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Edwin Haslam
Excerpt
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Introduction

> Et voyez ici (dans le cas du vin ...) comme la science pure, dans ce qu'elle a de plus élevé, ne peut avancer d'un pas sans faire profiter tôt ou tard les applications de ses preceiux résultats.
>
> *Louis Pasteur, 1862*

> ... the future ... is to use the chemical information as the starting point for questions that lie in the realms of biology.
>
> *T. A. Geissman, 1965*

> According to Albert Einstein we live in an era of perfect methods and confused aims. For example in Organic Chemistry the known synthetic methods allow us to prepare an astronomical number of compounds; the gap between the **possible** and the **relevant** becomes larger every day.
>
> *V. Prelog, 1986*
> *18th Solvay Conference*

Liebig's *Handbuch der Organische Chemie* of 1843 listed around 2000 substances derived from natural sources. Amongst these were several phenolic compounds ranging from gallic acid, isolated towards the end of the eighteenth century by Scheele from gall nuts, to morphine obtained from the opium poppy by Séquin in 1804 and a year later by the apothecary's assistant Serturner. The *raison d'être* for the growth of organic chemistry as a scientific discipline can be traced to this point at the beginning of the nineteenth century and to the desire of chemists to comprehend more fully these 'children of nature'. For the century and more which followed the elucidation of the chemistry of natural products – their structure, chemical properties and ultimately their synthesis – were the dominant themes and goals of organic chemists. In this same period the number of known natural products, including phenolic compounds, increased exponentially. Today this 'stamp collecting' emphasis has, quite rightly, changed – a fact which might well be considered appropriate in view of the percipient observation of Prelog made just over a decade ago.

In the case of plant polyphenols (*syn.* vegetable tannins) the surge in knowledge of a fundamental chemical and biochemical nature which has taken place over the past 40 years has now paved the way, as predicted by Geissman, to an interest in questions which lie in the realms of biology (not least the questions of the purpose or function which these forms of metabolism serve in the organisms concerned). Equally it has given rise to a burgeoning concern with practical polyphenolic phenomena and an understanding of the importance of plant polyphenols in areas as diverse as agriculture, ecology and chemical defence in plants; foodstuffs, nutrition and beverages; fruit and floral pigmentation; natural glues, varnishes and exoskeletons; the efficacy of traditional and herbal medicines in the treatment of certain pathological conditions; traditional processes for the making of leather and the generation of important industrial chemicals. Whilst this change in emphasis might be considered timely it also perfectly illustrates the seminal observation of Pasteur that the proper understanding of complex applied scientific problems invariably follows periods when substantial advances in fundamentals have first been accomplished. Underlying a great number of the practical phenomena in which polyphenols are intimately involved is their distinctive ability to form intermolecular complexes with each other and with other molecules, large and small – the property of *molecular recognition*. In the case of polyphenols such non-covalent interactions are not only important in their own right but are often an indispensable prelude to further chemical reactions in which new chemical bonds are formed. The fundamental basis of molecular recognition is provided by the potential energy surface that represents the interaction of two or more molecules in a cluster as a function of their mutual separation and orientation. The number of independent variables upon which this intermolecular energy depends increases as the molecular size increases; for molecules the size of typical polyphenols in an aqueous medium the number of variables is astronomical and rationalisations are, necessarily, macroscopic and qualitative in their nature. Nevertheless understanding how this facet of the physical and chemical behaviour of polyphenols is achieved through their particular chemical structures has become a question of paramount importance in the understanding of the wider practical issues noted earlier and this particular question is given prominence in the ensuing discussion.

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'E.C.' – A personal memoir

'E.C.' on his Eightieth Birthday
(reproduced by kind permission of Jeffery Harborne).

E. C. Bate-Smith ('E.C.' as he was almost universally known) was born at the beginning of the century and died near its end in 1989. His academic training was as a physiologist but it is probably for the period when he became Director of the Low Temperature Research Laboratory (L.T.R.C) in Cambridge from 1947 to 1965 that his career will be best remembered. The immediate post-war period was an exciting time in the plant sciences and 'E.C.' became one of the great pioneers of comparative plant biochemistry. His studies of plant phenolics and his collaborations with Tony Swain were acknowledged world-wide and were seminal in the development of this area of science. One of the characteristics of the truly great scientist is his or her ability to judge when the time is ripe and propitious to press ahead in a particular field, and equally when it is certainly not provident to do so. The demonstration by Partridge in the L.T.R.C. of the potential of paper chromatography for the separation of sugars stimulated him to show very quickly that it was an equally beautiful method with which to explore the chemistry and biochemistry of plant phenolics. There was an explosion of activity in this field, none more so than his own, much of it carried out by himself, often in his spare time; his surveys of the distribution of the commoner phenolic constituents from over 1500 species of plants from nearly one-half of the known plant families, published in the Journal of the Linnaean Society (Botany) in 1962 and 1968, is a monument to that endeavour. It remains a work for all ages.

'E.C.' always preferred his contributions to be referred to as Phytochemistry and, with Tony Swain, he was able to demonstrate that the field of vegetable tannins was now, and for the first time, open to serious and detailed biochemical and plant physiological study. Over a period of some thirty years his researches gave rise to a constant flow of ideas and theories. These he illuminated with a clarity and wit and presented with a boyish enthusiasm which bordered upon a passion, unequalled amongst most professional scientists of my acquaintance. He possessed a huge intellectual curiosity, a need to know and understand; his objectives were encapsulated in a letter written as late as September 4th 1985:

> I myself started with the 'information' that tannins were waste products deposited in the wood of trees because the plants had nothing else they could do with them: a horrible thought! Convinced that they had a function I set out to try and find it by way of their systematic distribution linked to the idea of their astringency.

On a personal level the author owes 'E.C.' an everlasting debt for his unfailing kindness and thoughtfulness for he, amongst others, prompted me to participate in the investigation of the vegetable tannins in the 1950s, when it seemed a fathomless black hole, and moreover one which in its time had engulfed many eminent scientists and their endeavours. He had sensed that the time was now ripe to systematically examine these recalcitrant children of nature with the new armoury of methods which were becoming available. His shrewd comments and continual support were a constant theme to my own work and that of my many colleagues. It is exemplified by a correspondence which lasted some twenty five years until just before his death; a correspondence beginning 'Dear Haslam', it ended 'Dear Eddie'. He understood the need to constantly give encouragement to the young; how else might one judge the

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significance of one's own contributions? How could one's spirits fail to be lifted by the following letter (October 17th 1977) which commenced:

> By one of those coincidences I have long since ceased to regard as remarkable the last thing I wrote as I went up to bed last night was ...
> 'There is now ample evidence that evolutionary dispersal of woody dicots is accompanied by progressive loss of hydroxyl functions from proanthocyanidins and flavonols and by many other transformations of these regular constituents.'... And behold your letter was waiting for me in the lab this morning!

Or again (January 8th 1979):

> How immensely encouraging the chapter you've written for Eric Conn's book is! I miss having a kindred spirit to discuss my bits of research with and I often think how nice it would be to float ideas and pass on idle thoughts – some of which might even prove to be useful ones.

Thoughtful and frequently stimulating comments and criticisms flowed unceasingly from his pen and typewriter (now in the AFRC Institute in Babraham) well into his eighties. His continuing interest in Phytochemistry, Function and Taxonomy and his humorous asides upon life are illustrated by a brief selection of extracts from these letters.

Taxonomy, metabolism and function

> Your letter and enclosure have given me a good deal of pleasure during an otherwise dismal week.... Your paper on Secondary Metabolism fits in oddly with this question. In your first paragraph you ask the question 'Why?' You unwittingly answer it yourself in your postscript; you quote Hoppy as saying living matter becomes dead when the chemist touches it and Robert Robinson as saying, virtually, chemistry comes alive when it touches living matter. I knew both of these people, the former very well. ... I think the truth is simply that neither chemistry nor biology can answer the question 'Why'; both in their particular way can answer the question 'How'. Between one and the other there is a bridge to be crossed, a metaphysical one. I call it 'The bridge over the River Why'.
>
> But the two months have not been entirely wasted. I have written a long letter to Dahlgren (copy herewith) about his revised system, which I dont like so much as his original one. I regard the galloyl esters as the key components in the systematics of the dicots, and am relying on your work to bring some coherence to their distribution. By now you must have a good deal more than that belatedly published in Phytochemistry. I haven't written to you before because I wanted to get a reply from Dahlgren to my (Friendly!) criticism and I not only got this, but also a visit last week from Dahlgren himself. I hadn't previously met him, and we got on splendidly. I have also had a visit from Kubitzki, and have written to Thorne, who had sent me his latest phylogenetic tree, not yet published. It agrees more closely with the chemical data than his earlier one did.

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Summing all this up, I think your speculations about the biosynthesis of the gallo- and ellagitannins are well founded, especially the need to dispose of them by blocking hydroxyl groups or by complete elimination when they are not needed. This can happen at the fringe of evolving genera (e.g. Geranium) or when they are replaced by more efficient deterrents. This seems to be what happens with the iridoids… An interesting situation is when the galloyl esters occur with the deterrent which it is assumed to be an improvement upon them and may eventually replace them. This co-occurrence represents the kind of evidence which suggests that the iridoid-containing taxa have evolved from what were formerly galloyl ester-containing ancestors.

As I said I have been making good use of what otherwise would have been a tedious break by reading your paper and pondering about it. … The next question involves function. This is my own particular province and was, in fact, the starting point of my whole interest in phenolics. Their function as astringents has, I think never been questioned, and so far as I know no other function has yet to be assigned to them. 'Astringency', of course includes their inhibition of enzyme action and other consequences of their association with proteins which you point out. As 'broad spectrum' repellents one would not expect a high molar efficiency – such as one might get in an alkaloid with a specific predator target. Your evidence of the wide variation in structure and ability to associate has now to be seen in the context of systematic distribution. My reaction is to try and identify a limited number of **groups** of structures which as such can be treated as taxonomic characters, like, in fact you have done with the *Rubus* and *Quercus* groups, and see how they organise systematically.

It is the significance of the distribution of the different classes of tannins that is the ultimate reason for working on their chemistry. As I said there is immensely important taxonomic material emerging from your present work and your future intentions. Okuda's work too is important. I am glad to have the xerox copy of his paper which you sent and which I would otherwise not have known about. It's interesting to reflect that after the very first chemotaxonomic symposium in Paris in 1962 I suggested that as an exercise people interested in different chemical fields should combine forces and have a go at Casuarinaceae. The idea didn't catch on. My contribution would have been the tannins – but what a hope in those days! Later, perhaps Seikel and Hillis might have got the answers, with the advantage of access to the species where they are indigenous. Until casuarinin turns up again there wont be any clue as to the possible relationships of this family, which Melchior describes as 'Ein recht isolierten Formenkreis', which is why I wanted to have a go at it.

I have been reading Gottlieb's book which he kindly sent me, and he has a categorical philosophy of micromolecular evolution from the simplest to the more complex, mostly by oxidations and dehydrogenations. This may well be true of particular cases, but those I know anything about seem to proceed from more complicated to less complicated forms with evolutionary advance, e.g. the flavonoids, which lose B-ring hydroxyls as they become more advanced (but they do of course acquire 6 and 8-hydroxyls). So in the case of the iridoids I prefer Robert Hegnauer's view of the aucubin glycosides as occupying a central position (Naturwiss, 1971) evolutionary changes taking place both by oxidations and reductions. The truth is, as you point out, we don't know enough yet about the systematic distributions of most of these constituents, let alone their evolutionary history. . . . The condensed tannins are an interesting case, and probably a special one, because they were presumably inherited as such from the gymnosperms, complete with myricetin and prodelphinidin. Evolution in the angiosperms seems to have involved loss of these constituents concomitant with loss of arboreal habit or by replacement with hydrolysable tannins, or, as many think, with more thrifty repellents. . . . I haven't any reason to question the Schmidt and Mayer biosynthetic route to gallic and acid synthesis, but it had to begin somewhere and be confined to the dicots, and that is where I'm struggling to find the answer.

Tannins, proteins and haemanalysis

Your letter is an answer to a prayer! A thorough investigation of the 'galloyl esters' of fresh, green plant tissues is urgently called for, and I'm so glad you have been and are, doing this. I am not surprised you are finding none of the substances so laboriously studied by Schmidt and Mayer in commercial extracts. I have long been convinced that these are mostly artefacts of dead tissues.

My third point (and my last, so as not to make this letter too long), is to come back to using blood as the substrate for determination of astringency. To me its first advantage is the ease with which precipitation can be measured. This is equalled by its availability, if only a few measurements are to be made and people can bring themselves to pricking their finger occasionally. . . . It is a protein omnibus! I think it is necessary, when considering the tannins generally in a biological context, to have simple **agreed** methods of evaluating astringency.

Beautiful!
Your results have certainly given me something to think about. . . . What especially pleases me is that the RA of the procyanidins is much the same as I get by haemanalysis. . . . I'm sorry that you can't bring yourself to shed a little blood in a good cause. The method's so simple and so very quick – 10 minutes from door to door!

As regards your paper on the association of proteins with polyphenols, I have to confess that I find up-to-date chemistry very difficult to cope with. (The last chemistry lesson I had was in 1922!). However I got some help with the Scatchard diagrams from Denis Sharman, and I can find nothing in your paper with which, translated into my own arcane idiom, I couldn't, from my own experience, agree. In fact you are saying, in more precise and elegant fashion, what I have been thinking . . . and trying to say . . . about 'haemanalysis' for several years.

Plant phenolics and the Phytochemical Society of Europe

Very modestly he acknowledged his role in the 1950s in setting up the Plant Phenolics Group which was eventually to become the now thriving Phytochemical Society of Europe; few of its members may be aware of the immense debt which they owe, amongst others, to 'E.C.''s foresight, enthusiasm and intuition.

Reading the names and the Institutes represented I have a sensation of 'déjà vue'. It recalls as I mentioned in a letter to Ron a week ago, the beginnings of our work on tannins and the formation of the Plant Phenolics Group. This started with an Interlaboratory Symposium between Long Ashton and ourselves, the name being suggested by Wilson Baker. It developed several years later into the Phytochemical group and then into the Phytochemical Society. I wrote an article in 'Nature' entitled 'Plant Phenolics into Phytochemistry' of which I have no reprints and have lost the reference.

The group idea came from the Society of Chemical Industry which has a number of subject groups, . . .

Life

I am enclosing a reprint of the paper which has the distinction of being the first 'Review Article' in Phytochemistry! It is a concession by the editors, in consideration of my senility, and is a compromise with my first proposal to publish the 1964 memorandum entire.

Incidentally I'm learning a lot of chemistry on Sunday mornings listening to the Open University. I shall soon be able to understand some of the papers you send me! Even some of Gottlieb's too.

I'm very frustrated by my inability to get to the Botany School Library. Without a car life is very difficult and I can only ask for transport when the situation is urgent. Otherwise my life as a villager is very pleasant and also is in the height of fashion!

It seems to me the time has come to withdraw (not retire) from phytochemistry. The message came with the last number of the Bot.J.Linn.Soc. which had a paper on computerised systematics which was written in a language which might have been Ancient Egyptian or Martian so far as I was concerned. I shall be leaving the board of Phytochemistry, but still getting my copy of the journal. I shall hope to keep up a lively correspondence with a select company of interesting people.

And finally and very sadly shortly before his death:

I had a slight stroke about a fortnight ago. My writing's not very good but it's improving. As regards mental activity I'm not aware of any deficiencies, but that's not for me to judge. I enjoy the usual occupations. I'm led to expect improvement. Hope to hear from you.

In today's high-powered business-like and business-oriented laboratories, manned by the proverbial 'big battalions' of researchers we shall be fortunate if we see again such an individualist and pioneer as 'E.C.'. He was unfailingly courteous and gentlemanly, thoughtful and erudite. His work truly belongs to the ages.

The abiding recollection which I have of 'E.C.' is from one of the very first meetings of the Plant Phenolics Group. It was at Englefield Green in the spring of 1960. 'E.C.' came into the opening session as chairman clutching in his arms a freshly picked, gorgeous bunch of pink, white and red camellias. As he wisely remarked they were the focus of many of our scientific interests and it was therefore highly apposite that they should also give, throughout the duration of the meeting, such aesthetic pleasure as well.

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Polyphenols – structure and biosynthesis

1.1 Phenolic metabolism in plants

Studies of the chemical basis of flower colour variation comprised one of the first successful scientific excursions into phenolic metabolism in plants. Its success depended on the collaboration of geneticists, biochemists and chemists who established unequivocally that genetic variation in anthocyanin colour was mainly due to simple structural modifications (hydroxylation, methylation) of a basic pigment molecule – the anthocyanidin (Harborne, 1960; Scott-Moncrieff, 1981). It remained an isolated example for some time for although it was recognised that a very wide variety of phenolic compounds were to be found in plant tissues it was the advent of two techniques – chromatography and the use of radioisotopic labelling – which permitted wide-ranging and successful experimentation in this field. Thereafter interest in the biochemical and physiological aspects of phenolic metabolism literally mushroomed (scientific progress indeed hinges on the continuing discovery and extension of new laboratory techniques). Biosynthetic pathways to the principal classes of plant phenols were established and, more recently, enzymes which catalyse various steps in these pathways have been isolated and characterised. It seems likely that further progress in this field will follow the more widespread use of the techniques of molecular biology in this area of plant secondary metabolism.

The kind of substances that a plant contains depends on the kind of plant that it is.

Writing in 1964, E. C. Bate-Smith (a true pioneer in this field and a giant on whose shoulders we now stand) looked forward to a phytochemistry that increasingly recognised the biological relationships between the substances that plants elaborate. He continued: