than the other three. The mean values are very similar to those found by Tristram[2] and slightly higher than those of Miller[22] for the same grass species.

Discussion

It would be of considerable importance in animal nutrition if there were any variation in the amino-acid composition of the protein of the commonly-used grasses from season to season or at different stages of growth. The present results, in agreement with those of Chibnall[1] and Lugg & Weller,[4] suggest that in the protein as extracted there is little or no such difference in the basic amino-acids. The view that the protein extracted by the present and similar methods is representative of the protein of the whole plant, including that remaining in the residue, has already been discussed by Lugg & Weller,[3] Lugg[20] and Chibnall.[1] Recently Smith & Agiza[23] have published results for the amino-acid content of a large number of grass samples, for one of which, an Italian rye-grass, the values are given at three different stages of growth. In general, the figures given by these authors for arginine and lysine (histidine was not determined) are lower than those reported here, although the lack of data for individual samples makes comparison difficult. Their results for the Italian rye-grass, and to a lesser degree for red clover and barley, show a marked decrease in lysine and arginine content in the older samples. Their method of protein hydrolysis, however, 30–36 hours in 6N-hydrochloric acid in a boiling-water bath, may not have been rigorous enough to break down completely the more impure proteins (8·9–15% nitrogen). A comparison using the hydrolysis conditions of Smith & Agiza and the present method on four proteins is given in Table III, where it can be seen that hydrolysis by refluxing resulted in lower values when the purity of the protein was not high. This agrees with the experience already described which led to the choice of hydrolysis conditions for the present work.

Table III

A comparison between the lysine and arginine content of protein hydrolysed with 6N-hydrochloric acid under reflux and in a sealed tube

Protein	N, % dry protein	36 hours' refluxing at 100°		48 hours' hydrolysis in a sealed tube at 105°	
		Lysine	Arginine	Lysine	Arginine
		(% of dry protein of 16% N)			
Timothy A	12·3	5·2	5·9	6·0	8·6
Timothy B	13·5	4·5	5·9	5·5	7·7
Fescue	15·0	4·4	7·6	5·3	7·5
Rye-grass	14·9	5·5	8·3	6·0	8·2

If herbage grown at different seasons of the year is shown to have a varying value for milk production, it seems possible that constituents other than the protein are responsible. Non-protein nitrogen compounds,[24] oestrogens[25] and the general balance of carbohydrates to nitrogen compounds may all contribute to such an effect.

Summary

1. Protein has been extracted from perennial rye-grass, meadow fescue, timothy and cocksfoot cut at the following four stages of growth: young leafy spring, young leafy autumn, before heading and after unchecked growth from March to September.

2. The basic amino-acid content of these proteins has been determined by a method employing electrodialysis and one-dimensional paper chromatography. The values obtained varied little despite the difference in stage of growth or season of cutting. The range of the mean values for the four grasses were: lysine, 5·2–6·3, arginine 6·9–7·7, and histidine 1·3–1·5 expressed as a percentage of each acid in moisture-free protein containing 16% nitrogen.

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THE EFFECTS OF METHOXY-*p*-BENZOQUINONE AND SOME RELATED COMPOUNDS ON BREAD QUALITY

By E. N. GREER, P. HALTON, J. B. HUTCHINSON and T. MORAN

Following the identification of (I) methoxy-*p*-benzoquinone and (II) 2 : 6-dimethoxy-*p*-benzoquinone in the chloroform extract of yeast-fermented wheat germ, the bread-improving properties of natural and synthetic preparations of these compounds have been studied. A positive effect of (I) on loaf volume and an adverse effect of both on loaf colour have been found. Preliminary feeding trials on rats have shown no toxic effects at five times the levels of (I) which might occur in over-fermented wholemeal bread.

Examination of a range of quinoids has revealed some capable of improving loaf volume but none free from adverse effect on colour which is probably due to chemical instability.

Introduction

The adverse effects of wheat germ on the baking quality of flour are well known and are usually ascribed to the presence of –SH-containing bodies, especially glutathione, both because of the effect of this substance on baking quality, which is qualitatively similar to that of wheat germ (Sullivan, Howe & Schmalz, 1936; Ford & Maiden, 1938), and because it has actually been isolated (Sullivan & Howe, 1937) from aqueous extracts of wheat germ. Hullett & Stern (1941) have shown that these effects can be much reduced by previous fermentation of the germ with yeast before the addition of the flour required for bread making, and they have suggested that the beneficial effects of this treatment are due to the inactivation of the glutathione by the yeast, since the nitroprusside reaction given by raw germ extracts could no longer be obtained from the ferment. Smith & Geddes (1942) have confirmed the beneficial effects of fermentation upon fresh germ, but they have reported appreciable adverse effects when the proportion of germ used in the dough exceeded 10% of the flour, despite the absence of a nitroprusside reaction in the aqueous germ extracts. The adverse effects of fermented wheat germ used at high dosages have also been noted by one of us (P. H.). Evidence of possible yeast poisoning by the products of fermentation was obtained, and further tests showed that the adverse effects on bread quality could be eliminated by the addition of fresh yeast at the dough-making stage. Another undesirable feature of the process, observed by all three groups of workers, was the formation of pink and reddish-brown discolorations, both in the germ ferment and in the crumb of the bread, whenever fermentation of the germ was unduly prolonged, an effect sometimes observed by bakers accustomed to the use of flours containing substantial quantities of germ.

Vuataz (1950) has taken the general problem a stage further by the isolation of two substances, one of which (comprising about four-fifths of the yield) reacted rapidly with cysteine and glutathione in aqueous solution to destroy their capacity to give the nitroprusside reaction. In common with extracts of fermented wheat germ, this capacity was not restored by reducing agents as would have occurred if the glutathione had only been converted to its oxidized form.

The active body described by Vuataz has been identified in these Laboratories (Cosgrove, Daniels, Greer, Hutchinson, Moran & Whitehead, 1952) as monomethoxy-*p*-benzoquinone, and the accompanying substance as the corresponding 2 : 6-dimethoxy-*p*-benzoquinone. The close connexion between the various likely mechanisms of flour improvers and oxidation phenomena has suggested that these substances might act as flour improvers, and the present paper describes such experiments on these two substances and a number of related compounds.

Experimental

Preparation of methoxy-p-benzoquinone from wheat germ

Freshly-milled wheat germ was defatted with light petroleum (b.p. 37°) and fermented with bakers' pressed yeast according to the directions of Vuataz (1950), using 150 g. of germ per batch, plus 50 g. of yeast suspended at 30° in 1500 ml. of water containing 0·5 g. of potassium bromate. In our experience it was desirable to reduce the initial pH of the suspension from 5·6 to 5·0 by the addition of 15 ml. of N-hydrochloric acid. The mixture was stirred continuously throughout, but although the active phase of gas production and the destruction of the substances adverse to baking quality were virtually complete within two hours, no methoxy-*p*-benzoquinone could be extracted at this stage and fermentation was allowed to continue preferably for another 14 hours. Yields of the order of 80 mg. of methoxy-*p*-benzoquinone and 10 mg. of the 2 : 6-dimethoxy-*p*-benzoquinone were finally obtained from 150 g. of germ. After the chemical identity of these substances with authentic specimens of the two quinones synthesized from vanillin had been established (Cosgrove, Daniels, Whitehead & Goulden, 1952), the ample

supplies of the synthetic material available were used for further study of their effects in preference to the minute amounts obtained from the fermentation process.

Assessment of a flour-improving action

A baking test was used as an indication of any improving properties upon fresh, commercially-milled, unbleached and untreated National flours (81% extraction). For each loaf a flour sample (140 g.) containing the improver being tested was doughed with the necessary water containing 1·75 g. of yeast and 1·75 g. of salt, the mixture was fermented for four hours at 27° and baked in the oven for 24 minutes at 218°.

The loaves were examined for external crust characteristics, loaf volume, crumb texture and crumb colour. In the results quoted, loaf volume is given as the percentage increase over that of a loaf made from untreated flour. The improver potassium bromate was also used as a standard of comparison; at optimum dosage (about 20 p.p.m. of flour) this substance increased loaf volume by 20%. Crumb texture and colour were noted by inspection of the loaf cut when 24 hours old.

Preparation of samples of flour for text

When very small quantities of flour improvers of the powder type are required to be brought into intimate mixture with the freshly milled flour, it is convenient to make a more concentrated mixture or pre-mix which could then be added to the bulk of the untreated flour. Two methods of preparing such a concentrate were used: (1) The substance was dissolved at the requisite dosage in light petroleum (b.p. 37°) containing about 10% by volume of chloroform, and added to 20 g. of the flour to give a well-mixed thick paste which was then air-dried, gently powdered and sifted several times to aerate it and thus remove the solvent. It remained exposed to the air in a thin layer for at least 16 hours before being mixed into the remaining 120 g. of flour and doughed up within a few minutes. (2) The substance was dry mixed into 20 g. of flour by grinding it into the flour with a pestle and mortar. Unless otherwise stated this was mixed immediately into the remainder of the flour and mixed into dough.

Results

Methoxy-p-benzoquinone and 2 : 6-dimethoxy-p-benzoquinone

In comparative trials (see Table I) the synthetic preparations had the same effects upon bread quality as the substances isolated from the wheat-germ ferments. Methoxy-*p*-benzoquinone improved both volume and texture of the bread, and for improvement was only slightly inferior at its optimum level (60 p.p.m. of flour) to bromate (20 p.p.m.). 2 : 6-Dimethoxy-*p*-benzoquinone, on the other hand, was less effective, and in general the crust was adversely affected, showing a ragged appearance reminiscent of over-treatment (Table II).

Table I

Comparison of synthetic methoxy-p-benzoquinone (A) with that isolated from the fermentation of germ (B)

Rate of addition, p.p.m.	Loaf volume, % increase		Improvement	Colour
	A	B		
15..	5	—	Fair	Slight change
30..	12	9	Fairly good to good	Pink tinge
60..	22	22	Very good	Decided pink tinge
120..	18	18	Over-treated	Very pink
240..	6	—	Badly over-treated	,, ,,

Table II

Effect of 2 : 6-dimethoxy-p-benzoquinone upon bread quality

Rate of addition, p.p.m.	Loaf volume, % increase	Improvement	Colour
44..	2	—	
87..	6	Progressive improvement up to 700 p.p.m., but loaves had ragged crusts	Pink tinge increasing with dosage
175..	10		
350..	11		
700..	12		

With the monomethoxy compound the pre-mix, prepared by either method, began to develop a pinkish colour within a few hours, and this continued to increase in intensity for several days. With freshly made mixtures, the pink colour developed in the dough and

persisted in the crumb of the finished bread. The dimethoxy-compound was more stable in flour, but imparted the same colour to the bread.

The effect of methoxy-p-benzoquinone on the growth rate of rats

Although it seemed most unlikely that either mono- or di-methoxybenzoquinone or their decomposition products could have any adverse nutritional effects at the maximum levels likely to occur in bread, a number of feeding tests are in progress to check this point. In a preliminary investigation* three groups of five weanling rats, selected from comparable litters of an inbred stock, were compared for growth when fed *ad lib.* on the basic diet shown in Table III.

Table III

Diet	%	Supplement	mg./kg. of diet
White flour	91·5	Choline chloride	1000
		Thiamine hydrochloride	5
Sucrose	2·5	Riboflavin	10
		Nicotinic acid	5
Arachis oil	2·0	Pyridoxine hydrochloride	5
		Calcium pantothenate	25
Salt mixture (Jones & Foster, 1942)	4·0	*p*-Aminobenzoic acid	150

Using one group as control, the remaining two groups received the same diet supplemented with 0·0025% and 0·0125% of methoxy-*p*-benzoquinone respectively (0·0025% is likely to exceed the concentration of this substance in over-fermented wholemeal bread). The diet became pink within a few days of preparation, indicating the formation of decomposition products.

The trial was continued for six weeks, over which time no significant differences could be observed between the growth-rate curves of any of the three groups, which treated as a single group showed a rate well below optimum, as would be expected from the diet used.

Activity of some related compounds

As a preliminary to a search for analogues which would be more effective as flour improvers, the following substances, which had the merit of being readily obtainable, were purified by recrystallization or sublimation and tested for bread-improving properties: (*p*-)benzoquinone, 1 : 4-naphthaquinone, toluquinone (2-methyl-*p*-benzoquinone), quinone dioxime, hydroquinone, quinhydrone, 2 : 5-dihydroxy-*p*-benzoquinone, chloroquinol, menaphthone (2-methyl-1 : 4-naphthaquinone), acetomenaphthone, triacetoxybenzene and chloranil.

Benzoquinone

With a pre-mix of this compound a marked pink colour developed within a few hours. The bread, however, showed no trace of this colour, presumably because it was destroyed during the fermentation and baking processes. The improvement of volume and texture was considerable and comparable to the improvements obtained with the methoxy-derivative, but the optimum was at a higher level of addition (Table IV). At dosages of 200 p.p.m., however, the benzoquinone almost completely inhibited gas production by the yeast, an effect not observed with the methoxy-derivatives.

As shown in Table IV, all the quinones used produced varying types of discoloration in the bread crumb, and most caused colour development when their mixture with flour was kept for a few days. It was also noted, as with benzoquinone, that inhibition of gas production by yeast occurred to a particularly marked extent when toluquinone or 1 : 4-naphthaquinone was present in the dough. Disregarding colour, an appreciable improving effect was found with chloranil and triacetoxybenzene.

Effect of storage on flour–quinone mixtures

The colour formation, described in the last paragraph, which developed in dry mixes with flour, was presumably due to modification of the added substance. Similarly, the improving effects produced on crumb structure and toxicity to yeast were found to decrease after a few days' storage of the pre-mix; these changes are exemplified in Table V.

In view of the instability of all these substances in flour, dough and bread, we have not persevered with the search for a more satisfactory improver of the quinonoid type.

* A repetition of this trial, using supplements of 0·025% of methoxy-*p*-benzoquinone and 0·003% of 2 : 6-dimethoxy-*p*-benzoquinone respectively, again showed no significant change in growth-rate curve, as compared with the unsupplemented diet

Table IV

Effects of quinones on bread

Substance added	Rate of addition, p.p.m.	Volume increase over control, %	Improvement	Colour
1 : 4-Naphthaquinone	25	6	Fair	Brown
Toluquinone	50	7	Fair	Faint grey
,,	100	11	Fairly good	Deeper grey
Quinone dioxime	100	−1	Nil	Grey
Benzoquinone	100	13	Good	Faint grey-brown
,,	200	—	Yeast killed	—
Hydroquinone	100	4	Slight	Faint green
,,	200	4	,,	,, ,,
Quinhydrone	100	6	Fair	Faint grey
,,	200	8	,,	,, ,,
Dihydroxybenzoquinone	100	3	Slight	Faint pink
Chloroquinol	100	3	Slight	Nil
Chloranil	100	9	Fairly good	Faint green
,,	200	18	Good	,, ,,
,,	400	11	Over-treated	,, ,,
Menaphthone	100	−2	Nil	Yellow
Acetomenaphthone	100	−1	Nil	Yellow
Triacetoxybenzene	100	10	Fairly good	Faint pink
,,	200	8	,, ,,	,, ,,
,,	400	13	Good	,, ,,

Table V

Effect of storage in flour on activity of quinones

Substance added	Age of pre-mix, days	Rate of addition, p.p.m.	Volume increase over control, %	Improvement	Colour
Methoxy-*p*-benzoquinone	0	50	12	Good	Faint pink
,, ,,	6	50	5	Fair	Deeper pink
p-Benzoquinone	0	100	13	Good	Faint grey-brown
,,	0	200	—	Yeast killed	—
,,	8	100	5	Fair	Grey brown
,,	8	200	12	Good	Deeper brown
,,	8	400	0	Over-treated	,, ,,
Toluquinone	0	50	7	Fair	Faint grey
,,	0	100	11	Good	,, ,,
,,	8	50	3	Slight	Grey pink
,,	8	100	7	Fair	,, ,,

Conclusions

To summarize the results obtained in these trials, it may be concluded that:

(1) Methoxy-*p*-benzoquinone, obtainable from fermented germ, is potentially capable of acting as a flour improver, but its tendency to form coloured compounds makes it useless for this purpose.

(2) It is likely that the end products from methoxy-*p*-benzoquinone are responsible for the known discoloration which can occur in over-fermented bread containing appreciable quantities of wheat germ.

(3) Of the quinonoid substances tried, all suffer from the same objection, that they are unstable under the conditions required.

(4) These substances are toxic to yeast in concentrations not far removed from those required for optimum flour improvement.

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THE COMPONENT GLYCERIDES OF STILLINGIA OIL

By A. CROSSLEY and T. P. HILDITCH

The glycerides of stillingia oil have been studied by resolution into a series of fractions by crystallization from acetone. Deca-2 : 4-dienoic acid, which forms about 8% (mol.) of the total acids of the oil, is shown to occur almost exclusively as monodecadienoic di-C_{18}-polyethenoid glycerides with the short-chain acyl group in the β- or symmetrical position. The chief constituents of the oil are linoleodilinolenins, decadienolinoleolinolenins and decadienodilinolenins, with lesser proportions of saturated-linoleolinolenins, saturated-dilinolenins, oleolinoleolinolenins and oleodilinolenins. Owing to the presence of the decadienoic acid (8%) with linolenic acid (51%) and linoleic acid (22%), the total proportion of tripolyethenoid glycerides (95%) in the oil is greater than in linseed oil and comparable with that in conophor oil. This accounts for the relative behaviour of stillingia oil and linseed oil as drying oils in paint films.

Introductory

Stillingia oils (the seed oils from *Sapium sebiferum* Roxb. and *S. discolor*) have recently been shown to contain about 5% (wt.) or 8% (mol.) of the short-chain conjugated deca-2 : 4-dienoic acid, together with major proportions of linolenic and linoleic acids and smaller amounts of oleic and saturated acids, in their total fatty acids (Hilditch, 1949 ; Huang, Holman & Potts, 1949 ; Crossley & Hilditch, 1949, 1950 ; Devine, 1950). The proportions of the component acids in a commercial stillingia oil, in three authentic specimens of the oil from seeds of *S. sebiferum* and in two similar specimens from *S. discolor* were recorded by Crossley & Hilditch (1950). The unusual presence of deca-2 : 4-dienoic acid in addition to the acids commonly present in a drying oil pointed to the desirability of examining the glyceride structure of stillingia oil. The present communication gives an abbreviated account of studies which were carried out to this end at the University of Liverpool in 1950–51.

Methods

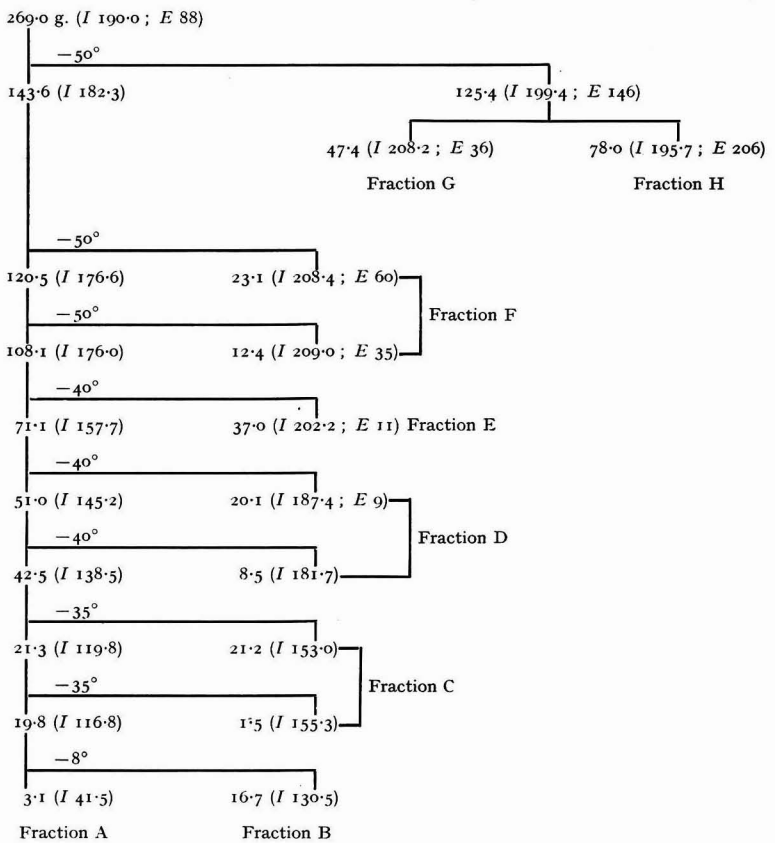
The general method employed was broadly the same as that used in the past few years in this Laboratory in connexion with the component glycerides of other mainly unsaturated fatty oils. It has been described in full detail in previous publications (Barker & Hilditch, 1950 ; Crawford & Hilditch, 1950 ; Hilditch & Seavell, 1950). Briefly, it consists in systematic crystallization of the neutral fatty oil from acetone or from ether at low temperatures in such a way that ultimately the oil is separated into a number of groups of glycerides which differ widely in composition ; each of these groups, although still a mixture of mixed triglycerides, is much more simple in composition than the original oil. The component acids in each separated glyceride group are quantitatively determined, and from the results the approximate glyceride composition of each group can be deduced. The procedure, except with relatively simple fatty oils, rarely indicates the precise proportions of each individual mixed glyceride present ; but it affords a measure of the amounts of different categories of glycerides that may be present (e.g.

trisaturated, mono-, di-, or tri-unsaturated, glycerides with one, two, or three groups of a specific acid such as oleic, linoleic or linolenic, etc.).

Two specimens of neutralized stillingia oil were studied : the commercial specimen used for component-acid determination by Crossley & Hilditch (1950), and the oil from *S. sebiferum* seeds sent to us from Hong Kong in 1947. The results were of a similar nature in both instances and it is proposed here to illustrate the specific properties of stillingia-oil glycerides by results obtained with the oil extracted from the authentic 1947 specimen of *S. sebiferum* seeds.

Acetone (10 ml. per g. of glycerides) was found to be the most suitable solvent, and it was also found that a preliminary crystallization of the neutral stillingia oil (269 g.) from acetone at — 50° left in solution most of the glycerides containing decadienoic groups together with much of those in which linolenic and linoleic groups predominated. Further crystallization of this fraction of the oil (soluble in acetone at — 50°) from acetone at a lower temperature (down to — 70°) effected a further concentration in solution of the decadienoic-containing glycerides, leaving a deposit of glycerides of somewhat higher iodine value than those which remained in solution (owing to the lower concentration of decadienoic groups, and the greater concentration of linolenic groups in this deposited glyceride fraction). The glycerides originally deposited from acetone at — 50° were further recrystallized, first at — 50°, then successively at — 40°, — 35° and — 8°, with the results shown in Fig. 1.

In some instances, soluble fractions of closely similar iodine values were combined (as



(*I* = iodine value ; $E = E^{1\%}_{1\,cm.}$ at 260 mμ in *cyclo*hexane)

FIG. 1.—*Crystallization of seed oil of* Sapium sebiferum

shown in Fig. 1) before determination of their component acids. These determinations were in each instance carried out exactly as described by Crossley & Hilditch (1950) for the determination of the component acids of a number of stillingia oils. In this paper the full numerical details of the complicated analyses necessary for each fraction are not included, but only the final compositions thus deduced for the acids present in the eight glyceride fractions into which the oil had been resolved by the crystallization procedure.

Results

The weights and general characteristics of the eight fractions of glycerides into which the original neutral stillingia oil had been separated by low-temperature crystallization are given in Table I, together with the molar percentages of the component acids in each group of glycerides and the increments of the various categories of glycerides in each group, deduced from the component-acid analyses. The final column of Table I shows the amounts of the various components estimated to be present in the original stillingia oil.

Anomalous equivalents of glyceride fractions F, G, H.—We drew attention in an earlier paper (Crossley & Hilditch, 1950, p. 300) to an anomaly between the observed saponification

Table I

Component glycerides of stillingia oil (from S. sebiferum *seeds)*

Fractions obtained by low-temperature crystallization from acetone (Fig. 1)

	A	B	C	D	E	F	G	H	Total
Weight, g.	3·1	16·7	22·7	28·6	37·0	35·5	47·4	78·0	269·0
Iodine value	41·5	130·5	153·0	185·3	202·2	208·6	208·2	195·7	190·0
$E^{1\%}_{1\,cm.}$ @ 260 mμ	2	3	6	8	10	51	36	206	
Saponification equivalent									
Observed	not detd.		289·0	289·9	288·3	276·1	280·3	243·8	270·2
Calc. (from component-acid analyses)	281·4	288·6	288·8	289·4	289·3	285·0	286·4	272·9	283·1
Glycerides, % (wt.)	1·2	6·2	8·4	10·6	13·8	13·2	17·6	29·0	100·0
,, % (mol.)	1·1	5·8	7·9	10·0	12·9	13·0	17·1	32·2	100·0
Component acids (% mol.)									
Palmitic	53·4	26·3	19·1	10·0	8·4	7·6	8·4	2·0	8·8
Stearic	17·8	14·9	8·8	4·6	3·9	3·5	3·8	1·0	4·1
Deca-2 : 4-dienoic	—	—	0·8	1·0	1·1	5·7	4·0	20·8	8·4
Oleic	18·3	6·3	11·0	8·0	3·1	0·9	1·1	8·6	5·7
Linoleic	4·7	17·4	20·5	27·2	26·4	20·1	20·3	23·0	22·3
Linolenic	5·8	35·1	39·8	49·2	57·1	62·2	62·4	44·6	50·7
Component glyceride categories (increments % mol.)									
I. Trisaturated	0·2	—	—	—	—	—	—	—	0·2
Mono-unsaturated	0·9	1·4	—	—	—	—	—	—	2·3
Diunsaturated	—	4·4	6·6	4·4	4·8	4·3	6·3	2·9	33·7
Triunsaturated	—	—	1·3	5·6	8·1	8·7	10·8	29·3	63·8
II. *No* decadienoic	1·1	5·8	7·7	9·7	12·5	10·8	15·0	12·1	74·7
Monodecadienoic	—	—	0·2	0·3	0·4	2·2	2·1	20·1	25·3
III. *No* linoleo-	1·0	2·8	3·0	1·8	2·7	5·2	6·7	9·9	33·1
Monolinoleo-	0·1	3·0	4·9	8·2	10·2	7·8	10·4	22·3	66·9
IV. *No* linoleno-	0·9	—	—	—	—	—	—	—	0·9
Monolinoleno-	0·2	5·5	6·4	5·2	3·7	1·7	2·2	21·3	46·2
Dilinoleno-	—	0·3	1·5	4·8	9·2	11·3	14·9	10·9	52·9
V. *No* C_{18}-polyethenoid	0·8	—	—	—	—	—	—	—	0·8
Mono-C_{18}- ,,	0·3	2·5	1·5	—	—	—	—	—	4·3
Di-C_{18}- ,,	—	3·3	6·4	7·1	6·4	6·9	8·9	31·3	70·3
Tri-C_{18}- ,,	—	—	—	2·9	6·5	6·1	8·2	0·9	24·6
VI. *No* polyethenoid *	0·8	—	—	—	—	—	—	—	0·8
Monopolyethenoid	0·3	2·5	1·3	—	—	—	—	—	4·1
Dipolyethenoid	—	3·3	6·6	6·8	6·0	4·7	6·8	11·2	45·4
Tripolyethenoid	—	—	—	3·2	6·9	8·3	10·3	21·0	49·7

* I.e. C_{18} polyethenoid + decadienoic glycerides

equivalents of stillingia oils and those calculated from the results of our determinations of their component acids. The present work reveals that this anomaly is connected with the presence of the decadienoic glycerides, for it is seen (Table I) that good accordance obtains between the observed and calculated values except in fractions F, G and H, into which the short-chain glycerides are concentrated. Indeed, in fraction H, which contains the bulk of these glycerides, the discrepancy becomes extreme, the directly determined equivalent of the fraction being nearly 30 units lower than that calculated from the determined component-acid figures.

One of three possible explanations might account for this peculiarity: (*a*) the presence of an alcohol other than glycerol in the oil, (*b*) the attachment of the short-chain acid group to a long-chain acid group instead of directly to glycerol as an ordinary mixed glyceride, and (*c*) the presence of small proportions of acids of very low molecular weight either in the original oil or produced during saponification with alkali.

(*a*) Stillingia oil (280 g.) was hydrolysed with alcoholic alkali and the product (after removal of alcohol by distillation) was poured into water, then made acid, and fatty acids were removed by repeated extraction with ether. The ether extracts were twice washed with water, and the aqueous washings added to the ether-extracted acid liquor, which was then neutralized with excess of calcium carbonate and evaporated to dryness. The dry calcium salts were extracted in a Soxhlet apparatus with alcohol, which removed 28·7 g. of a thick viscous liquid. Distillation of this at 0·2 mm. pressure through a fractionating column showed that (except for the first few drops and for about 10% of non-volatile residue, evidently polyglycerols) it was homogeneous; it boiled at 136° (0·2 mm.) and had a refractive index at 25° of 1·4725–1·4729 (α-naphthylurethane derivative, m.p. 192°, unchanged when mixed with pure glyceryl α-naphthylurethane). The observed yield of glycerol was 10·3% (calculated for stillingia oil from the determined fatty acid composition, 10·9%).

These figures are conclusive evidence that glycerol is the only alcoholic constituent in stillingia oil.

(*b*) The possibility that decadienoic acid in stillingia oil might be present as an ester derivative of a hydroxy-unsaturated C_{18} acid instead of in an ordinary mixed triglyceride was considered. It was, however, found that (although the unsaturated acids, in which linolenic acid was present, exhibited variable acetyl values of a low order) the hydrogenated esters of the long-chain unsaturated acids possessed negligible acetyl values and also showed no indication of the presence of keto or amino groups.

Moreover, it was observed, employing cryoscopic measurement of molecular weights in *cyclo*hexane as solvent, that the molecular weight of a 'decadienoic concentrate' similar to fraction H (Table I) accorded with the presence of mixed triglycerides of decadienoic and the C_{18} polyethenoid acids and not with that of a complex decadieno-C_{18} polyethenoid derivative. [With about 20% of decadienoic acid in the total fatty acids of the concentrate, the molecular weight of the fraction as ordinary mixed glycerides would be 50–70 units less than that of an ordinary drying oil (870–880), whereas if it contained, in effect, three C_{18} radicals with a decadienoic group attached to one of these, the molecular weight would be 70–100 units higher than that of an ordinary drying oil. The observed molecular weights tended uniformly towards the lower values.]

This evidence suggested that the decadienoic acid entered into mixed glycerides in the same way as the linolenic, linoleic and other more familiar acids in the stillingia oil.

(*c*) Acids of low molecular weight and volatile in steam were shown to be absent from stillingia oil. A specimen of the oil (200 g.) was first submitted to steam-distillation (the distillate being completely neutral) and then saponified with alcoholic alkali, and the product was again distilled in steam until all alcohol had been removed. The soaps were then made acid and the product distilled in steam for six hours, when 3·5 l. of aqueous condensate was obtained. This contained traces of oily material (0·3% of the weight of oil saponified) which, on removal of ether, was found to have an equivalent of 190 and to possess an absorption band with maximum ($E^{1\%}_{1\,cm.}$ 816) at 252 mμ in alcohol: it appeared to be either impure deca-2 : 4-dienoic acid or a derivative thereof, possibly produced by oxidation. The ether-extracted aqueous condensate was completely neutral, so that acids from acetic up to hexoic or octanoic were not present in the saponified stillingia oil.

Very small amounts of oxalic acid were, however, detectable in the saponified oil. A portion (30 g.) of the residue of calcium salts left after extraction of glycerol from saponified stillingia oil [see (*a*) above] was acidified with dilute sulphuric acid and extracted once with ether to remove any traces of higher fatty acids. The aqueous solution was heated and filtered, and the residual calcium sulphate thoroughly washed with hot water. The filtrates and washings were titrated at 80° with standard potassium permanganate solution, the amount of which

consumed corresponded to 0·34% (calculated as oxalic acid) of the original oil. The production of this small amount of oxalic acid during alkaline hydrolysis will affect appreciably the observed equivalent of the oil and it seems likely that extra alkali consumed in this way is the main cause of the anomaly. The production of yellow solutions during saponification of decadienoic esters has been observed (Hilditch, 1949; Crossley & Hilditch, 1949), and more recently the considerable extent to which migration of the unsaturated system takes place when deca-2 : 4-dienoic acid is heated at 180° with excess of alkali has been reported (Crossley & Hilditch, 1952). Specific spectrophotometric tests to determine the apparent loss of deca-2 : 4-dienoic acid in the conditions to which the oil and a decadienoic glyceride concentrate of it are subjected during the determination of saponification equivalent indicated in both instances that the deca-2 : 4-dienoic acid content of the mixed fatty acids was about 5% less than that shown spectroscopically by the corresponding glycerides before saponification.

This loss, however, corresponds to only about 0·2–0·3% expressed on the total fatty acids of stillingia oil. We therefore conclude that the proportions of deca-2 : 4-dienoic acid and of the higher fatty acids in stillingia oil, or its segregated fractions, are determinable with reasonable accuracy by the detailed methods of component-acid analysis employed, and that the true saponification equivalents of the glycerides are more closely represented from the component-acid figures than by saponification equivalents determined directly on the glycerides.

Constitution of the decadienoic glycerides in the glyceride concentrate H.—A portion of the glycerides left in solution at − 67° in acetone (see Fig. 1) was hydrogenated as far as possible at 110° in presence of Raney nickel. The hydrogenated product (15·5 g., iodine value 3·2) was crystallized from ether (600 ml.) at room temperature, when 1·7 g. of solids were deposited, consisting almost wholly of tristearin. The material left in solution (13·8 g.) was further crystallized from ether (275 ml.) at − 5°, when 6·4 g. of solids (A, iodine value 0·6) separated, leaving in solution 7·4 g. which, on crystallization from ether (110 ml.) at − 5° deposited a further 4·5 g. of solids (B, iodine value 0·9). The residual material left in solution (2·9 g., iodine value 15·3) was not further examined.

The component acids in the crystalline fractions A and B were determined by ester fractionation and were found to consist of:

	Decanoic acid, % (mol.)	Stearic acid, % (mol.)
Fraction A	31·4	68·6
Fraction B	28·4	71·6

[Ester-fractionation analysis of a mixture of methyl decanoate and methyl stearate corresponding to a molar mixture of 33·3% decanoic and 66·7% stearic acids gave values (found) of 32·0% decanoic and 68·0% stearic acid, indicating a slight loss of decanoic acid during the procedure.]

The melting and transition points of the hydrogenated fractions A and B were determined and compared with those recorded for symmetrical and unsymmetrical decanodistearin by Malkin & Meara (1939) and Carter & Malkin (1939), with the following results:

	Fraction		*n*-Decanodistearins	
	A	B	Symmetrical	Unsymmetrical
1st transition point (α form)	45°	45–46°	47°	42·5°
2nd ,, ,, (β′ form)	51–52°	53°	53°	46°
Final melting point (β form)	57°	57–57·5°	57°	49°

These results show that the deca-2 : 4-dienoic acid of stillingia oils is present only once in any triglyceride molecule in which it occurs, and that in these it occupies the central or β-position, so that the symmetrical β-deca-2 : 4-dienoic di-C_{18}-polyethenoid glycerides are the only forms present.

Discussion

Triunsaturated glycerides form the greater part (64%) of the oil, the remainder consisting almost entirely of glycerides with two unsaturated groups (34%), except for very small amounts of disaturated glycerides and traces of trisaturated glycerides: it is possible that the latter arise from contamination with traces of the fruit-coat fat, which is made up of 77% of disaturated and 21% of trisaturated glycerides (Gupta & Meara, 1950).

Inspection of the component glyceride categories in Table I suggests *prima facie* that the glycerides of stillingia oil are assembled on the conventional lines of ' even distribution '. Thus no trilinolenin was observed, and no evidence of the presence of dilinoleoglycerides was obtained, the respective molar contributions of linolenic and linoleic acids to the total fatty acids being

51% and 22%. The distribution of a given acid or group of acids may be more rigorously examined by plotting the percentages of glycerides containing one, two or three radicals of the acid against the proportion of the acid in the total fatty acids of the oil. The points thus obtained for the glyceride distribution of decadienoic, linoleic or linolenic acid, or the C_{18}-polyethenoid acids or total polyethenoid acids (i.e. C_{18} + decadienoic) as groups, fit closely on the curves obtained by plotting points drawn in a similar manner for oils containing different proportions of linolenic and linoleic acids (Barker & Hilditch, 1950; Hilditch & Seavell, 1950). These curves represent the approximation to 'even distribution' which characterizes the glycerides of nearly all seed fats.

It is to be noted that the decadienoic components of stillingia oil follow precisely the same mode of distribution as that of the long-chain acids in the glycerides. Moreover, the decadienoic acid [which enters only once into any triglyceride molecule owing to its relatively small proportion (8% mol.) of the total acids of stillingia oil] has now been shown to be present wholly as symmetrical or β-decadieno-C_{18}-polyethenoid glycerides. In this respect it resembles other seed-fat mixed glycerides which contain two groups of a major component acid and one group of an acid present in smaller proportions in the fat as a whole: the acid present in the whole fat in lesser proportions is attached to the β-hydroxyl group of the glycerol molecule (cf. Meara, 1945*a*, *b*, 1947, 1949; Gupta & Meara, 1950). It is interesting to note that this is the first occasion on which it has been feasible to determine the configuration of a mixed glyceride containing three unsaturated acyl groups—a circumstance rendered possible by the difference in carbon content of the unsaturated acids concerned.

The practical interest of stillingia oil as a drying oil is connected of course with its very high content of di- and tri-polyethenoid glycerides. It contains about 25% tri-C_{18}-polyethenoid (linoleno- and linoleo-)glycerides with about 70% of glycerides which include two C_{18}-polyethenoid groups—95% in all. If, however, the total content of polyethenoid acyl groups (decadienoic as well as linolenic and linoleic) is considered, the oil consists of about 50% of tripolyethenoid glycerides and 45% of dipolyethenoid glycerides, with a conjugated decadienoic group present in about 25% of the whole oil. This is the largest proportion of tripolyethenoid groups observed in any seed fat which has yet been studied, exceeding even that in conophor oil (Hilditch & Seavell, 1950). It is accordingly not surprising to find that stillingia oil has received favourable reports in the paint industry, and that it has been considered to be superior for some purposes to linseed oil.

The similar glyceride analysis (not reported in detail) of a commercial specimen of stillingia oil gave closely analogous results, having regard to the somewhat different proportions of its component acids (palmitic 7·0, stearic 4·2, decadienoic 5·8, oleic 13·9, linoleic 26·8, linolenic 41·2, elaeostearic 1·1% mol.). The observed content of triunsaturated glycerides was 69%, with 29% of diunsaturated glycerides; about 19% of tri-C_{18}-polyethenoid and about 71% of di-C_{18}-polyethenoid glycerides were present. About 17% of the glycerides contained a single deca-2 : 4-dienoic group. The total polyethenoid glycerides (C_{18} + deca-2 : 4-dienoic) amounted to about 35% tripolyethenoid and about 55% of dipolyethenoid glycerides.

It is not possible to define with great exactitude the amounts of individual categories of mixed glycerides in stillingia oil. Of the tripolyethenoid glycerides about half is probably linoleodilinolenins, the other half being decadienodilinolenins and decadienolinoleolinolenins. Nearly all the decadienoic acid seems thus to be in combination with linoleno- (and linoleo-)-glycerides. The 33% of the oil which consists of monosaturated glycerides is made up largely of saturated linoleolinolenins and saturated-dilinolenins, and the 16% of mono-oleoglycerides of oleolinoleolinolenins and oleodilinolenins; in both categories the glycerides containing both a linoleic and a linolenic group appear to predominate over those containing two linolenic groups. The presence of the decadienoic glycerides in stillingia oil renders the correlation of its structure and properties with those of the more usual type of drying oils somewhat complicated and difficult. As regards glyceride structure, however, it is noteworthy that the short-chain conjugated acid behaves exactly similarly to the long-chain acids that are the more usual components of seed-fat glycerides.

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THE COMPONENT ACIDS AND GLYCERIDES OF REFINED NEEM (*MELIA INDICA*) OIL

By S. S. GUPTA and C. R. MITRA

The refined oil obtained after the successful removal of the bitter and odoriferous constituents of neem oil has been studied for its component acids and glycerides. Palmitic (16·2%), stearic (14·6%) and oleic (56·6%) acids were found to be the major component acids, with subordinate proportions of linoleic (9·0%) and arachidic (3·4%) and probable traces of myristic acid. The glyceride composition is in fair accordance with the ' rule of even distribution '. The utility of the refined oil for soap-making purposes and as a good source of technical oleine and stearine has been pointed out.

Introduction

The fatty acids of neem oil (margosa oil) and its glyceride composition have been a subject of critical study by a number of workers since 1917, the investigations being very often prompted to isolate the special constituents regarded to be responsible for its long reputed therapeutic value. Mixtures of fatty acids contaminated with hydrolytic-degradation products of the bitter or odoriferous constituents had been reported[1] to be active pfinciples of neem oil thus attributing medicinal value to the oil (glycerides) itself. Newer fatty acids, some with an odd number of carbon atoms, were also claimed to have been isolated.[2] The subsequent investigations of Roy & Dutt,[3] Child & Ramanathan,[4] Hilditch & Murti[5] and Dasa Rao & Sheshadri[6] have since satisfactorily disproved the existence of any new acids in neem oil ; thus it became evident that the medicinal properties of the oil were due to the non-fatty components and not to any peculiar acids comprising the glycerides.

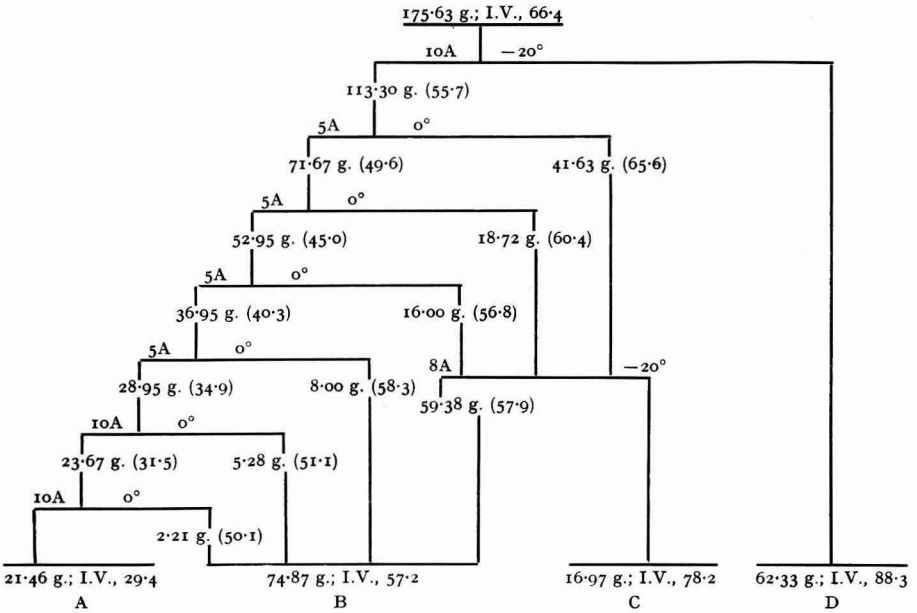
Recently, a series of therapeutically-active amorphous and crystalline bitter constituents have been isolated from neem oil by a method of mechanical separation with the aid of solvents,[7] and these have been characterized as separate chemical entities. The oil left after extraction of the ' bitters ' has since been freed of the odoriferous sulphur-containing compounds and has been further refined.[8] The refined oil thus obtained was of a pale-yellow colour having a very faint characteristic smell and no bitter taste. Little difficulty was encountered in hydrogenating this refined oil, in contrast with the observations of Hilditch & Murti,[5] who reported consistent interference of the non-fatty components during their study of neem oil. It was therefore considered of interest to reinvestigate the composition of this refined oil, using the recently developed analytical methods of low-temperature crystallization and absorption spectroscopy.

The sample of oil used during the present study was obtained from Gwalior (Madhya Bharat) through the Government Agricultural Department.

Methods and results

The general procedure followed in this investigation was essentially the same as that adopted by Hilditch and collaborators[9, 10] at the Liverpool laboratories during the past few years. The refined oil, which was rendered neutral by washing with aqueous alkali, had the following characteristics : specific gravity at 30°, 0·9087 ; refractive index n_D^{30}, 1·4612 ; iodine value, 66·4 ; saponification equivalent, 290·9 ; free fatty acids, 0·2% (as oleic), and unsaponifiable 0·8%.

The oil was submitted to a series of crystallizations from acetone (10 ml./g. of oil) at temperatures ranging from — 20° to 0° as shown in Fig. 1, and thus resolved into four final fractions having a range of iodine values between 29·4 and 88·3. In this connexion, it is of importance to add that no further separation of crystalline glycerides from fraction A (I.V., 29·4) in ether at 0° was found possible, showing the absence of any appreciable quantity of fully saturated glycerides. This observation is in full accordance with the results obtained by Hilditch & Murti,[5] who used the alternative method of acetone–permanganate oxidation.



5A, 8A, 10A = Acetone (5, 8, 10 ml./g. of oil)
Figures in parentheses are iodine values

FIG. 1.—*Crystallization of 'refined' neem oil*

The respective glyceride fractions were then saponified and the mixed fatty acids obtained were converted into methyl esters in the usual manner. Sufficient material was available from fraction B to permit further resolution of the mixed acids into solid and liquid groups by the lead salt–alcohol method.

	Fraction	Wt., g.	%	I.V.
BA	Lead salt–alcohol insoluble	16·61	39·9	5·8
BB	Lead salt–alcohol soluble	25·02	60·1	94·2

The methyl esters in each case were then fractionated through an electrically heated and packed column and the composition of the individual fractions calculated from their iodine values and saponification equivalents. Acids from liquid fractions of highest iodine values (BB_6, C_4, D_6) were examined in a Beckmann spectrophotometer, after isomerization as recommended by Hilditch, Morton & Riley.[11] The respective extinction coefficients and the calculated composition of these fractions are given in Table I. Spectrophotometric examination did not reveal the presence of any linolenic acid in these fractions.

Table I

Fraction	I.V.	$E^{1\%}_{1\,cm.}$ at 234 mμ	Percentage composition		
			Linoleic	Oleic	Sat.
BB_4	98·0	87	9·7	89·5	0·9
C_4	91·3	145	16·0	69·2	14·8
D_6	92·5	143	15·7	71·1	13·2

The final acid composition of the individual glyceride fractions was calculated from the combined ester-fractionation and spectroscopic data and the total acid composition of the oil was deduced (Table II). Further, assuming that exhaustive crystallization has resulted in the segregation of the oil into binary categories of mixed saturated-unsaturated glycerides, the composition of each glyceride fraction was computed with respect to the distribution of the total

Table II

Component glycerides of 'refined' neem oil

	Glyceride fractions obtained by low-temperature crystallization					
	A	B	C	D	Total	
Wt., g.	21·5	74·8	17·0	62·3	175·6	
I.V., obs.	29·4	57·2	78·2	88·3	66·4	
Sap. equiv., calc.	290·2	290·2	290·7	293·9	291·5	
Glycerides, % (wt.)	12·2	42·6	9·7	35·5	100·0	
,, % (mol.)	12·3	42·8	9·7	35·2	100·0	
Component acids (increments, % mol.)						
					% (mol.)	% (wt.)
Myristic	—	0·3	—	—	0·3	0·2
Palmitic	3·6	9·4	1·6	3·0	17·6	16·2
Stearic	3·7	5·8	0·9	4·0	14·4	14·6
Arachidic	1·0	2·0	—	—	3·0	3·4
Oleic	4·0	22·9	5·8	23·1	55·8	56·6
Linoleic	—	2·4	1·4	5·1	8·9	9·0
Component acid categories (increments, % mol.)						
Palmitic (with C_{14})	3·6	9·7	1·6	3·0	17·9	
Stearic (with C_{20})	4·7	7·8	0·9	4·0	17·4	
Oleic	4·0	22·9	5·8	23·1	55·8	
Linoleic	—	2·4	1·4	5·1	8·9	
Component glyceride categories (increments, % mol.)						
(i)						
Trisaturated	0·2	—	—	—	0·2	
Disaturated-mono-unsaturated	12·1	9·8	—	—	21·9	
Monosaturated-diunsaturated	—	33·0	7·5	20·9	61·4	
Triunsaturated	—	—	2·2	14·3	16·5	
(ii)						
Tripalmitin	—	—	—	—	—	
Dipalmito-	—	—	—	—	—	
Monopalmito-	10·8	29·0	4·8	8·9	53·5	
No palmitic group	1·5	13·8	4·9	26·3	46·5	
(iii)						
Tristearin	—	—	—	—	—	
Distearo-	1·8	—	—	—	1·8	
Monostearo-	10·5	23·6	2·7	12·0	48·8	
No stearic group	—	19·2	7·0	23·2	49·4	
(iv)						
Triolein	—	—	—	—	—	
Dioleo-	—	25·8	7·8	34·1	67·7	
Mono-oleo-	12·1	17·0	1·9	1·1	32·1	
No oleic group	0·2	—	—	—	0·2	
Probable component glycerides (increments, % mol.)						
Fully saturated (0·2%)						
Palmitodistearin	0·2	—	—	—	0·2	
Disaturated-mono-unsaturated (21·9%)						
Oleopalmitostearin	10·5	9·8	—	—	20·3	
Oleodistearin	1·6	—	—	—	1·6	
Mono-unsaturated-diunsaturated (61·4%)						
Palmito-oleolinolein	—	3·6	1·9	1·1	6·6	
Palmitodiolein	—	15·6	2·9	7·8	26·3	
Stearo-oleolinolein	—	3·6	—	—	3·6	
Stearodiolein	—	10·2	2·7	12·0	24·9	
Triunsaturated (16·5%)						
Linoleodiolein	—	—	2·2	14·3	16·5	

saturated, palmitic, stearic and oleic acids. By a simultaneous consideration of these four modes of distribution, the final probable content of individual glycerides was arrived at and is shown in Table II.

Identification of individual acids and glycerides

(*a*) *Arachidic acid.*—The residue obtained after fractionation of solid esters from fraction B had saponification equivalent 322·2 and was free from unsaponifiable matter. Repeated crystallization of the acids from ethyl acetate yielded fairly pure arachidic acid (m.p. 73–74°; mixed m.p. with an authentic sample of arachidic acid, 73–74°).

(*b*) *Oleopalmitostearin.*—An aliquot portion of fraction A was hydrogenated using platinum catalyst and the product, on crystallization from ether, gave palmitodistearin (m.p. 66–68°), indicating the presence of oleopalmitostearin in the original oil.

Discussion

Component acids.—The fatty acid composition of neem oil as observed during the present study is in general conformity with the results obtained by earlier workers; palmitic, stearic and oleic acids have been confirmed to be the only major component-acids of the oil. The minor differences in respect to the content of these acids, observed on comparing the results of the different analyses (Table III), are in all probability due to variations in climatic conditions, etc. The results obtained in the present case are, however, more dependable; for, contrary to earlier observations[5] at no stage during the analysis was any difficulty experienced, and the refined oil was practically free from the interfering non-fatty components. Further, it is now fairly well established that the spectroscopic method, adopted in the present study, affords a more accurate estimation of the linoleic acid content.

Table III

Component acids of neem oil

	Investigators				
	Roy & Dutt [3]	Child & Ramanathan [4]	Hilditch & Murti [5]	Dasa Rao & Seshadri [6]	Present study
I.V.	74·3	71·5	67·9	69·2	66·4
Component acids, % wt.					
Myristic	—	—	—	—	0·2
Palmitic	14·2	13·6	14·9	13·8	16·2
Stearic	24·1	19·1	14·4	18·2	14·6
Arachidic	0·8	2·4	1·3	1·8	3·4
Oleic	58·6	49·1	61·9	52·6	56·6
Linoleic	2·3	15·8	7·5	13·6	9·0

The only uncertain feature of the present analysis is perhaps the recorded trace-content of myristic acid, which could not be further confirmed, since the quantity of the particular fraction was insufficient to permit its direct isolation and identification.

Component glycerides.—From the data recorded in Table II, neem oil is found to be fairly simple in composition. The recorded contents of various glycerides can therefore be viewed with less uncertainty than the results obtained with more complex fats. The computed values of individual glycerides are unequivocal except for mixed saturated-oleo-linoleo-glycerides, for which an arbitrary equal partition of linoleic acid molecules between palmitic and stearic acids has been assumed, because these two solid acids occur in almost equivalent amounts in the oil.

Our results differ substantially from those of Hilditch & Murti[5] in certain respects. These discrepancies are due partly to slight differences in the acid composition of the two samples and partly to the comparatively exhaustive segregation of the glycerides that has been effected in the present work. Nevertheless, the general findings in regard to the glycerides of neem oil embodied in these two investigations are broadly similar and in conformity with the idea of widest distribution of acid molecules among the glycerides. Within moderately comparable proportions, the major acids are found to be present in each glyceride fraction. As envisaged by Hilditch,[5] a major portion, though not the whole, of the linoleic acid is present as linoleo-diolein (16·5%), the remainder being distributed between palmitic and stearic acids as described above. Palmitodiolein (along with palmito-oleolinolein) and stearodiolein (with stearo-oleo-linolein) constitute the bulk (61·4%) of the glycerides; this is in fair accordance with the 'rule of even distribution', as enunciated by Hilditch.[12] Fully saturated glycerides are found to be virtually absent, as would be expected for a fat having only 35% of saturated acids. The

appearance of some palmitic acid and stearic acid in the form of disaturated glycerides (21·9%) is a natural consequence of their being absent from a similar proportion of triunsaturated ones whose presence, as stated above, is in conformity with the preferential integration of linoleic acid with oleic acid molecules to form linoleodiolein.

The easy and simple method developed for the removal of the bitter and odoriferous constituents from neem oil and its subsequent refining should render this cheap and abundant indigenous material a very valuable ingredient for making high-quality soaps in India. Further, the high content of oleic acid and the presence of palmitic and stearic acids as the only saturated components suggest a convenient separation of these two fractions; simple crystallization of the mixed acids from alcohol was found to yield fairly good samples of technical oleine (I.V. 85–88) and stearine (m.p. 50–54°). As stated above, removal of the bitter and, more important, of the sulphur-containing odoriferous components, rendered the oil suitable for hydrogenation, and the product thus obtained could serve as a very important source of high-grade stearine (m.p. 64–65°).

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BEHAVIOUR OF FUMIGANTS DURING VACUUM FUMIGATION. I.—Penetration of Methyl Bromide into Boxes of Dates

By W. BURNS BROWN and S. G. HEUSER

The penetration of fumigants into packages during treatment by various methods employing reduced pressures, as well as at atmospheric pressure, can be studied by measuring gas concentrations. A 'penetration factor' is defined, which allows comparison of the effectiveness of penetration in different methods of treatment. Preliminary laboratory tests with methyl bromide have been carried out, mainly on boxes containing tightly compressed dates. The 'sustained-vacuum' method, as normally used, gave higher penetration factors at the centres of boxes of dates in three hours than the method in which an air–fumigant mixture is introduced to the evacuated chamber. Very low penetration factors were obtained in this period in tests at atmospheric pressure. It was found that, after a period of fumigation at reduced pressure, release of the vacuum produced very high concentrations at the centres of boxes. It is suggested that utilization of this effect in a modified vacuum-fumigation procedure should result in increased efficiency. The 'air-washing' technique for the removal of fumigant at the end of a treatment has also been examined.

In a number of countries, notably the U.S.A., Canada, France and the French colonies, reduced pressure or 'vacuum' techniques of fumigation are in common use for the disinfestation of stored products. Various techniques are employed, of which the following have been most frequently described:

1. The sustained-vacuum ' method is that most widely used in the U.S.A. and Canada and a number of other countries. The absolute pressure in the loaded chamber is first reduced to, say, 5 cm. of mercury. The dose of fumigant is then introduced, causing a small rise in pressure, but no further increase in pressure is allowed until the end of the fumigation period. At that stage air is allowed to enter until the pressure is atmospheric, and then the chamber is usually re-evacuated to remove residual fumigant. This ' air-washing ' may be repeated once more before the pressure is finally raised to atmospheric, and the chamber is opened and unloaded.

2. The ' released-vacuum ' or ' dissipated-vacuum ' method. In the usual form of this method, as tested, for instance, in the U.S.A. and Canada, the fumigant is introduced to the previously evacuated chamber as in the method described above, but immediately afterwards the pressure is raised until it is about atmospheric by allowing the entry of air. In comparative tests it has usually been concluded that this method is less efficient than the sustained-vacuum method.

3. A variation of the released-vacuum method is in use in France and the French overseas territories. In this method air is removed from the chamber as in other methods until the pressure is between 5 and 10 cm. of mercury. A mixture of air and the vaporized fumigant in the correct proportion is then introduced until the pressure is raised almost to atmospheric. This technique was developed by Lepigre, whose researches in Algeria[1, 2] led him to the conclusion that it was more efficient than either of the techniques described above.

It is usually claimed that the fumigation of commodities in bags, boxes or other types of package is more efficient by vacuum methods than by the method at atmospheric pressure. It is said that penetration of fumigant into the commodity is much more rapid under reduced pressure. It has also been supposed that in the methods in which, early in the treatment, the vacuum is released, the fumigant is driven into the bulk of commodity, thus quickly establishing an even distribution. The important effects of sorption by the commodity on the behaviour of the fumigant have not always been appreciated. The efficiency of the sustained-vacuum method has also been thought to be due in part to increased susceptibility of insects at greatly reduced pressures. The published data on this point are, however, meagre.

From the published results of comparative tests on a wide variety of commodities, it seems evident that effective treatment has been obtained in a much shorter time by use of a vacuum method than is considered practical in a treatment at atmospheric pressure. Exposure periods of two to four hours are commonly used in commercial-scale vacuum fumigations, whereas periods of 18 or 24 hours, or even longer, are usual for treatments at atmospheric pressure. A higher dose of fumigant is usually necessary in the short-period vacuum fumigation.

In a treatment at atmospheric pressure in a well-equipped chamber, the complete cycle of operations—loading, fumigation, airing and unloading—can usually be fitted into a 24-hour period, allowing 16 to 18 hours overnight for the fumigation. With a vacuum plant the cycle of operations may be compressed into a period of, say four hours, allowing perhaps three complete cycles in the 24-hour period if normal hours are worked. To deal with a given weight of commodity in each 24 hours, the chamber working at atmospheric pressure would, in this case, need to have a capacity three times that of the vacuum-fumigation chamber. In such circumstances, to judge which system of fumigation is the better, practically and economically, comparable data are required on the initial cost of each installation, and on operating and maintenance costs, together with observations on the convenience and safety of operation. A search of the literature has not revealed evidence of this kind.

For a number of commodities fumigation is especially difficult by reason of the nature of the material or of the manner in which it is packaged, and it has been claimed that these can be fumigated effectively by a vacuum method, whereas treatment at atmospheric pressure is unpractical, if not impossible.

Vacuum-fumigation methods have been very little used in Great Britain, and in view of the inconclusive evidence on the relative merits of the various techniques of vacuum fumigation for the treatment of different types of commodity, there has been no clear opinion on whether the vacuum methods should be more widely adopted.

As a contribution to the better understanding of the conditions in which these different techniques of fumigation are of advantage, a series of tests has been carried out in which the behaviour of the fumigant was studied by the measurement of concentrations. This method of investigation has been of great value in studying fumigation techniques at atmospheric pressure, but there are special difficulties in applying it to treatments at reduced pressure, and there have been few previous attempts to do so. Page & Lubatti[3] referred briefly to some preliminary experiments in which this procedure was presumably adopted. Some other workers, e.g. Johnson, Becker & Hawkins,[4] have taken concentration measurements in tests of vacuum-

fumigation techniques, but only after the pressure in the chamber has been allowed to return to atmospheric. The results in such tests may be misleading.

Since the work described in the present paper was completed, the report has become available of an extensive series of tests by El Nahal.[5] The aims of this worker were similar to those of the present authors, namely a comparative study of the sustained-vacuum method, the French released-vacuum method and the atmospheric pressure method. He has worked with a single product, wheat in bags, and has used three fumigants, ethylene oxide, methyl bromide and hydrogen cyanide, and has studied the effects of method, dose and moisture content on penetration, residual bromide and biological effect on insects.

In the present series of tests methyl bromide has been used as fumigant and its penetration into a number of products has been studied.

Experimental

The first three experiments were carried out in a cylindrical, steel, vacuum-fumigation chamber of approximately 3000-l. capacity controlled at a temperature of 20°. All subsequent tests were made in a similar chamber of approximately 1700-l. capacity, situated in a room controlled at 15° and 70% R.H.

In the tests at atmospheric pressure, the pressure in the chamber was first reduced by a small amount (about 5 cm. of mercury), then the required dose of methyl bromide was measured from a cooled burette into a small glass vessel connected to the chamber. This vessel was heated by hot water while a small stream of air was drawn through it to dilute the vapour entering the chamber. When vaporization was complete, the pressure in the chamber was immediately raised to atmospheric. A fan, which provided only very gentle stirring, was kept running in the chamber for 30 minutes.

In all the vacuum-fumigation tests vaporized methyl bromide was drawn from a simple counterpoised gas-holder in which it was confined over pure glycerol. In the tests by the sustained-vacuum method the absolute pressure in the chamber was first reduced to 5 cm. of mercury, and the required volume of fumigant vapour was then drawn in, the pressure rising to about 7 cm. This operation was completed in about two minutes. When testing the air-washing process, in each cycle the pressure was reduced to 5 cm. of mercury in a period of 11 minutes.

For tests by the French released-vacuum method the initial pressure was again 5 cm. of mercury. The methyl bromide vapour was drawn from the gas-holder and mixed with air at a mixing valve correctly set as a result of several trial dosings. The constant mixture of air and fumigant was introduced until the pressure in the chamber was atmospheric. This operation was completed in about three minutes.

A few tests were made of the penetration of fumigant into 140-lb. bags of wheat, and 140-lb. bags of decorticated groundnuts, but most tests were made using 70-lb. wooden cases of compressed dates measuring 19 in. × 11 in. × 10 in. For the withdrawal of gas samples, use was made of stiff hollow spears 12 in. long with points at the centre of each package. In some tests with the boxed dates an additional spear was used for the withdrawal of samples from a point 2 in. below the surface of the block. The least depth to the centre of the bags was 11 in Fine-bore copper tubing was attached to the outer end of the spear and led out of the chamber This type of tubing was also used for sampling the gas in the free space of the chamber. At the outer end of each sampling tube a rubber connexion was fitted, closed by a screw clip.

Samples were collected in evacuated flasks of various types. Removal of a series of gas samples from a point inside a package, at a time when movement of gas into or out of the package is taking place, inevitably disturbs the distribution. This effect is reduced if there is appreciable sorption of the gas by the commodity. It is also minimized by taking samples which are as small as practicable. In the tests at atmospheric pressure, 150-ml. samples were taken for the determination of concentrations in the free space, and in the bags of wheat and groundnuts. For other measurements in the tests at atmospheric pressure, and for all the sampling when the chamber was at reduced pressure, 20-ml. sampling vessels were used.

In the experiments where it was necessary to draw gas samples when the chamber was at atmospheric pressure, flasks containing 1 or 2 ml. of *cyclo*hexylamine and evacuated to a pressure of 2 cm. of mercury were used. These flasks have a short capillary tube and tap detachable by means of a standard ground-glass joint. A factor is applied in calculating the amount of gas drawn to allow for the residual pressure in the flask. After sampling, the flasks are left overnight before washing out the contents into a 250-ml. conical flask.

For sampling from the chamber when it is under reduced pressure (generally 5 to 7 cm. of mercury), completely evacuated flasks have been used, the reagent being introduced after the

gas sample has been drawn. This procedure avoids a number of errors which are difficult to allow for. If a sampling flask containing a reagent with an appreciable vapour pressure is used, the correction which is necessary for the residual pressure in the flask is greater, the lower the pressure in the chamber. Thus, using a flask containing *cyclo*hexylamine evacuated to a pressure of 2 cm. of mercury to sample from a chamber at a pressure of 6 cm. of mercury, the correction to be applied to the flask volume is approximately 4/6. Furthermore, any absorption of gas in the reagent in the time the sampling flask is connected to the chamber increases the volume of the sample taken. This is usually negligible when sampling from a chamber at atmospheric pressure as the proportion of fumigant to air is small. In a vacuum fumigation this proportion may be large. Thus if the chamber is evacuated to 5 cm. pressure and fumigant is introduced to bring the pressure up to 7 cm., the concentration of fumigant in air is about 30%. If an evacuated flask of volume V containing reagent is connected by a fine-bore tube to the chamber and left until sorption of fumigant is complete, the volume of the sample taken will be approximately $1{\cdot}43V$. In practice the volume taken will depend on the speed of sorption of the gas in the reagent and on the time the sampling flask is left connected to the chamber. It has been found difficult to obtain consistent results from samples taken in this way.

A completely empty sampling flask can readily be evacuated to a pressure below 0·1 mm. of mercury, and provided the pressure in the chamber is not below 1 cm., the correction for the residual pressure in the flask can be neglected. The concentration in the flask, expressed in mg./l., is equal to that at the point sampled irrespective of the pressure in the chamber.

The flasks used for sampling from the chamber when it is under reduced pressure were of a type devised by Winteringham, and similar to the sampling bulb of the two-chamber micro-sorption apparatus described by him.[6] Each consists of a bulb having two capillary outlets, one closed by a tap and the other by a small ground cap. To sample, the flask with cap removed is fitted to the rubber connexion on the sampling tube with the screw clip still closed, and is evacuated through the tap which is then closed. The screw clip is then opened. After about three minutes the clip is again closed, the flask removed and the cap quickly placed in position. For absorption and decomposition of the methyl bromide, use is made of *cyclo*hexylamine as suggested by Lewis.[7] After drawing the sample, approximately 1 ml. of this reagent is introduced into the flask from a graduated cylinder by removing the ground cap, dipping the capillary below the surface of the reagent, and cooling the bulb with cotton wool swabs soaked in ether. The cap is then quickly replaced and the flask left overnight. Before opening the flask for washing out the contents for analysis, the pressure is reduced by cooling in a refrigerator to prevent any loss of the contents as the cap is removed. About 50 ml. of water is drawn in and then expelled through the capillary in washing the contents into a 250-ml. conical flask. After making alkaline, the contents of the flask are boiled to expel the *cyclo*hexylamine, and estimation of the bromide present is completed by the oxidation method described by Kolthoff & Yutzy[8] and modified by Lewis.[9]

Results

For each experiment the nominal concentration has been calculated from the weight of fumigant applied and the volume of the chamber. The concentrations found are plotted against time in Figs. 1–8. Curves have been drawn showing the probable variation of concentration with time. Areas below these curves have been measured to provide estimates of the concentration–time products in mg.hr./l. obtained in selected fumigation periods. These products are shown in Table I. If each product is expressed as a percentage of the product obtained by multiplying the nominal concentration by the selected time, a figure is obtained which is a measure of the efficiency of penetration of fumigant to the point sampled. This figure has been termed the 'penetration factor'. These factors are also shown in Table I.

Discussion

The first three experiments were carried out at about the same nominal concentration, to allow a comparison of the rates of penetration of methyl bromide into three products when fumigated at atmospheric pressure and by the French released-vacuum method. Rates of penetration during the first three hours of each treatment can be compared, but since treatments at atmospheric pressure are not usually attempted in this short period, the fumigations at atmospheric pressure were continued for 24 hours.

With the coarsely granular products, wheat and decorticated groundnuts, penetration of methyl bromide to the centres of bags is so rapid that it is difficult to foresee circumstances in which it would be worth while employing vacuum-fumigation chambers to secure more rapid penetration. Even in a three-hour period, penetration factors exceeding 70 were obtained

Table I

Summary of results

Expt. No.	Method	Products	Sampling position	Nominal concn., mg./l.	Period hr.	Period min.	Concn.-time product, mg.hr./l.	Penetration factor
1	Atmospheric	Wheat, 1 bag	Centre	30	3	—	65	72
		,, ,,	,,		24	—	520	72
		Groundnuts, 1 bag	Centre		3	—	64	71
		,, ,,	,,		24	—	470	65
2	Atmospheric	Dates, 2 boxes	Centre, box 1	37	3	—	3	3
			,, ,,		24	—	240	27
			Centre, box 2		3	—	11	10
			,, ,,		24	—	290	33
3	French released-vacuum	Wheat, 1 bag	Centre	36	3	—	83	77
		Groundnuts, 1 bag	,,		3	—	93	86
		Dates, 1 box	,,		3	—	34	32
4	French released-vacuum	,, 2 boxes	Centre, box 1	96				
			,, box 2					
5	Sustained vacuum*	,, 1 box	2 in. deep	96	3	—	195	68
			,, ,,		4	10	400	100
			Centre		3	—	130	45
			,,		4	10	450	113
6	Sustained vacuum†	,, ,,	2 in. deep	96	3	—	145	51
			,, ,,		6	—	560	98
			Centre		3	—	110	38
			,,		6	—	650	113
7	Sustained vacuum‡	,, 12 boxes	Centre of one box	96	3	—	65	23
					4	10	220	55
					6	—	340	59

* Vacuum released after 3 hr. 10 min.
† ,, ,, ,, 3 hr. 30 min.
‡ ,, ,, ,, 3 hr. 15 min.

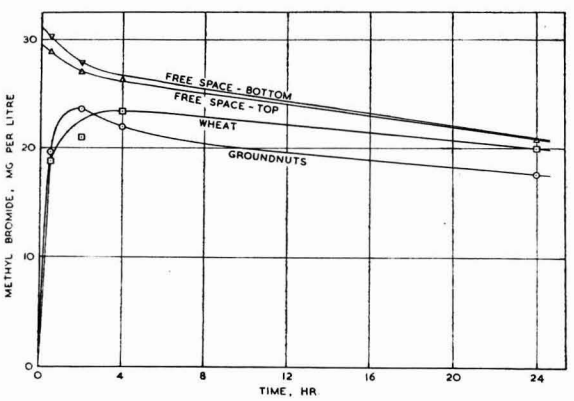

FIG. 1.—*Atmospheric fumigation of wheat and groundnuts*

(Expt. 1, Fig. 1). With tightly compressed blocks of dates in boxes, penetration during the first three hours in a test at atmospheric pressure (Expt. 2, Fig. 2) was very small, giving penetration factors of 3 and 10 at the centres of two boxes. The concentrations at these points rose (probably continuously) during the 24-hour period, and the concentration–time products obtained in this period confirmed that under favourable conditions and with adequate dose and exposure time this product can be effectively fumigated with methyl bromide at atmospheric pressure. In fact dates are commonly disinfested in this manner in Great Britain. However, it is clear that this is a product which might be more efficiently fumigated by methods employing reduced pressures.

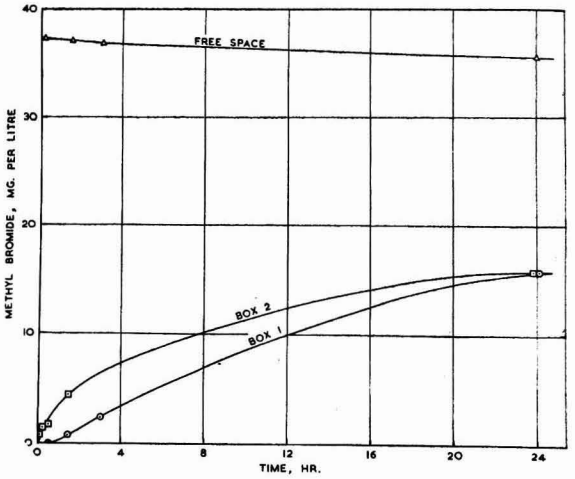

FIG. 2.—*Atmospheric fumigation of dates*

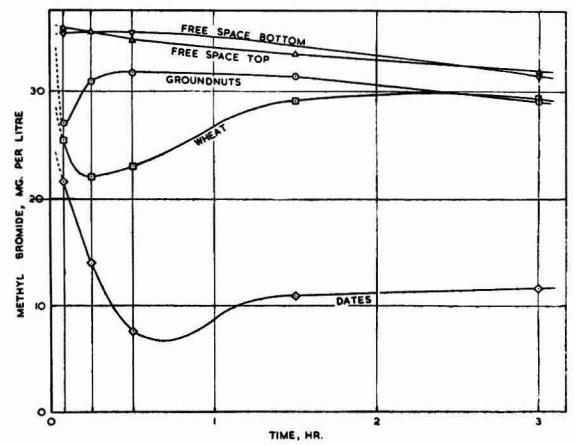

FIG. 3.—*Fumigation of wheat, groundnuts and dates by the French released-vacuum method*

In Expt. 3 (see Fig. 3), employing the French released-vacuum method, the curves show that the introduction to the evacuated chamber of fumigant–air mixture until the pressure was almost atmospheric caused the fumigant to be carried rapidly to the centre of each package. In the bags of wheat and groundnuts the concentrations at the centres probably approached the nominal concentration. Immediately after introduction of the mixture, however, sorption of fumigant by the product caused a rapid fall in concentration at these points. Later the concentration rose again by normal diffusion at atmospheric pressure. With groundnuts diffusion through the comparatively large intergranular spaces is so rapid that this rise in concentration appears to have begun about five minutes after the start of the fumigation, and the initial peak was not detected, but its occurrence has been inferred from the behaviour of the fumigant in the other products.

The curves for wheat and groundnuts cross after about two hours (as they also do in the test at atmospheric pressure). This is probably due to two factors, namely the lower rate of sorption of methyl bromide into wheat grains than into the kernels of the groundnuts, and the lower rate of diffusion through the smaller intergranular spaces of the wheat. The penetration factors obtained in three hours by the French method were higher than in the same period in the atmospheric test, but the difference for wheat was small.

Diffusion to the centre of the block of dates was very much slower, so that sorption continued to reduce the concentration for more than 30 minutes before this began to rise again. The

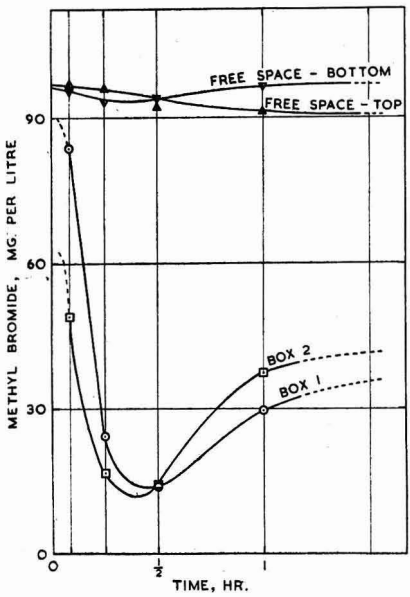

FIG. 4.—*Fumigation of dates by the French released-vacuum method*

penetration factor in three hours was 32, which, when compared with the figures obtained in the treatment at atmospheric pressure (Expt. 1), was much higher than those obtained in three hours and similar to those obtained in 24 hours. In such a case of equal penetration factors in a three-hour fumigation and a 24-hour fumigation, the dose of fumigant required in the three-hour period will be very much larger than in the 24-hour fumigation. If Haber's rule applied, and there were no special effects on the resistance of the insects due to the pressure changes, then the dose in the shorter fumigation would be eight times greater.

In Expt. 4 (see Fig. 4) two boxes of dates were fumigated by the French released-vacuum method at a higher dose. Measurements of concentration within the dates were not obtained after one hour, but up to that time the curves were similar in shape to that obtained in Expt. 3. The concentrations were higher by an amount corresponding to the increased dose, and it is probable that the penetration factor in three hours was similar.

During this experiment, temperature measurements were taken by means of thermocouples. No change was detected at the centre of a box, but in the free space during introduction of the fumigant–air mixture the temperature rose by 6° to 21°. On cooling to room temperature the pressure in the chamber was in consequence slightly reduced. This might cause an outward movement of fumigant from the package, but the effect would be small, since the lowering of pressure was only about 2% of atmospheric and would account for only a small part of the fall in concentration which occurred at the centre.

In Expt. 5 (see Fig. 5) penetration into a single box of dates was tested in a fumigation by the sustained-vacuum technique. Concentrations were measured 2 in. below the surface of the box as well as at the centre. At the reduced pressure (about 7 cm. of mercury, absolute pressure) diffusion of methyl bromide into the box was much more rapid than in the test at atmospheric pressure (Expt. 2). The concentration reached at the centre in three hours was about 73% of the nominal concentration, compared with 6·5% and 16·5% in the test at atmospheric pressure (Expt. 2), and 32% in a test by the French released-vacuum method (Expt. 3). The penetration factor in three hours was 45 compared with 32 by the French method. In a repeat of this test (Expt. 6, Fig. 6) the penetration factor in three hours was 38. Thus it appears that with this particular commodity in a three-hour treatment, the sustained-vacuum method as ordinarily applied results in rather better penetration of methyl bromide than the French released-vacuum method.

After the release of the vacuum in Expt. 5, further measurements of concentration were made, and it was found that a considerable increase in concentration occurred at the centre of the box, the maximum recorded being more than four times the free space concentration. The increase 2 in. below the surface was about half this amount. These increases result from a contraction of the air–fumigant mixture towards the centre of the box as the pressure is raised by allowing air to enter the free space. If, before the pressure is raised, there is an even distribution of fumigant throughout the box, then in the absence of sorption and mixing within the intergranular spaces, the concentration could be expected to increase by a factor equal to the increase in pressure. If the pressure rises from 7 cm. to 77 cm. an eleven-fold increase in concentration would be expected. In practice considerable mixing of air–fumigant mixtures must occur, and sorption causes a fairly rapid fall in concentration, so that the biggest increase recorded in our tests has been about 5·5 times (Expt. 5). These large increases to figures well above the free-space concentration can occur only when there has been a substantial penetration of fumigant throughout the package before the release of the vacuum. Although sorption and outward diffusion then cause the concentration to fall, a large concentration–time product is

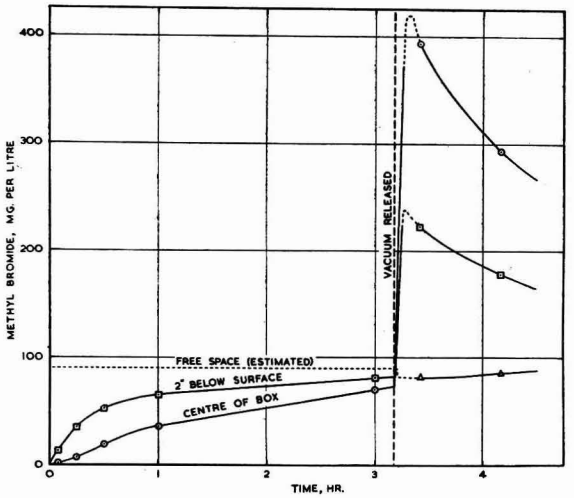

FIG. 5.—*Fumigation of dates by the sustained-vacuum method*

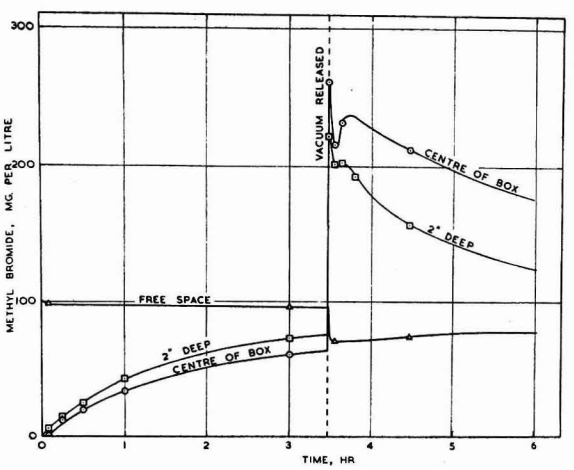

FIG. 6.—*Fumigation of dates by the sustained-vacuum method*

obtained in the ensuing period, which is greatest at those points for which the lowest products have been measured in the period before releasing the vacuum. In current fumigation practice, after release of the vacuum the chamber is immediately re-evacuated and then air is again allowed to enter. This process may be repeated once more. This is the so-called ' air-washing ' technique. No advantage is taken of the increased penetration demonstrated in the experiment described above.

It appeared possible that a marked increase in efficiency might result if, at the end of a period of treatment under reduced pressure, air is admitted and the treatment continued for a further period before any fumigant is removed from the chamber. It was decided to study further this modified technique. Expt. 6 was a repeat of Expt. 5 with, however, more detailed examination of the changes of concentration following the release of the vacuum. Similar high peaks were obtained, but the more frequent gas-sampling in the period immediately following gave results which appeared to require a double inflexion of the curves. Similar results have been obtained in other experiments, e.g. Expt. 7, Fig. 7, but an explanation of them is at present uncertain. The effect might arise if the point at the geometric centre of the box from which gas-samples were drawn was not the point to which maximum contraction occurred, and at

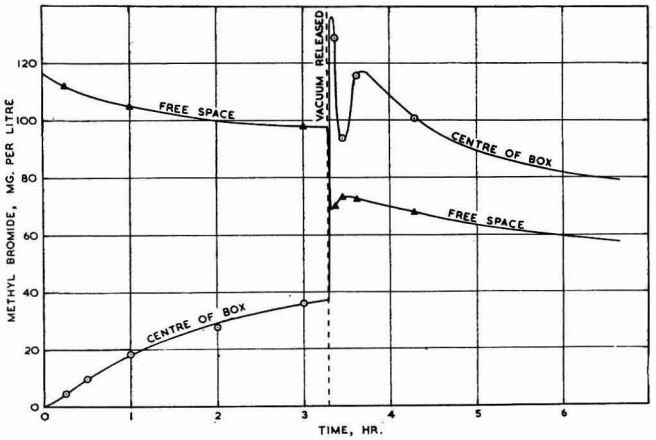

FIG. 7.—*Fumigation of 12 boxes of dates by the sustained-vacuum method*

which the highest concentration was produced. Whatever the explanation, the effect is of very short duration and is unlikely to be of practical importance.

An indication of the increase in efficiency of penetration under the conditions of these experiments is obtained by comparing the penetration factors calculated from the concentration–time products obtained in the periods of 4 hours 10 minutes or 6 hours, with the penetration factors which would have been obtained in the same periods if the vacuum had not been released. The latter figures were estimated after extrapolation of the concentration–time curves beyond the time when the vacuum was released. The figures obtained are shown in Table II. In some cases the penetration factor is more than doubled by release of the vacuum.

Table II

Penetration factors obtained by two techniques at the centres of boxes of dates in equal periods of time

Expt. No.	Load, boxes	Total period		Penetration factors	
		Hr.	min.	Sustained-vacuum only (estimated)	With delayed release of vacuum
5	1	4	10	54	113
6	1	4	10	46	75
		6	—	55	113
7	12	4	10	28	55
		6	—	34	59

It might be expected that the increase in the efficiency of penetration would be less marked under the conditions of commercial fumigation, when it is usual for a much larger proportion of the chamber volume to be occupied by commodity than in the experiments which have been described. That the increase should still be substantial is suggested by the results of Expt. 7, in which 12 boxes of dates were treated in the 1700-l. chamber. The boxes were arranged in three layers of 2 × 2 boxes, and the box sampled was in the middle layer and thus had only two surfaces exposed. Penetration to the centre of this box was slower than in Expts. 5 and 6 and in three hours the concentration at this point was little more than one-third of the free-space concentration. However, when the vacuum was released, the concentration at the centre increased more than threefold, and the estimates in Table II indicate that in a period of 4 hours 10 minutes the result of releasing the vacuum was to double the penetration factor.

Expt. 8 was designed to throw light on the changes of concentration inside a package during the air-washing treatment. Two boxes of dates were treated at a nominal concentration of about 85 mg./l. Sufficient intervals were allowed between successive operations to allow concentrations to be measured in the free space and at the centre of each box. In normal commercial practice the procedure is completed without any such delays. The concentrations found are shown in Fig. 8, and curves have been drawn showing the probable variation of concentration.

After the first release of the vacuum the usual sharp rise in concentration at the centres of boxes was noted, and this was followed by a fall in concentration as further sorption took place at these points. On re-evacuating the chamber there was the expected sharp fall in concentration, though the concentrations at the centres of boxes probably did not fall below 20 mg./l. and there was an increase in concentration during the ensuing period. Similar changes of concentration occurred during the second cycle of operations, the concentrations remaining at the centre of the two boxes immediately after the second re-evacuation being about 6 and 11 mg./l., and after the final release of vacuum about 13 mg./l. The final concentration measured in the free space was 1·2 mg./l. It is probable that rather less fumigant would have been removed from the boxes by the two air-washing cycles, if these had been carried out in the normal manner without delay between operations. In practice care must be taken to ensure that work-people unloading chambers are not exposed to methyl bromide, which may be desorbed from the fumigated product even after two air-washing cycles have been carried through.

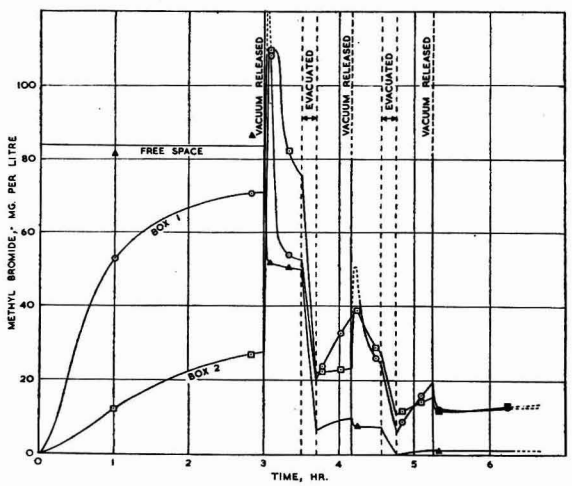

FIG. 8.—*Fumigation of dates by the sustained-vacuum method ; tests during air-washing process*

The wide difference between the results obtained in the two boxes in this last test illustrate the difficulty of attempting more detailed tests with this product. For further tests of vacuum-fumigation methods, and, in particular, of the proposed method employing delayed release of the vacuum, it was decided to use a product which might be expected to provide more reproducible results from one test to another, and bagged wheatfeed has been selected. These tests will be the subject of a further communication.

Acknowledgment

This work has been carried out as part of the programme of research of the Pest Infestation Laboratory, and this account is published by permission of the Department of Scientific and Industrial Research.

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MANUFACTURE OF ALGAL CHEMICALS. V.*—Laboratory-scale Isolation of D-Glucose from Laminarin

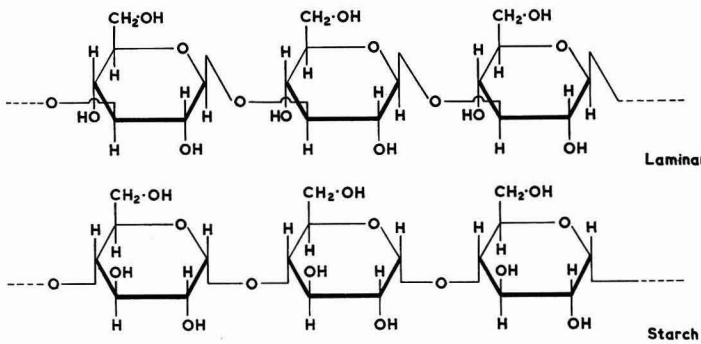
By W. A. P. BLACK, E. T. DEWAR AND F. N. WOODWARD

A method for the isolation of D-glucose from laminarin has been worked out on the laboratory scale, with a view to the ultimate development of a process suitable for large-scale production. Laminarin is hydrolysed quantitatively to D-glucose by heating an 18% (w/w) solution of the polysaccharide in 0·05N-hydrochloric acid at 135° in an autoclave for one hour. The resulting hydrolysate is neutralized and decolorized, and the glucose is crystallized from water as the monohydrate.

The production of glucose from laminarin is closely related to the commercial production of crystalline dextrose by the acid hydrolysis of starch.

Introduction

Laminarin is a glucose polysaccharide occurring exclusively in brown marine algae. It is a glucan containing β-D-glucopyranose units linked through the 1 : 3-positions,[1, 2, 3] and thereby differing fundamentally from the starch of land plants, in which the repeating units are α-D-glucopyranose residues linked through carbon atoms 1 and 4.



Laminarin isolated from *Laminaria cloustoni* frond is insoluble in cold water, whereas that isolated from *L. digitata* frond and many other species is water-soluble in the cold, and the preparation of these forms has recently been investigated in some detail.[4] Studies by Connell, Hirst & Percival[2] and Percival & Ross[3] have failed to detect any major chemical differences in the two forms, methylation and hydrolysis giving a yield of tetramethyl glucose corresponding to a chain length of about 20 glucose units in each case.

The conversion of laminarin to glucose by treatment with acids has been investigated by numerous workers. Kylin[5] found that laminarin from *L. cloustoni*, in solution (1·6%) in 5% sulphuric acid, was hydrolysed completely on heating at 100° for 4 hours, a 90% conversion to glucose being determined polarimetrically and by reducing power towards Fehling's solution. He also showed[6] that laminarin from *Desmarestia aculeata* gave 90% of glucose on heating a 2·0% solution in 3% sulphuric acid on the boiling-water bath for 4 hours. Gruzewska[7] carried out a number of experiments to determine the optimum conditions of hydrolysis of laminarin both by acids and by diastases. She found that laminarin from *L. flexicaulis* gave a 97% conversion to glucose on heating a 0·45% solution in 6% hydrochloric acid in an autoclave at 120° for 30 minutes. The hydrolysate, on neutralization, concentration and treatment with phenylhydrazine, yielded the characteristic crystals of glucosazone. Colin & Ricard[8] obtained glucose in crystalline condition from the hydrolysis of laminarin (from *L. flexicaulis*) with 5% sulphuric acid at 120° in an autoclave for 30 minutes. They carried out a series of hydrolyses with 5% sulphuric acid and 2% hydrochloric acid at 120° for various times, the course of the reactions being followed polarimetrically and by reducing power with Fehling's solution. The specific rotations of the hydrolysates varied from 48·8 to 53·0°, but were generally slightly less than that of pure glucose.[9] Barry[10] hydrolysed laminarin with 5% sulphuric acid on the boiling-

* Part IV : *J. Sci. Fd Agric.*, 1952, **3**, 122 ; Part III : *J. appl. Chem.*, 1951, **1**, 505 ; Part II : *J. appl. Chem.*, 1951, **1**, 414

water bath to constant rotation, but the glucose estimated polarimetrically never exceeded 90% of the yield calculated from the equation:

$$(C_6H_{10}O_5)_n + nH_2O = nC_6H_{12}O_6$$

With N-hydrochloric acid at 100° for 2½ hours, however, a 98·5% conversion to glucose was obtained.

Barry[11] isolated the disaccharide, laminaribiose, by heating laminarin with N-oxalic acid until the hydrolysis was about two-thirds complete (7 hours, 100°), neutralizing and destroying the glucose formed with yeast. The optically active liquid remaining was dissolved in methanol, and the laminaribiose was obtained as a powder by fractional precipitation with ethanol and ether. Recently, Connell, Hirst & Percival[2] have obtained the disaccharide by partial hydrolysis of laminarin with oxalic acid, and separation of the sugars on a cellulose column.

Proof of the structure of laminaribiose as 3-β-D-glucosyl D-glucose has been obtained by its synthesis from 1 : 2–5 : 6-di*iso*propylidene glucose and tetra-acetyl glucosyl bromide.[12, 13]

Cameron, Ross & Percival[14] found that laminarin from *L. cloustoni* in 1% solution was completely hydrolysed with N-sulphuric acid at 100° in 4½ hours. Connell, Hirst & Percival[2] showed that hydrolysis at 95° in 0·85N-hydrochloric acid was complete in 2½ hours, giving glucose, 96% (polarimetric estimation) and 95% (hypoiodite). No sugar other than glucose could be detected on the paper chromatogram. Soluble laminarin from *L. digitata* was completely hydrolysed with N-sulphuric acid at 95° after 4½ hours to give a 95·3% conversion to glucose.[3]

The hydrolysis of laminarin by enzymes has also been studied by various workers, although the early results are somewhat conflicting. Torup[15] stated that laminarin was not attacked by ptyalin, nor by diastase from pancreas or malt. Kylin[5] claimed that the polysaccharide was slowly hydrolysed by malt diastase, but he has since withdrawn this claim;[16] in this more recent work, malt diastase, takadiastase, ptyalin and emulsin were found to be without action, and the laminarin was precipitated unchanged after three days at 33°. Gruzewska[7] showed that the action of various animal and vegetable diastases was very slow, but that the juice of the snail, *Helix pomata*, was very effective, the hydrolysis to glucose being almost complete in 1 hour. This was confirmed by Colin & Ricard[8] and by Barry;[11] Barry found that a glycerol extract of the limpet (*Patella*) was also capable of hydrolysing laminarin to glucose.

Dillon & O'Colla[17] found that wheat β-amylase slowly hydrolyses laminarin, yielding glucose (70%) and a disaccharide after several days. Since malt diastase was inactive, they attributed the action to some factor ('laminarinase') other than the small amount of α-amylase in the crude wheat enzyme. The occurrence of a similar laminarinase has since been demonstrated in extracts of oats, barley, potato and hyacinth bulbs.[18] Recently, Peat, Thomas & Whelan,[19] in their studies on the enzymic synthesis and degradation of starch, have isolated a Z-enzyme from the soya bean, the apparent function of which is to supplement the action of β-amylase or phosphorylase on the amylase fraction of starch. They found that stock soya β-amylase, which still contained the Z-enzyme, hydrolysed laminarin, the limiting yield of glucose (72·3% after 142 hours) agreeing closely with the value of 70% reported by Dillon & O'Colla[17] for wheat laminarinase. There is thus a strong indication that the Z-enzyme of soya bean and the laminarinase of wheat are identical. Dillon & O'Colla also showed that laminarin is slowly attacked by emulsin (72·8% conversion to glucose after 46 days at 35°), but is not hydrolysed by pure crystalline β-amylase nor by freeze-dried salivary α-amylase.

In the present investigation, the complete hydrolysis of laminarin with acids has been studied under various conditions to determine the optimum conditions for the production and isolation of glucose from this polysaccharide. Commercial glucose (dextrose) is manufactured by the acid hydrolysis of starch, chiefly corn starch at the present time. The usual practice is to suspend the starch in water at a concentration of 15–20%, add hydrochloric acid until the normality is about 0·03, and then hydrolyse in an autoclave at 150° for about 30 minutes. The converted liquor is neutralized with sodium carbonate, passed through skimming tanks to remove floating impurities, and decolorized with bone char and activated carbon. The liquor is finally evaporated to a syrup containing 75–78% dry matter, and crystallized slowly over a period of days at about 41°. The crystalline mass of dextrose monohydrate (α-D-glucose monohydrate) is centrifuged, washed with water and dried in a stream of air. The commercial production of crystalline dextrose has recently been reviewed by Dean & Gottfried.[20]

Experimental; discussion of results

The laminarin used in these investigations was the insoluble form extracted from *L. cloustoni* frond. It was 'recrystallized' twice from water, and contained ash, 0·7%; $[\alpha]_D^{16}$ − 12·7° in water (*c*, 2·134). The pure glucose used was AnalaR anhydrous α-D-glucose, which gave

$[\alpha]_D^{14}$ + 107·6° (5 minutes) → + 52·4° in water (*c*, 4·165). Pigman & Goepp[21] quote $[\alpha]_D^{20}$ + 112·2° → + 52·7° in water (*c*, 4) for pure α-D-glucose. All rotations were measured in a 2-dm. tube.

Rate of hydrolysis of laminarin with acids

The rate of liberation of glucose from laminarin was followed by estimating the reducing power in the hydrolysate by Shaffer & Somogyi's method.[22] In Expts. 1–8 (Table I), the polysaccharide was heated under reflux on a boiling-water bath with the appropriate acid, and 2-ml. portions of the hydrolysate were withdrawn at intervals and allowed to cool. Portions of solution (1 ml.) were then transferred by pipette to a standard flask (250 ml.), water (20–30 ml.) was added, the solution neutralized with 0·1N-sodium hydroxide, using one drop of phenol red as indicator, and made up to the mark. The reducing power of 5-ml. portions of this solution was then estimated with Shaffer and Somogyi reagent 50 against a 5-ml. water blank as described by Cameron, Ross & Percival:[14]

$$\% \text{ Conversion to glucose} = \frac{\text{Titration difference} \times 250 \times 25 \times 162{\cdot}1 \times 26{\cdot}7 \times 100}{9{\cdot}02 \times 5 \times 1 \times 180{\cdot}2 \times 25 \times (\text{wt. of polysaccharide}) \times 1000}$$

The factor of 26·7/25 was introduced to allow for the increase in volume on solution. When anhydrous glucose (2·749 g.) was dissolved in exactly 25 ml. of N-sulphuric acid, the resulting volume was 26·7 ml.; roughly the same increase was observed with the other acids used. Laminarin (2·480 g.) on hydrolysis should yield 2·757 g. of glucose according to the equation:

$$(C_6H_{10}O_5)_n + nH_2O = nC_6H_{12}O_6$$

Two experiments (Expts. 2 and 5) were carried out with potato starch in order to compare the relative rates of hydrolysis of laminarin and starch.

In Expts. 7 and 8, the laminarin was dissolved in the hydrochloric acid in a Lintner pressure-bottle with a rubber stopper, which was enclosed in a metal frame to prevent the stopper being blown out, and the hydrolysis was carried out at 135° in a Pentecon autoclave (gauge pressure, 33 lb./sq. in.). The period of hydrolysis was taken as the time between the thermometer reaching 135° and the pressure being released, the times taken to reach pressure (about 15 minutes) and release pressure (about 5 minutes) being neglected. The technique was slightly altered in Expt. 9, where the concentration of polysaccharide, normality of acid and temperature of hydrolysis are similar to those employed in the commercial production of glucose from starch. The laminarin was dissolved in a known weight of 0·05N-hydrochloric acid, and the solution hydrolysed at 135°. Portions (2 ml.) were withdrawn at intervals and allowed to cool, 1 ml. was transferred by pipette to a weighed beaker, the weighed solution made up to 500 ml., and the reducing power determined as before on 5 ml.

The results in Table I show that laminarin is hydrolysed to glucose only slightly less rapidly than potato starch. Hydrochloric acid is to be preferred to sulphuric acid, 0·29N-hydrochloric acid (Expt. 6) being more efficient than 0·5N-sulphuric acid (Expt. 3). Expt. 9 indicates that the conditions used commercially for the hydrolysis of starch will also apply to laminarin, with the exception of the time of conversion, which may have to be increased slightly for laminarin.

Specific rotation of laminarin on hydrolysis

As a check on the rate of liberation of glucose determined by reducing power, the hydrolysis of laminarin was followed polarimetrically. Laminarin (3·600 g.) was dissolved in 0·057N-hydrochloric acid (16·86 g.) to give a 17·60% (w/w) laminarin solution (i.e. 19·57% as glucose, $C_6H_{12}O_6$), and the solution was hydrolysed at 135° as in Expt. 9 (Table I). Portions (5 ml.) were withdrawn at intervals, weighed, filtered, made up to 50 ml. in Expts. 1 and 2, and to 200 ml. in Expt. 3, and the rotations measured in a 2-dm. tube. The percentage conversion to glucose was calculated assuming the $[\alpha]_D^{20}$ of D-glucose at equilibrium to be + 52·7°.[21] The results are recorded in Table II.

These results are in good agreement with Expt. 9 (Table I) and with the figures quoted by Colin & Ricard.[8]

Rate of destruction of glucose by N-sulphuric acid

There are several references in the literature to the destruction of glucose during the determination of starch by hydrolysis with acids, and factors have been introduced to compensate for these losses.[23–25] Since most of the results in the literature concern rather dilute solutions, it was decided to investigate the degrading effect of acid in a glucose solution similar to those

formed in Table I. Anhydrous glucose (2·758 g., equivalent to 2·482 g. as $C_6H_{10}O_5$) was heated under reflux at 100° with N-sulphuric acid (25 ml.), and the reducing power determined at intervals as described previously. The results are shown in Table III. When the results after 7·5 hours in Expts. 1 and 2 (Table I) are corrected for a 5% destruction of glucose, the percentage conversions for laminarin and starch are increased to 96·2 and 99·4 respectively.

Isolation of β-penta-acetyl glucose from laminarin

In an attempt to show that glucose could be isolated quantitatively from laminarin on hydrolysis, it was decided to prepare the β-penta-acetate which is almost insoluble in water. Gruzewska[7] and Barry[10] confirmed the presence of glucose in laminarin by preparing glucosazone, but their experiments were not quantitative. Colin & Ricard[8] state that they obtained glucose in crystalline condition but give no details of the method used nor yield obtained. Laminarin (4·524 g.) was hydrolysed at 100° with N-sulphuric acid (50 ml.) for 5 hours with occasional stirring, the hydrolysate was centrifuged to remove a small brown residue, diluted to about 100 ml., passed through a Zeo-Karb 225 column to remove any cations derived from the ash, and the effluent neutralized by adding Amberlite IR-4B-OH (40 ml.) and stirring for about 10 minutes. The resin was filtered off, washed thoroughly with water, and the filtrate and washings evaporated *in vacuo* at 50° to a yellow syrup, which was dissolved in boiling methanol (25 ml.) and the solution centrifuged. The centrifugate was evaporated to dryness, and the resulting syrup dried *in vacuo* over phosphorus pentoxide (4·993 g.).

Acetylation was carried out with acetic anhydride and sodium acetate.[26] The syrup was heated with powdered anhydrous sodium acetate (2·5 g.) and acetic anhydride (25 ml.) at 100° with frequent shaking until completely dissolved, and then heated for a further 2 hours. The brown solution was poured into ice-cold water (250 ml.), when the viscous oil solidified almost immediately. The acetate was filtered, washed thoroughly with water, and dried *in vacuo* over phosphorus pentoxide (6·974 g.). Yield, 64·0%. On recrystallization from 67% ethanol, the yield dropped to 49·6%. M.p. 128–129°, not depressed on admixture with authentic β-penta-acetyl glucose (m.p. 129–130°); $[\alpha]_D^{15} + 7{\cdot}9°$ (*c*, 6·11 in glacial acetic acid). Hudson & Dale[27] quote m.p. 132° and $[\alpha]_D^{20} + 3{\cdot}7°$ (*c*, 6·267 in glacial acetic acid) for pure β-penta-acetyl glucose.

When potato starch (4·530 g.) was hydrolysed and acetylated under identical conditions, the yield of crude acetate was 67·6%, which is not significantly different from laminarin. With pure glucose (5·005 g.), however, the crude acetate was formed in 84·2% yield, which fell to 70·6% on recrystallization from 67% ethanol. It is evident that the small amount of impurity in hydrolysed laminarin or starch seriously lowers the yield of this compound.

Crystallization of glucose from various solvents

Before attempting to isolate glucose itself from laminarin hydrolysates, preliminary experiments were carried out to find the most suitable conditions for crystallizing small amounts of glucose on the laboratory scale in relatively quantitative yield.

Water.—Glucose is very soluble in water, 100 g. of saturated solution containing 45·0 g. of glucose at 15° and 84·9 g. at 91°.[28] When glucose (27·6 g.) was dissolved in hot water (10 ml.) to give a 73·4% (w/w) solution and the syrup placed in the refrigerator at 5°, crystals began to form after 3 days. After 7 days, the crystalline material was centrifuged, washed with water (5 ml.), ethanol (2 × 25 ml.) and ether (25 ml.), and dried in the atmosphere (9·65 g.). Yield, 35·0%; $[\alpha]_D^{16} + 101{\cdot}3°$ (5 minutes) → $+ 52{\cdot}4°$ in water (*c*, 4·15). The crystals were therefore anhydrous, and chiefly the α-form. In view of the high solubility of glucose in water, this solvent was abandoned for small-scale quantitative work.

When glucose is crystallized from concentrated aqueous solutions at 40° in the presence of a large amount of seed crystals, the monohydrate is formed.[20]

Methanol.—Glucose is relatively soluble in hot methanol. Methanol (100 g.) dissolves 1·5 g. of glucose at 0° and 11·1 g. at 76°.[29] Glucose (27·5 g.) was dissolved in water and evaporated *in vacuo* to a syrup, which was dried *in vacuo* over phosphorus pentoxide. The syrup was dissolved by refluxing with methanol (150 ml.), and the solution cooled, seeded with glucose, and kept at 5° for 3 days with occasional stirring. Crystallization was slow, particularly during the first day. The crystalline glucose was filtered, washed with ethanol and ether, and dried at 100° for 45 minutes (23·22 g.). Yield, 84·5%. The crystals were an αβ-mixture: $[\alpha]_D^{16} + 62{\cdot}1°$ (6 minutes) → $+52{\cdot}6°$ in water (*c*, 4·214).

When a syrup (28·1 g.), obtained by the hydrolysis of laminarin (24·75 g.) with N-H_2SO_4 (250 ml.) as described for the preparation of β-penta-acetyl glucose, was dissolved in methanol (150 ml.), seeded and kept at 5° for 4 days, glucose was isolated as a white crystalline powder

Expt. No.	Polysaccharide hydrolysed, g.	Acid used *	Concn. of polysaccharide (w/w), %	Temp. of hydrolysis, ° c.	% conversion to glucose after time (hr.)							
					0·5	1·0	1·5	2·0	3·0	5·0	6·0	7·5
1 ..	Laminarin, 2·478	N-H_2SO_4	9·0	100	29·4	58·0	—	85·3	91·1	92·7	—	91·4
2 ..	Starch, 2·480	N-H_2SO_4	9·0	100	40·0	71·7	—	91·3	93·6	95·2	—	94·3
3 ..	Laminarin, 2·484	0·5N-H_2SO_4	9·0	100	11·4	19·9	—	42·2	59·5	82·8	—	92·5
4 ..	,, 2·485	0·25N-H_2SO_4	9·0	100	5·4	—	—	21·7	30·8	52·2	61·7	70·4
5 ..	Starch, 2·485	0·25N-H_2SO_4	9·0	100	8·1	—	—	36·0	50·3	71·5	79·9	85·1
6 ..	Laminarin, 2·485	0·29N-HCl	9·0	100	14·9	30·9	—	59·9	77·5	91·5	92·5	93·9
7 ..	,, 2·487	0·1N-HCl	9·0	135	92·8	94·2	—					
8 ..	,, 2·483	0·05N-HCl	9·0	135	90·4	98·2	95·7					
9 ..	,, 3·599	0·05N-HCl	17·62	135	83·3	92·9	92·0					

* 25 ml. used for Expts. 1–8; 16·83 g. for Expt. 9

Table II

Specific rotation of laminarin on hydrolysis

Expt. No.	Time of hydrolysis, hr.	Concn., g. of $C_6H_{12}O_6$/100 ml.	$[\alpha]_D^{17}$	Conversion to glucose, %
1 ..	0·5	2·090	+ 49·3	93·5
2 ..	1·0	2·086	+ 49·8	94·6
3 ..	1·5	0·523	+ 49·7	94·3

Table III

Action of N-*sulphuric acid on glucose*

Time of heating, hr.	0·0	1·0	2·0	3·0	7
Glucose in soln., %	100·3	98·6	97·7	97·1	95

Table IV

Crystalline glucose fractions from hydrolysed laminarin

Fraction	Crystallization time at 5°, days	Equilibrium $[\alpha]_D^{17}$ in water (*c*, 4·0)	Purity (calc. from rotation), %	Yield, %

Table V

Crystalline glucose fractions from hydrolysed laminarin

Fraction	Moisture, %	Dry wt. (corr. for added seed),	Yield, %	$[\alpha]_D^{20}$ (5 min.) in water (*c*, 3·7 on	Equilibrium $[\alpha]_D^{20}$ in water	Purity (calc. fro rotation),

(11·30 g.). Yield, 41·1%; $[\alpha]_D^{15}$ + 69·4° (6 minutes) → + 52·0° in water (*c*, 4·04). M.p. 145–146°.

Ethanol, 92% (*v/v*).—This is a very convenient medium for crystallizing small quantities of pure glucose (Dr. D. J. Bell, private communication). When glucose (4·998 g.) was dissolved in water (4 ml.) and ethanol (46 ml.), crystals formed almost immediately. After 2 hours at room temperature, and overnight at 5°, the glucose was isolated and dried (4·163 g.). Yield, 83·3%; $[\alpha]_D^{14}$ + 62·4° (6 minutes) → + 52·3° in water (*c*, 4·165). The product was again an $\alpha\beta$-mixture. When, however, this solvent was applied to laminarin hydrolysates, the solutions failed to crystallize and syrups were deposited. Reducing the concentration of sugar in the solution still failed to give a crystalline product.

From these experiments, methanol was considered to be the most suitable solvent for the small-scale, quantitative crystallization of glucose, although the crystallization rate was very slow.

Hydrolysis of laminarin and crystallization of glucose from methanol

The conditions used in Expt. 9 (Table I) were employed. Laminarin (49·67 g.) was dissolved in 0·057N-hydrochloric acid (220 ml.) to give an 18·0% (w/w) laminarin solution, and the solution hydrolysed at 135° for 1 hour in a flask plugged with cotton wool. The hydrolysate was centrifuged to remove a small brown precipitate, the centrifugate passed through a Zeo-Karb 225 column, and the effluent neutralized by adding Amberlite IR-4B-OH and stirring for about 10 minutes. The resin was filtered off, washed thoroughly with water, and the filtrate and washings evaporated *in vacuo* at 50° to a brown solution (300 ml.), which was decolorized with charcoal (25 g.), filtered, and the filtrate evaporated to a colourless syrup and dried *in vacuo* over phosphorus pentoxide. The syrup (56·1 g.) was dissolved by refluxing with methanol (300 ml.), the solution centrifuged to remove a small residue, the centrifugate seeded with α-D-glucose (2·00 g.) and kept at 5° for 7 days with occasional stirring. The crystalline glucose A was filtered, washed with ethanol and ether, and dried at 100° for 45 minutes to a pure white powder.

The mother liquor was evaporated to a syrup (40·1 g.), which was dissolved in 0·057N-hydrochloric acid (160 ml.) and the solution ' reconverted ' at 135° for 1 hour. The hydrolysate was again neutralized, treated with charcoal (20 g.) and worked up to a syrup (37·6 g.), which was dissolved in methanol (150 ml.) and seeded (1·00 g.). After 7 days at 5°, the crystalline glucose B was isolated as before.

The mother liquor on evaporation was again hydrolysed with 0·057N-hydrochloric acid, and the solution worked up as described above to give crystalline glucose C. Two further crops of crystals were obtained by evaporating the mother liquor and crystallizing from small volumes of methanol. The analysis of these crystalline fractions is shown in Table IV.

The total yield of crystalline glucose therefore amounts to 75·8% of the theoretical yield. However, in view of the extremely slow rate of crystallization of glucose from methanol, an attempt has been made to crystallize from water using the conditions employed in the commercial production of dextrose from starch.[20]

Hydrolysis of laminarin and crystallization of glucose from water

Laminarin (49·67 g.) was dissolved in 0·057N-hydrochloric acid (220 ml.), and the solution hydrolysed, treated with ion-exchange resins, decolorized with charcoal and evaporated to dryness exactly as described in the previous experiment. The resulting colourless syrup was dissolved in water to give a solution containing 77·5% (w/w) dry matter, and the solution seeded with α-D-glucose (13·9 g.) and allowed to stand at room temperature (20°), when the viscous solution solidified overnight. After 2 days at room temperature, followed by 2 days at 5°, the crystalline glucose A was filtered, washed with ethanol and ether, and dried *in vacuo* over phosphorus pentoxide. The results in Table V show that this fraction is α-D-glucose monohydrate (Calc. for $C_6H_{12}O_6.H_2O$: H_2O, 9·1%).

The filtrate on evaporation gave a syrup (17·5 g.) which was dissolved in water to give a 76·1% dry matter content and seeded with α-D-glucose (4·4 g.). After 1 day at 20° and 1 day at 5°, the glucose B was isolated as before. This fraction was found to be a mixture of the monohydrate and the anhydrous material.

The analysis of these fractions is shown in Table V.

The yield of crystalline glucose therefore amounts to 67·3% of the theoretical yield after a crystallization time of only 6 days. The yield of 56·9% after the first crystallization is in good agreement with the figure of about 60% generally obtained for starch.[20] Greater efficiency could no doubt be obtained by crystallizing under the carefully controlled conditions of time

and temperature employed in the commercial production of dextrose, and also by a more thorough working up of the mother liquors.

Summary

Optimum conditions have been worked out for the complete conversion of laminarin to glucose. Hydrolysis is effected by heating an 18% (w/w) solution of the polysaccharide in 0·05N-hydrochloric acid at 135° in an autoclave for 1 hour, the reducing power and specific rotation of the hydrolysate indicating a 93–95% conversion to D-glucose. Laminarin is hydrolysed by acids only slightly less rapidly than potato starch.

β-Penta-acetyl glucose has been prepared from hydrolysed laminarin in 64·0% yield (crude product), which compares favourably with a yield of 67·6% from hydrolysed starch under the same conditions. Pure glucose, however, gives a much higher yield (84·2%) of crude acetate, indicating that impurities in the hydrolysates of laminarin and starch seriously lower the yield of this compound.

Methanol has been found to be a satisfactory solvent for crystallizing relatively small amounts of glucose, although solutions must be allowed to stand for long periods to obtain maximum yields of crystals. Pure glucose can be recrystallized from methanol in 84·5% yield after a crystallization time of 3 days at 5°. Much longer times are required for laminarin hydrolysates. In a hydrolysis using 50 g. of laminarin, crystalline glucose has been prepared in 75·8% yield. The glucose was obtained in 5 fractions with purities ranging from 99·6 to 95·1%.

When glucose is crystallized from concentrated aqueous solution at 5° in the absence of any seed, crystallization is slow and the yield is poor. When, however, the solution is treated with a large quantity of seed crystals, and allowed to stand at 20°, crystallization sets in rapidly and a good yield of α-D-glucose monohydrate is obtained. In a hydrolysis using 50 g. of laminarin, pure crystalline glucose has been isolated in 2 fractions in 67·3% yield, after a crystallization time of only 6 days. The yield of the first crop of crystals (56·9%) compares favourably with approximately 60% obtained in the commercial production of dextrose from starch.

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