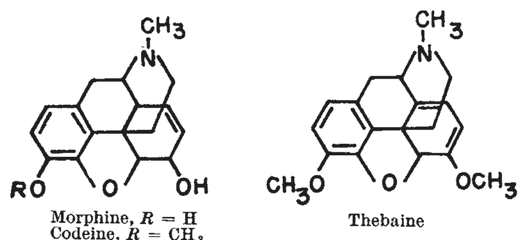

Morphine, $R = H$
Codeine, $R = CH_3$

Thebaine

in this case is the incorporation of total activity. Based on ring skeleton activities derived from the above-mentioned degradations, a 13 per cent incorporation was achieved from codeine to morphine in plant 2, while the incorporation from thebaine to morphine and codeine in plant 3 was 7·5 and 5·0 per cent, respectively. The values listed in Table 1 for the amount of alkaloid absorbed are based on loss of activity from the feeding solution. Experiments now under way show that a rapid adsorption or absorption of the alkaloids by the root hairs occurs, but that the further translocation is relatively slow. After the 42-hr. biosyntheses reported here, these root hairs had been removed and were not included in the total plant analysis. Therefore, the values for the percentage incorporation of total activity given above are undoubtedly minimal.

The proved conversion of thebaine to codeine and codeine to morphine clearly establishes O-demethylation as a metabolic pathway. This is in contrast to the commonly held concept[2] that the O-methylated alkaloids are metabolic end-products. Because of the relatively rapid synthesis and interconversion of alkaloids which has been found in this and the previous work[1], such demethylations could be a significant part of the total plant metabolism and perhaps provide one answer to the century-old problem of alkaloid function.

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[1] Rapoport, H., Stermitz, F. R., and Baker, D. R., *J. Amer. Chem. Soc.*, **82**, 2765 (1960).

[2] For example, Schröter, H.-B., "Encyclopædia of Plant Physiology", **8**, 844 (Springer, Berlin, 1958).

Phenolase and Pectic Enzyme Activity in the Chocolate Spot Disease of Beans

THE chocolate spot disease of beans (*Vicia faba* L.) caused by *Botrytis fabae* Sard. is so named because an early result of infection by the fungus is a small brown lesion on leaf or stem. In the course of studying the factors controlling the development of these lesions, it was found that the latent phenolase of bean leaves could be activated by certain pectic substances and by a soluble cellulose derivative in a way similar to that described by Kenten[1] for anionic wetting agents. The result of phenolase action, with the naturally occurring leaf phenols as substrate, is an intense brown coloration.

Kenten[1,2] showed that aqueous extracts of bean leaves had very little phenolase activity, measured by oxygen uptake or by coloured end-products, unless extracts were first exposed to acid or alkaline conditions, precipitated by ammonium sulphate, or incubated with certain anionic wetting agents. In the present experiments, mixtures of dialysed leaf extracts, citrate/phosphate buffer and activators were incubated for appropriate periods. They were then added to catechol or dihydroxyphenylalanine (DOPA) at *p*H 7·0. Phenolase activity was measured in an EEL colorimeter with green filter 623. Kenten's results were confirmed with sodium dioctyl sulphosuccinate as the activating agent; the optimum *p*H for activation was 5·5, and at this *p*H the buffer alone had little effect. Solutions of sodium polypectate, polygalacturonic acid and pectin had an optimum *p*H for activation of 4·5, and the buffer alone had a slight activating action at this *p*H. Monogalacturonic acid also activated the phenolase, but was less effective than the polymers; with carboxymethylcellulose, activation was most rapid between *p*H 4·0 and 4·5.

It is likely that soluble pectic substances are liberated from walls during the invasion of tissues by *Botrytis fabae* because the fungus readily produces polygalacturonase in culture. Enzymatic hydrolysis of sodium polypectate and pectin to short-chain polymers did not destroy the ability to activate the phenolase.

Filtrates from cultures of *B. fabae* on certain media containing pectic substances rapidly reduce the viscosity of pectic substances in solution. These filtrates also disintegrate bean stem tissue, but if leaf extracts are included in the original media the filtrates have little or no polygalacturonase activity, and do not disintegrate stem tissue. Other results suggested that the enzyme was inactivated by substances formed following the oxidation of phenols in the leaf extract, and direct evidence for this was obtained in the following experiment. Phenolase was activated by carboxymethylcellulose at *p*H 4·0 and allowed to oxidize DOPA at *p*H 7·0 for 6 hr.; red-brown products had now developed. Equal volumes of oxidized DOPA and *B. fabae* culture filtrate were mixed at *p*H 7·0. Viscosity-reducing activity changed little over 2 hr. in the control with DOPA, but in the presence of oxidized DOPA, although there was no immediate effect, activity was markedly reduced after 10 min., and, after 1 hr., polygalacturonase activity was very low.

From these and other experiments, it would appear that the formation of a chocolate spot results from an interaction of the polygalacturonase systems of the fungus and the phenolase systems of the host. Fungal polygalacturonase probably kills host cells rapidly[3] and liberates the phenolase system. It also degrades cell walls and so liberates pectic substances which have been shown to activate host phenolase between *p*H 4·0 and 5·0. This will be followed by the oxidation of host phenols and the formation of dark brown polymeric products, which now inactivate the polygalacturonase of the pathogen.

A hypothesis based on interactions of this sort could explain why chocolate spot lesions are generally limited in size and why the tissue of the lesion remains coherent for some time.

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