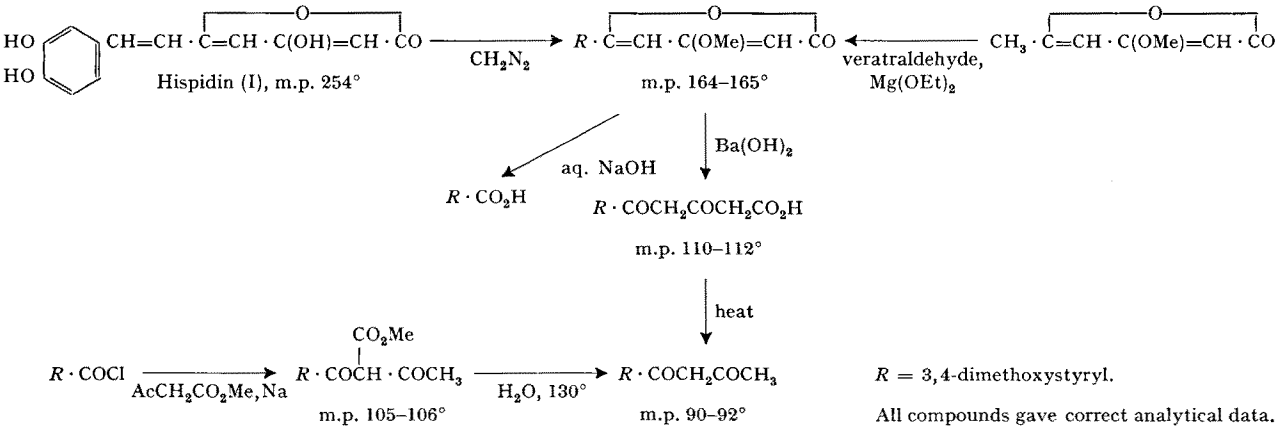


pigment from a fungus described as *Inonotus obliquus* (Pers.) ('chagi') can be broken down to coniferyl and sinapyl derivatives which it is thought derive originally from the lignin of the host tree[5]. We ourselves have studied fruit-body constituents of *Polyporus hispidus* (Bull.) Fr., a lignin-attacking white rot with annual bracket-shaped fruits, found in Britain almost exclusively upon ash trees but occurring less selectively elsewhere.

Alcohol-soluble pigments constitute up to 4% of the dry weight of immature *P. hispidus* fruits and comprise a mixture of methoxyl-free phenols with absorption maxima at 255 and 370 mμ; the principal constituent with this spectrum we have named hispidin, and the degradative and synthetic routes summarized here show it to be the enollactone of 3,4-dihydroxycinnamoylacetoacetic acid (I)[6]. As the fruits mature they become rather darker in colour but meanwhile the amount of alcohol-soluble pigment decreases considerably and hispidin and other monomeric phenols disappear altogether. The pigment becomes polymeric and firmly bound to the structural material and simultaneously the fruit-bodies become tougher and their fibrous 'woody' structure more pronounced. This polymerisation and cell-wall-binding of the phenols is effected *in situ* by oxidase enzymes; water extracts of the immature fruits (which darken rapidly when bruised) have very strong catechol oxidase activity and acting on hispidin or crude pigment extracts bring about rapid oxidative polymerisation *in vitro*. No great amount of quinonoid material is formed but the characteristic absorption spectrum of the monomeric pigments becomes broader and more diffuse.

The pigmented structural material of mature *P. hispidus* fruits can be described as lignin-like in that it is non-quinonoid and results from the oxidative polymerisation of substances such as hispidin based on a phenylpropane skeleton. From preliminary results this also seems to be true for various related species. The material differs from plant lignins in that the monomer phenols are not methylated and may contain other structural elements (e.g. the pyrone ring in hispidin). Whether the C_6C_3 group in hispidin is derived from breakdown products of the host lignin, or from monomeric phenols of the host (e.g. coniferin, aesculin, etc., in *Fraxinus*), or from *de novo* synthesis by the fungus, is as yet uncertain, but it may be significant that vegetative *P. hispidus* mycelium, which is non-pigmented on most culture media, contains hispidin etc. when grown on blocks of ashwood.

Zusammenfassung. In den Früchten des höheren Pilzes *Polyporus hispidus* bildet sich *in situ* ein ligninähnlicher Stoff durch Oxidation-Polymerisation von Phenolen des Phenylpropantyps, hauptsächlich Hispidin (I), dessen Struktur als Enollacton der 3,4-Dihydroxycinnamoylacetessigsäure erkannt wurde.

J. D. BU'LOCK and H. G. SMITH

Department of Chemistry, The University, Manchester (England), August 21, 1961.

[5] E. V. LOVIAGINA, A. N. SHEVRINA, and E. G. PLATONOVA, Biokhimiia *25*, 640 (1960).

[6] J. D. BU'LOCK, Folia Microbiologica *5*, 64 (1960). – J. D. BU'LOCK and H. G. SMITH, J. chem. Soc., in press.

Cotton Wilt and Calcium Accumulation

In earlier communications[1,2] we had discussed the derangement in metallic status (K, Ca, Mg, and Mn) in wilt-resistant (Co 2) and wilt-susceptible (K 2) cotton plants grown in soils inoculated with the wilt fungus *Fusarium vasinfectum*. Data on the percentage loss or gain of the elements were presented together with figures for the actual quanta of these in leaf samples from 18 day old healthy and infected plants. Further critical study of the calcium lines revealed that, in addition to the changes in calcium content in these plants as indicated by the spectra at 4226.7 Å (U_1-neutral atom), there was a more interesting difference in the amounts of ionized calcium, as was evident from the spectra at 3933.7 Å (V_1-ionized atom). The most important facts can be summarized as follows:

In the healthy state, the resistant plants had greater amounts of ionized calcium than the susceptible; following infection, there was little change in the resistant plants while in the susceptible ones the quantum increased, particularly in the infected but apparently healthy plants where very strong lines were noted (Figure). (The leaf analysis was carried out with a Medium Quartz Spectrograph, employing Lundegårdh's Spark-in-flame technique as detailed earlier[1].) As no comparable ionized calcium lines were present in the standard spectra, the actual quantities could not be calculated. Nevertheless, the

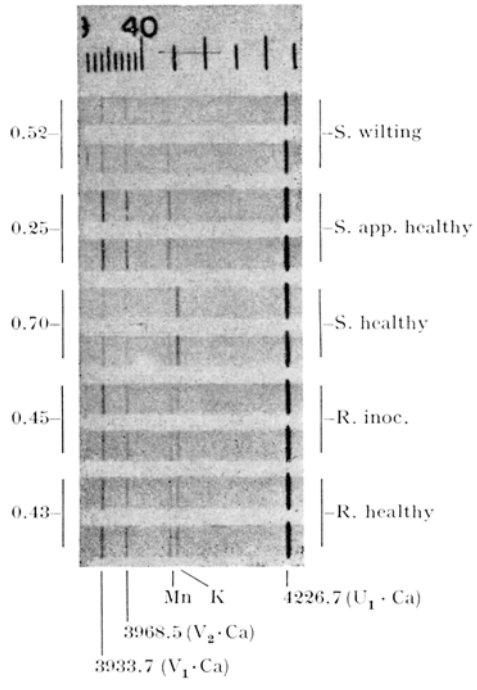
[1] T. S. SADASIVAN and R. KALYANASUNDARAM, Proc. Indian Acad. Sci. *43* B, 271 (1956).

[2] T. S. SADASIVAN and L. SARASWATHI-DEVI, Curr. Sci. *26*, 74 (1957).

transmission values (calculated from microphotometer readings and given on the left side of the spectra in the Figure) give us an idea of the relative amounts present, the two being inversely proportional to each other.

The insignificant change in ionized calcium in the resistant plants following infection, as against the marked one in infected susceptible plants, falls in line with the trend in general ionic pattern reported earlier[2]. It was noted that a considerable loss of potassium, and an increase in the amounts of other metals generally, brought about an ionic imbalance in the infected susceptible plants, whereas the ionic ratios in the resistant plants remained little affected. As was observed in the case of magnesium and manganese, the strongest lines for ionized calcium also were in the infected susceptible plants looking healthy (non-wilting). Although at present it is not possible categorically to state the reasons for these *in vivo* ionic changes, a few possibilities are suggested.

Two groups of substances implicated in many vascular wilts are extracellular toxins and enzymes produced by the pathogens concerned. In the *Fusarium* wilt of cotton, both the phytotoxin fusaric acid and pectic enzymes are considered to play a significant role[3].



Duplicate spectra of leaf ash from 18-day old cotton plants, resistant (R) and susceptible (S) to *Fusarium* wilt.

One of the possibilities suggested here is that the extra calcium may be transported along the conduction stream by fusaric acid as a complex to the leaves where it may dissociate releasing the metal, as proposed for iron in wilting tomato plants by GÄUMANN et al.[4]. The second is that the disintegration of the middle lamella by fungal pectic enzymes may release the metallic ions. It is well known that pectic materials in the middle lamella occur mostly as calcium and magnesium pectates. Studying the pectic enzyme contents in leaves of the same varieties of cotton plants, SUBRAMANIAN[5] found that the resistant plants normally contained more of these enzymes than the susceptible (note the stronger lines for ionized calcium in these), and that the former did not show much change on infection while the latter showed increased amounts of enzymes.

A point to be noted here is that it is the infected but non-wilting susceptible plants that reveal the highest amounts of ionized calcium (Figure). What stage these plants represent during this wilt pathogenesis remains to be ascertained. It is obvious from their ionic imbalance[2] that they are only apparently healthy (on the 18th day after inoculation) but not really so. It is likely that these have outlived their contemporary wilting plants only because of a slower progress of pathogenesis, and they offer interesting material for further investigations[6].

Zusammenfassung. Blattanalysen von 18 Tage alten, gegen den Welkepilz *Fusarium vasinfectum* widerstandsfähigen oder anfälligen Baumwollpflanzen zeigten einen erhöhten Gehalt an Calcium-Ionen in den infizierten, anfälligen Pflanzen; am stärksten war der Effekt in denjenigen anfälligen Pflanzen, die infiziert worden waren, aber keine Welkesymptome zeigten. Die Steigerung kommt möglicherweise durch den Zerfall von Toxin-Metall-Komplexen oder durch den enzymatischen Abbau von Pektinstoffen zustande.

L. SARASWATHI-DEVI and T. S. SADASIVAN

University Botany Laboratory, Madras (India), July 18, 1961.

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[6] One of us (L. S.) is thankful to the National Institute of Sciences of India for the award of an I.C.I. (India) Fellowship.

Effect of Metabolic Inhibitors on the Release of Histamine by Anaphylatoxin and by Antigen *in vitro*

Previous results obtained in this laboratory[1], demonstrated that mast cell alterations and histamine release caused by compound *48/80*[2] in rat tissue *in vitro*, are readily inhibited by agents (dinitrophenol, salicylate, thiopental, anoxia, cyanide, etc.), which interfere with oxidative phosphorylation or Krebs cycle oxidations. The action of these inhibitors was shown to be markedly reduced by the presence of glucose in the incubation medium. This indicated that this substrate was able to supply the metabolites needed by the histamine-releasing process, even under conditions in which aerobic energy metabolism is fully blocked. A similar effect of glucose has been described for the action of compound 48/80 on cat tissue kept under anoxia[3], as well as for the action of several naturally occurring histamine liberators acting on rat tissue[4,5]. The present report extends these observations to conditions of histamine release by endogenous

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