The Brassins

A SERIES of papers[1-4] has described experiments purporting to show the existence of brassins from pollen of rape (*Brassica napus* Gaertn). It is claimed that they represent a novel group[1]; that they consist of five active components; that they have a fatty nature[1,2]; and that they have plant hormonal activity[1-4]. We consider that the evidence presented in the four papers is inadequate to support any of these conclusions.

Our major criticism is that all the results reported were obtained with a mixture. No attempt was made to correlate quantitative measurement of any physical property with the intensity of a biological response; consequently there is no reason to relate any physical determination with the active molecule(s). No two measurements need necessarily relate to the same component of the mixture. Even the simple and fundamental separation of the extract into ether-soluble acidic, basic and neutral compounds was not attempted.

To determine the constitution of the brassins a fraction was separated[1] from an ether extract of dry rape pollen by TLC of the crude extract on silica gel, developing with benzene–methanol–acetic acid (45 : 8 : 4); the active fraction (R_F 0.35 to 0.45) was then rechromatographed with *iso*propyl ether–acetic acid (19 : 1). The activity remained on the origin and the eluate was termed brassins.

The crude brassin fraction was subjected to NMR analysis in $CDCl_3$ solution[1]. No integration or multiplicity of observed resonances was given and yet from a few undefined signals in the δ 1.26 to 2.00 region, a "signal" at δ 3.8 ("CH_2 attached to oxygen"), and a "multiplicity character" at δ 5.3 ("olefinic protons") the authors decide that this crude fraction is fatty acid ester material and suggest a glyceride structure. This information is insufficient to warrant these structural conclusions. The active substance(s) may have been so dilute that it (they) escaped detection by NMR spectroscopy. In the NMR spectrum of tristearin[5] in $CDCl_3$ the glyceryl methylene and methine protons comprise an A_2B_2C system with resonances centred around δ 4.2 and δ 5.3 respectively. No saponification of these "fatty acid esters" was attempted to support the authors' structural hypotheses.

The brassin fraction was not tested in any of the familiar bioassays which are reasonably specific for auxins (oat coleoptile elongation), gibberellins (growth stimulation of dwarf maize or α-amylase synthesis in barley aleurone) and cytokinins (tobacco pith callus growth), therefore a contribution of these hormones to the activity of the brassin fraction has not been excluded.

Combined GLC-MS of the crude brassin fraction was carried out and "several components" were observed and some features of their mass spectra were recorded[1]. No attempt was made to correlate the biological activity with observed GLC peaks and no fractions were collected and tested. Apparently no derivatives were prepared (for example, by methylation or trimethylsilylation of acidic components) before GLC to render volatile any involatile and possibly active components and so several may have escaped detection.

'Sephadex LH-20' gel chromatography, monitored by ultraviolet absorption at 254 nm, gave five peaks, all containing active material; but the conclusion that five active substances were present is not justified. These five ultraviolet absorbing fractions could overlie a spread of one active material—it is necessary to demonstrate inactive areas between peaks of biological activity before the presence of more than one active component can be claimed; even then the possibility of an inhibitor would have to be excluded.

The brassins were originally extracted from rape pollen but no experiments have been carried out to determine their effect on the physiology of rape pollen, or even on a rape plant. Consequently there is no direct evidence for their having a hormonal role *in vivo*. The extract of rape pollen undoubtedly accelerates the growth of bean (*Phaseolus vulgaris*) test plants, but the activity may be mainly attributable to a gibberellin because the elongation of internodes is a characteristic gibberellin response. Gibberellin activity occurs in high concentrations in ripe anthers (4 μg GA_3 equivalents g^{-1} dry weight in *Vitis*)[6]. The dual cell division and cell elongation response of bean internodes to brassins was reported to be histologically different from that induced by GA_3, because GA_3 did not induce cell division[3]. GA_3, has, however, been reported to stimulate cell division and elongation in petioles of strawberry[7], stems of *Hyoscyamus*[8] and even of internodal cells of bean (*P. vulgaris*, c. var. Blue Lake)[9,10], so the claim of "a new kind of growth effect"[3] is unfounded. The greater responsiveness of small (slow-growing) bean plants to applications of brassins is similar to the more pronounced effects of gibberellins on dwarf plants[11]; furthermore, the presence of more open flowers on brassin-treated bean plants[4] than on controls is also a typical response to gibberellins (see illustration in refs. 12, 13 or 14). In the light of the foregoing comments, and those of Stowe and Dott[15], it is idle to criticize the postulated roles of the brassins, concepts such as "alpha hormone"[4] and the suggestion that " . . . brassins seem to function early in the development of the embryo, . . . "; suffice it to say that the only data reported from experiments which involved embryos were the weights of ripe bean seeds produced on plants treated with brassins. We consider that the claims advanced for brassins being a novel complex of fatty plant hormones are premature and unjustified on the basis of work published so far.

Note added in proof. Since this manuscript was completed Mandava and Mitchell[16] have published a further paper on the "brassins".

They conclude from NMR and mass spectroscopy data of derivatives that "brassins" are glucose esters of fatty acids; this conclusion is inconsistent with NMR data given and earlier NMR and UV data[1] cited as evidence for a glyceride structure. The authors have not correlated biological activity with any physical measurement, consequently the attribution of "brassin" activity to any specific substance is unjustified.

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1 Mitchell, J. W., Mandava, N., Worley, J. F., Plimmer, J. R., and Smith, M. V., *Nature*, **225**, 1065 (1970).
2 Mitchell, J. W., Mandava, N., Worley, J. F., and Drowne, M. E., *J. Agr. Food Chem.*, **19**, 391 (1971).
3 Worley, J. F., and Mitchell, J. W., *J. Amer. Soc. Hort. Sci.*, **96**, 270 (1971).
4 Mitchell, J. W., and Gregory, L. E., *Nature New Biology*, **239**, 253 (1972).
5 NMR Spectra Catalogue, **1**, spectrum 368 (Varian Associates, USA, 1962).
6 Coombe, B. G., *Plant Physiol.*, **35**, 241 (1960).
7 Guttridge, C. G., and Thompson, P. A., *Nature*, **183**, 197 (1959).
8 Sachs, R. M., Bretz, C. F., and Lang, A., *Amer. J. Bot.*, **46**, 376 (1959).
9 Feucht, J. R., and Watson, D. P., *Amer. J. Bot.*, **45**, 520 (1958).
10 Greulach, V. A., and Haesloop, J. G., *Amer. J. Bot.*, **45**, 566 (1958).
11 Phinney, B. O., *Proc. US Nat. Acad. Sci.*, **42**, 185 (1956).
12 Bukovac, M. J., and Wittwer, S. H., *Mich. Univ. Agr. Exp. Sta. Quart. Bull.*, **39**, 469 (1959).
13 Leopold, A. C., *Plant Growth and Development*, 118 (McGraw-Hill, New York, 1964).
14 Cleland, R. E., in *Physiology of Plant Growth and Development* (edit. by Wilkins, M. B.), 51 (McGraw-Hill, London, 1969).
15 Stowe, B. B., and Dott, M. A., *Plant Physiol.*, **48**, 559 (1971).
16 Mandava, N., and Mitchell, J. W., *Chem. Ind.*, 930 (1972).