J. Inst. Brew., March-April, 1978 Vol. 84. *pp.* 81-84

THE HORACE BROWN MEMORIAL LECTURE

RERUM COGNOSCERE CAUSAS

By Anna M. MacLeod

(*Heriot-Watt University, Edinburgh*)

To understand the causes of natural phenomena is a major objective of all scientists, and one in which Horace Brown succeeded to a greater degree than most. He was concerned with almost all aspects of the brewing process, and, as a bonus, his brewing studies led him to enunciate laws of general scientific applicability. His classic work on the physiology of germination, carried out in the 1890's, is still largely valid, and some problems which he recognized still await satisfactory solutions.

The motto of the Institute of Brewing, which can be seen on the President's Badge of Office and which is inscribed on the cover of every issue of the Institute's Journal, comes from Book II of The Georgics. 'To understand the causes of things'—in this case the contributions of the raw materials of brewing to the finished product, all of which are also featured on the Institute's crest—is a highly appropriate objective for a scientific body, and I am sure that it is one which Horace Brown, whose work we are commemorating today, would thoroughly have approved though the crest and the motto were not adopted by the Institute until 1937, some twelve years after Horace Brown's death. Moreover, it is instructive to consider this motto in context: the complete line from Virgil[22] reads *Felix qui potuit rerum cognoscere causes*—happy is the man who is able to understand the causes of things—and that sentiment too would certainly have been endorsed by Horace Brown.

In support of this, I commend to you the paper[4] which he delivered to the Institute in 1916 on the occasion of the presentation of his portrait. That paper, which occupies seventy-three pages of the *Journal*, is entitled *Reminiscences of Fifty Years Experience of the Application of Scientific Methods to Brewery Practise*, and it is permeated with enthusiasm. I think there can be little doubt that Horace Brown, more frequently than most scientists, experienced that intense intellectual excitement which comes from elaborating a theory, putting it to the test experimentally, and finding, apparently, that it works. All scientists, however humble their attainments, have such moments of elation. No matter that further work, their own or more frequently someone else's, shatters the beautiful hypothesis with a new, unwelcome fact: that is a different problem, to which I shall return later. The important point is that all creative scientists, and Horace Brown was certainly such a one, can most fully subscribe to the Institute's extended motto—they have their moments of joy.

So much for the title of this talk. Let me now remind you briefly of some of the achievements of Horace Brown in the context of *Rerum cognoscere causas*. After a grammar-school education in Burton-on-Trent—largely classical and mathematical—Horace Brown spent one year as a student at the Royal College of Chemistry in London before joining Worthington's brewery at the age of seventeen and a half, as a junior brewer—and a practical brewer he was for some twenty years, apparently working twelve hours a day, seven days a week. By present-day standards he had a fairly slim preliminary scientific education. But formal education is only a part, and possibly a minor part, of learning, and Horace Brown had the incalculable advantage of growing up in an environment permeated by the spirit of enquiry. As a schoolboy, and encouraged by his stepfather, he was a keen naturalist, specializing in the microscopic life of local ponds—a hobby which was to pay good dividends later during his work on yeasts and on barley. Furthermore, the chemist, Dr Henry Böttinger, manager of Alsopps Brewery in Burton and a pupil of Liebig's, was a family friend who sowed in the receptive mind of young Horace Brown the idea that brewing need not always be an empirical art, and that discoverable chemical principles underpinned the hallowed dogma dear to the heart of the practical brewer.

So Horace Brown started work in an enquiring frame of mind—an attitude not wholly approved by his superiors in the brewery, though they did buy him a balance, and, I suppose, the weights to go with it. His critical faculties were further stimulated by the presence in Burton in the 1870's of a quite remarkable galaxy of scientific talent—Peter Griess at Alsopps, Cornelius O'Sullivan at Bass, Adrian Brown as chemist at Salt's Brewery, to name but three. The brilliance of this Burton society is well attested by the fact that a small dining club of brewers called the Bacterium Club, which had no rules, no subscription and no formal lectures, produced from its fourteen argumentative members four Fellows of the Royal Society—among them, of course, Horace Brown himself.

The motto of the Royal Society is the slightly enigmatic one of *Nullius in verba* which means, roughly speaking, *Under nobody's word.* It really becomes meaningful only when one considers the whole sentence, this time from Horace,[13] which reads

> 'Nullius addictus iurare in verba magistri
> Quo me cumque rapit tempestas, deferor hospes.'

Being translated, that comes out as

'I am not bound to swear allegiance to any master
Where the wind carries me I put into port and make myself at home.'

I have no doubt at all that Horace Brown approved of, and abode by, that motto also.

Though Horace Brown's formal scientific training was perhaps minimal the range of subjects to which he applied his talents and to which he made major contributions was formidable: water analysis and brewing liquor, barley and its enzymic transformation into malt, starch and its degradation during mashing, the nitrogenous components of wort and their contribution via metal-catalysed interaction with tannins to yield haze, dry hopping and beer flavour, oxygen requirements of yeast, bacterial and wild yeast infections, pasteurization. It reads rather like chapter headings from a good modern textbook of brewing.

Many previous Horace Brown lecturers have taken one or other of these interests as starting points for their discourses. Thus, Dr Rainbow[21] last year gave us a masterly account of brewing microbiology as it has developed from its joint conception by Horace Brown and Louis Pasteur, and Dr Bishop[1] a few years ago brought us up to date with the nitrogen problem in brewing. I shall not refer to these topics again, because they will be fresh in your memories, but I should like to say just a little about most of Horace Brown's other investigations, in so far as they impinge on what I might call the text of this lecture.

Take first his preoccupation with dry hopping.[8] He was concerned to find an explanation of the fact that addition of hop cones to cask beer had what he described as a 'freshening' influence on the palate and was accompanied by improved after-fermentation with a desirable higher level of carbonation in the beer. These improvements he attributed to the presence of diastase in the hop bracts, yielding further supplies of fermentable sugars from the beer dextrins and so prolonging fermentation. This paper is a little masterpiece of logical experimentation, and, if my audience was a student one, I would say that it was required reading before the next lecture. You will find it in the Institute's Journal for 1893 on page 94.

His observations on hop diastase led him to wonder, and to investigate, whether all foliage leaves contained diastase. From this work came, also in 1893, his paper on *The Chemistry and Physiology of Foliage Leaves*,[3] a tour de force which won him the Longstaff Medal of the Chemical Society. Later, having left Burton for London, he extended this work still further at the Jodrell Laboratory at Kew. He was perturbed by the fact that the stomatal pores through which gases enter a leaf occupy only 1-2% of its surface area; this did not seem to be enough because, on making the necessary measurements he found that carbon dioxide enters an illuminated leaf about fifty times as rapidly as would be expected if the absorptive area was, say, dilute caustic soda.

To explain this anomaly he introduced,[6] with appropriate mathematical treatment, the concept of overlapping diffusion shells above each individual pore so that the leaf surface could be considered as an uninterrupted absorptive layer instead of a series of pinholes in a stretch of impermeable cuticle. This explained things nicely and in turn led to the realization that a gas could travel virtually unimpeded through a multiperforate septum provided the perforations, like those in the leaf, were suitably spaced—a finding of some engineering significance. So, starting from dry hopping, he ended with the enunciation of a new physical principle of general applicability which still holds good, as indeed does his work on gas absorption by leaves. Whether or not he provided a wholly satisfactory explanation of the beneficial effects of dry hopping is another question: I leave you to decide that, after you have read the recommended paper.

A somewhat similar tale could be told about Horace Brown's interest in the analysis of brewing liquor. Burton brewing wells (and, according to Dr Portno,[10] there are still fifty-two of them in use) draw their water either from the keuper marl or from sandstone, and the ionic composition of the water reflects the geological nature of the distant strata from which the water is ultimately derived. Not content with simple water analysis, Horace Brown pursued his investigations right back to the origins of the liquor salts, linking his work in the brewery with his earlier geological interests. All this, in conjunction with his other researches, brought him considerable personal renown—and an honorary degree from the University of Edinburgh. In his laureation address in 1898, the Dean of the Faculty of Law, Sir Ludovic Grant, said: 'Mr Brown now holds a pre-eminent place among the experimentalists whose labours have been crowned with fruitful results. . . . His published papers constitute a massive and important contribution to chemical, biological and geological literature.' Not, you will notice, to brewing literature—but no matter. This was *Rerum cognoscere causas* with a vengeance. And I cannot resist drawing your attention to the fact that it was only the University of Edinburgh, of which I have the privilege of being a member, which was percipient enough to recognize the merits of this unusual man, with an honorary degree.

But a continued, tenacious, dedication to elucidating the prime cause of any natural phenomenon which comes one's way, laudable though it is in the scientist involved, does pose problems for the paymaster of that scientist. What does a director of research do when he finds that one of his more brilliant scientists, whom he fondly imagined to be investigating dry hopping, is actually spending all his working hours devising equations to accommodate the flow of gases through multiperforate membranes? And another, who is reputed to be improving methods of water analysis, is using all his creative powers to explore problems of geomorphology? These are extreme cases, but I suspect that many of the ablest scientists in brewing research are going to feel intellectually frustrated if they are prohibited from going at least a little way along the path leading to the probably unattainable ultimate explanation of the problem they have been hired to study. And an intellectually frustrated scientist is not going to be wildly successful in the prosecution of even a fairly routine investigation: he must have the conviction that, scientifically speaking, the world is his oyster. But—who pays? I ask the question, and leave you to provide the answer.

Let us now return to Horace Brown's more industrial work and look at what I consider to have been his major contribution to brewing science and indeed to botanical science in general. I refer, of course, to that massive publication of work carried out in conjunction with G. H. Morris—the paper entitled *Researches on the Germination of some of the Gramineae.*[7] By 1890, when this paper appeared, the anatomy of the barley grain had been fairly fully described but virtually nothing was known about the metabolic changes which accompany—and indeed cause—germination. There was a static understanding of barley and malt—a state of affairs which Brown & Morris transformed into a dynamic appreciation of the malting process.

I suspect that this 1890 paper, which occupies seventy pages of the *Journal of the Chemical Society*, is more frequently cited in reference lists than it is actually read. This is unfortunate, because it is not only interesting in its own right but it is also a splendid source of ideas for further work. For example, and on a fairly topical note, Brown & Morris observed that, although there is no starch present in the embryo of a mature ungerminated grain of barley, the first faint beginnings of growth are accompanied by deposition of starch granules in the parenchymatous tissues of the rootlets and in the coleorhyza—a deposition which is detectable before the grain has fully chitted. Could this perhaps be used as a simple rapid diagnostic test for pre-germination?

Horace Brown's main preoccupation was with the nature and origin of the enzymes which are responsible for the breakdown of the food reserves of the endosperm. These enzymes, of course, are of outstanding importance in the process which maltsters refer to as modification. Brown was apparently the first to recognize that hydrolysis of starch is not really important in this context; what does matter to the maltster, and even more to the brewer, is the dismemberment of the endosperm cell walls, to leave in malt what he described as 'a residue of minute spindle-shaped fragments' whose interstices were permeable to amylolytic and other enzymes. Horace Brown also mapped out the three-dimensional pathway along which modification proceeds, rapidly on the dorsal non-furrowed aspect of the grain and rather slowly on the furrowed side. His explanation of this asymmetrical wave of modification—that the cells whose walls are first dissolved are developmentally younger ones, and therefore less tough, has now been largely superseded. Notice, I am not saying it is wrong. I am merely suggesting that today we have available new facts which fit better into the current explanation of modification. I shall return to this matter of acceptable hypotheses later.

In 1890, Horace Brown was completely confident that the tissue responsible for manufacturing what he called the cytohydrolytic enzymes (we would now call them endo-β-glucanases and pentosanases) was the embryo, and in particular the epithelial layer of its scutellum. The experimental work which he carried out fully supported this contention; thus, all metabolic changes in the endosperm, including wall dissolution, proceeded from the embryo towards the distal part of the corn, and no modification occurred if the embryo was removed from the grain. Furthermore, a transplanted embryo could induce the usual cell wall changes in an endosperm taken from grain which had previously been treated with chloroform vapour for twenty-four hours or immersed in absolute alcohol for six weeks, so that it could reasonably be assumed to be dead. From all these lines of evidence what other conclusion could he have reached: the embryo must be the effective organ. Incidentally, he did not ignore the aleurone, which he recognized as a living tissue and so as a potential source of enzymes, but in none of his experiments could he demonstrate enzyme formation and secretion from the aleurone.

There must, then, have been considerable consternation in the Burton laboratories a few days after the paper reporting the work I have just described had been despatched to the Chemical Society, because there appeared in *Berichte*[12] a paper from that eminent German plant physiologist, Haberlandt, in which he claimed, with good supporting evidence, that the aleurone of cereal grains was, contrary to Horace Brown's belief, an active enzyme-secreting region. Haberlandt had used the aleurone from rye which had been grown for some seven days, whereas Horace Brown had, apparently, always used aleurone from ungerminated grain or from grain which had been steeped just long enough to soften it. Sadly, neither of them appreciated the fact that they were working with two different tissues.

Having seen Haberlandt's work, Horace Brown went back to the bench, repeated some of his own experiments—and found no good reason to change his mind. He too had recognized that aleurone from seven-day grown grain contained hydrolytic enzymes but, in 1890, he attributed this quite reasonably to contamination from embryo-derived material. So he stuck to his guns or, one might say, clung to his Royal Society motto of *Nullius in verba;* he was not going to accept the word of anyone else, however eminent—and Haberlandt was very eminent indeed—unless it could be backed up experimentally.

But Horace Brown made one interestingly prophetic statement in his addendum to the 1890 paper rebutting Haberlandt. Referring to Haberlandt's comment that the secretory powers of the aleurone were manifested only when part of the embryo —say a root initial—was left attached to the endosperm he says,

'If we are to accept Haberlandt's explanation . . . we must assume that the presence of a germ has some mysterious power of influencing the aleurone cells and of so far controlling their metabolic processes as to cause active secretion of a special enzyme'.

Many a true word is spoken in jest and we now know that the mysterious power of the embryo is the plant hormone, gibberellic acid—though this mysterious power was not identified until seventy-two years after that passage was written.

However, Horace Brown did not let matters rest there. Despite his vehement rejection of Haberlandt's conjectures, a certain unease seems to have persisted—a frame of mind which must have been intensified by the appearance of a number of papers purporting to show that cereal endosperms were capable of self-digestion, regardless of the presence of the embryo. So, in conjunction with Escombe, he undertook a ruthless re-examination of the experimental methods used, including his own.

Fortunately Horace Brown was no mean microbiologist—rather a rarity at that time—and he had little difficulty in demonstrating that bacterial action was responsible for all of the reputed vital activity of the starchy endosperm. Nonetheless, there remained the problem of the living aleurone cells. A highly critical repetition of his own techniques eventually showed that the treatments with chloroform or absolute alcohol used previously to kill the aleurone were ineffective—so his conclusion that a living embryo grafted on to a dead endosperm could produce all the changes characteristic of normal germination was at best non proven. It is rather odd that barley grains have to be immersed in absolute alcohol for over seven weeks before they lose the power of germination, though they die very quickly in 50% ethanol, but there it is. When Horace Brown used more reliable methods to kill the aleurone, and repeated the earlier experiments several hundred times, it then became clear that when grafts were made on to endosperm with dead aleurone, root growth was restricted, the seedlings were flaccid, there was little dissolution of the endosperm walls adjacent to the embryo and no cytohydrolysis at all in the layers of the endosperm under the aleurone. On the other hand, when grafted embryos were associated with living aleurone, seedling growth was normal, cell walls adjacent to the embryo were rapidly dissolved, and cytohydrolysis in the sub-aleurone layer proceeded apace—just as in intact germinating grain.

The conclusion was inescapable: a living aleurone was essential to ensure the formation of cell-wall degrading enzymes.

I have dwelt at some length on this possibly minor part of Horace Brown's work on germination not only because I find it interesting but also because it illustrates his pre-eminence as a scientist. He was fully prepared to test his own cherished beliefs as rigorously as possible and, if necessary, to admit where he was wrong. This then opened the way to the enunciation of a new hypothesis—and science, surely, is basically concerned with the testing of hypotheses rather than with the simple accumulation of unexamined facts. To quote Karl Popper:[18]

'In the history of science it is always the theory and not the experiment, always the idea and not the observation, which opens up the way to new knowledge. I also believe that it is always the experiment which saves us from following the track which leads nowhere, which helps us out of the rut, and which challenges us to find a new way'.

The explanation of the differences between Horace Brown and Haberlandt now seems to us obvious, but one must remember that in the 1890's plant hormones were only a glimmer in the minds of a few highly imaginative plant physiologists. To put things in historical perspective we must recall that Darwin published his work on the influence of the cereal coleoptile tip on the bending of the whole organ towards light[9] only a few years before Brown & Escombe's paper on depletion of the endosperm—in 1880. With hindsight, Darwin's paper contained all the necessary experimental evidence for the inference that a defined chemical substance was concerned in differential growth—in this case, bending of a stem towards the light—but the reality of auxin was only established with the work of Went[23] in 1928, and its chemical nature as indolyl acetic acid, a relatively simple substance, was not unequivocally determined[15] until 1934–1935.

The gibberellins took even longer than the auxins to slot into place. It was in 1962 that the secretion of cell-wall degrading enzymes—endo-β-glucanase and pentosanase—by embryo-free aleurone was shown by MacLeod & Millar[16] to be a gibberellin-dependent phenomenon. A little later, various groups of workers established the key role of the embryo of barley as a synthesizer and exporter of gibberellins, and MacLeod & Palmer[17] correlated the path taken by the hormone, via the upper part of the scutellum, to the dorsal aspect of the grain, with the asymmetric pattern of cell wall lysis observed by Horace Brown eighty years previously—though Horace Brown's explanation of the differential rate of digestion has not, to my knowledge, been wholly disproved.

Armed with some understanding of the gibberellins, the differences between Horace Brown and Haberlandt are easily reconciled: Haberlandt used aleurone which had been naturally provided with gibberellin whereas Horace Brown appears to have preferred gibberellin-free aleurone. Quantitatively the two tissues differ by less than one part in a million—but what a world of difference that makes to their performance.

We can now fully endorse Horace Brown's early description of the barley embryo as equivalent to a parasite feeding on its host.[7] Indeed, this is a better analogy than he can have realized, because the embryo not only digests its host, the endosperm, but, through the agency of gibberellic acid, it induces the host to catalyze its own dissolution—an admirably economical arrangement, not uncommon in efficient parasites. Those of you who are familiar with Horace Brown's work on germination will realize that I have selected one small part of it for detailed examination—and that, perhaps, not his principal thesis. His major preoccupation in the 1890 paper was with the diastase, or as we would now say, the amylases, of germinating barley, and the cytolytic enzymes engaged his attention largely because the cell walls of the endosperm impeded the passage of diastase. What he says about the amylolytic enzymes of barley has a surprisingly modern ring, and one at least of the observations he made on the effects of these enzymes on starch granules merits re-investigation using modern techniques, such as electron microscopy.

Among the things he discovered, and I quote his results mostly from the chapter headings, I select six for your contemplation. He knew that slightly acid conditions stimulated the secretion of α-amylase from the scutellum—and, remember, the concept of pH had to await the work of Sørensen at Carlsberg. He knew that there were two amylases and that the diastase of raw barley, which we would now call β-amylase, was less heat stable than the diastase of secretion- α-amylase. He knew that β-amylase could not attack ungelatinized starch granules, and that α-amylase could cause corrosion of intact barley starch, though, as had previously been shown by Kjeldahl, it was apparently without effect on ungelatinized potato starch. This observation was completely overlooked for eighty years; its validity, for barley at least, has recently been confirmed.[10] The reason for its neglect is not only the authority of Kjeldahl, but also the fact that most investigators used potato starch which is readily available commercially. He knew that assimilable sugars, and, in particular, sucrose, strongly inhibited the secretion of both α-amylase and the cell-wall degrading enzymes—a finding which can perhaps be linked to Dr Radley's demonstration[20] that sucrose inhibits the formation of gibberellic acid. He knew that the maltose derived from starch hydrolysis in the endosperm was transformed into sucrose in the embryo—a fact rediscovered, in 1959, by Edelman.[11] I could go on, but I think I have made my point: though all these phenomena were hailed as new information when they were re-established, some of them quite recently, they were all available from 1890, to those who would read—and think.

There is one observation made by Horace Brown which, to my knowledge, has not been re-investigated. He reported, in the paper jointly with Escombe,[6] that the action of diastase from the aleurone caused a characteristic pitting of the starch granules, whereas the diastase from the scutellum, which, as Dr Briggs[2] has shown, is about 5% of the total α-amylase, did not; its action led to a smooth dissolution of the granule, similar to the changes in the transitory starch granules accumulated within the embryo in its preparation for germination. This suggests an unexplained major difference between the two α-amylases. When I reflected on this recently, I was vastly tempted to check it to see if it was correct and, if it were, then try to explain it. However, such a course of action would have put the presentation of this lecture in jeopardy, and, as Dr Johnson said to Boswell, 'When a man knows he is to be hanged in a fortnight it concentrates his mind wonderfully'. So I forswore the allurement of the microscope for the rigours of the desk, and I leave, perhaps to Dr Palmer, the pleasure of re-exploring this observation, electronmicroscopically.

Oddly enough, though Horace Brown was greatly interested in the metabolic changes undergone by starch, he was not really enamoured of starch *per se*. Indeed, he commented in his *Reminiscences*[4] on the 'disturbing influence which this puzzling colloid, starch, always exercises on those who are drawn into its viscid coils' and, after ascribing the birth of the 'starch problem' to Kirchof in 1814, he predicted that chemists would not be united upon it even in its bicentenary in 2014 A.D.—a prediction which bids fair to be fulfilled. It is pleasing to find some topic which completely baffled even Horace Brown.

In conclusion, let me generalize a little about Horace Brown's attitude to his scientific work. First and foremost he was essentially what we would now term an applied scientist, as indeed were many of the great figures of that time, including Louis Pasteur. He found his problems in the industrial processes with which he was familiar—and many such problems still remain to be solved. In pursuing the causes of things, and respecting only the authority of verifiable experimental facts, he wandered along pathways which led to discoveries in fields remote from his starting point—but he always returned to the challenge of the brewing materials and their processing. And Horace Brown was very much alive to the spin-off which came from technological research. As he said

'Brewing is an industry which is capable of yielding in kind a very high rate of interest on the scientific knowledge sunk in it'.

Might I suggest that this could prove an excellent theme for a future Horace Brown lecturer—*The Contribution of Brewing to Pure Science*. Or, perhaps, in these bureaucratic days, it might form a useful subject for a government enquiry. Brewing is so well accustomed to investigations—into beer composition, beer additives, beer prices, and brewery profits, that I think the industry would welcome a disinterested investigation of its contribution to fundamental scientific research. A fanciful thought, perhaps—but the result would be a very large volume indeed.

For myself, I do not really recognize any valid distinction between pure and applied scientists, but I would go along with Immanuel Kant where he says[14]

'To yield to every whim of curiosity, and to allow our passion for inquiry to be restrained by nothing but the limits of our ability, this shows an eagerness of mind not unbecoming to scholarship. But it is *wisdom* that has the merit of selecting, from among the innumerable problems which present themselves, those whose solution is important to mankind'.

Horace Brown had that wisdom.

References

1. Bishop, L. R., *Journal of the Institute of Brewing*, 1971, **77**, 12.
2. Briggs, D. E., In *Biosynthesis and its Control in Plants*. Ed. B. V. Milborrow. New York & London: Academic Press, 1973.
3. Brown, H. T., *Journal of the Chemical Society*, 1893, **60**, 604.
4. Brown, H. T., *Journal of the Institute of Brewing*, 1916, **22**, 267.
5. Brown, H. T. & Escombe, F., *Proceedings of the Royal Society*, 1898, **63**, 3.
6. Brown, H. T. & Escombe, F., *Philosophical Transactions of the Royal Society*, B, 1900, **193**, 223.
7. Brown, H. T. & Morris, G. H., *Journal of the Chemical Society*, 1890, **57**, 458.
8. Brown, H. T. & Morris, G. H., *Transactions of the Institute of Brewing*, 1893, **6**, 94.
9. Darwin, C. *The Power of Movement in Plants*. London, 1880.
10. Dunn, G. *Phytochemistry*, 1974, **13**, 1341.
11. Edelman, J. Shibko, S. J. & Keys, A. J., *Journal of Experimental Botany*, 1959, **10**, 178.
12. Haberlandt, G. *Berichte der Deutchen Botanischen Gesellschaft*, 1890, **8**, 40.
13. Horace (Quintus Horatius Flaccus, 65–8 B.C.) *Epistles*, I.i.14.
14. Kant, I., *Critique of Pure Reason, 1781*.
15. Kogl, F. A., Haagen-Smit, A. J. & Erxleben, H., *Zeitschrift fur Physiologische Chemie*, 1934, **228**, 113.
16. MacLeod, A. M. & Millar, A., *Journal of the Institute of Brewing*, 1962, **68**, 327.
17. MacLeod, A. M. & Palmer, G. H., *Journal of the Institute of Brewing*, 1966, **72**, 580.
18. Popper, K. *The Logic of Scientific Discovery*. London: Hutchison, 1959.
19. Portno, A. D., *Journal of the Institute of Brewing*, 1976, **82**, 6.
20. Radley, M., *Planta* (*Berlin*) 1969, **86**, 218.
21. Rainbow, C., *Journal of the Institute of Brewing*, 1977, **83**, 9.
22. Virgil (Publius Vergilius Maro, 70–19 B.C.) *Georgics*, **II**, 490.
23. Went, F. W., *Recueil des Traveaux Botaniques Neerlandais*, 1928, **25**, 1.