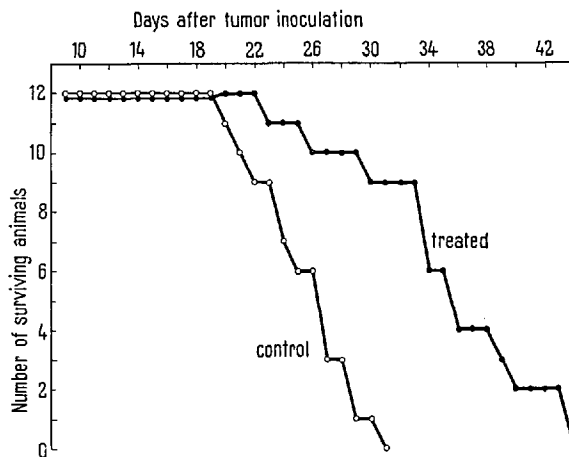Fig. 2

Fig. 3

dying later in each of the treated groups had recognizable ascites at autopsy. In the second group there were 6 indefinite 'survivors' which were observed for 2 months after the experiment was terminated.

Effect on transplantable epidermoid carcinoma DC 5. Twelve 6-months-old DC5 tumor bearing female MA/Sp mice were treated with 150 mg/kg purin-6-yltrimethyl-ammonium chloride daily intraperitoneally for one week. A fragment of DC5 carcinoma was implanted under sterile conditions with a trocar into the left axilla 10 days before treatment was started. Equal numbers of animals

Daily dose (mg/kg)	Survivors	Response – Test/Control (T/C)		
		Animal weight change (g)	Tumour weight (mg)	% (T/C)
165.00	06/10	01.7/04.4	10 /1478.0	
160.00	07/10	00.2/02.7	39.0/1716.0	02
80.00	08/10	−00.6/02.7	56.0/1716.0	03
40.00	10/10	00.3/02.7	67.0/1716.0	03
20.00	09/10	00.2/02.7	94.0/1716.0	05
20.00	04/10	−00.3/01.4	0.0/1499.0	
10.00	08/10	−00.4/01.4	22.0/1499.0	01
5.00	08/10	00.3/01.4	338.0/1499.0	22

served as controls. At the start of treatment tumors ranged from 2 to 5 mm in diameter. The growth of the tumor was progressive in all control animals during the duration of the experiment. In all treated animals the tumor decreased in size by the time treatment was stopped and was not palpable in two animals. However, 5 days later it became apparent that the tumor had resumed its growth in all animals. The survival of the treated and untreated animals is summarized in Figure 3.

To obtain further data, the compound was submitted to the Cancer Chemotherapy National Service Center for routine screening. The compound had no effect against transplantable sarcoma 180 or against leukemia 1210. However, it was effective against carcinoma 755. The dose response curve is summarized in the Table.

Zusammenfassung. Purin-6-yltrimethylammoniumchlorid wurde synthetisiert. Es erwies sich als ein hämopoetisch toxischer Antimetabolit, wirksam gegen Ehrlich Ascites-Tumor, DC5- und Ca 755-Mäusekrebs.

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Detroit Institute of Cancer Research and Henry Ford Hospital, Oncology Division, Detroit (Michigan), August 14, 1961.

A Fungus Pigment of Novel Type, and the Nature of Fungus 'Lignin'

The presence or absence of lignin in fungi has been disputed, disagreement arising partly over the constitution of certain fungal materials and partly over the definition of a lignin. It is well-known that the fruits of many higher fungi contain hydrolysis-resistant structural materials which are of aromatic nature and may be polymeric; a major difference between these and true plant lignins is the virtual absence of methoxy groups from the typical fungal samples[1]. In *Daldinia concentrica* and allied species of Ascomycetes the aromatic component of a hydrolysis-resistant structural substance in the fruits is formed by enzymic oxidation-polymerisation and is a partly quinonoid derivative of 1,8-dihydroxynaphthalene[2]. In other species similar 'melanizing' enzymes promote the deposition of structural materials in differentiated sexual structures, though the substrates have seldom been identified[3]. Materials of this kind resemble melanins rather than lignins.
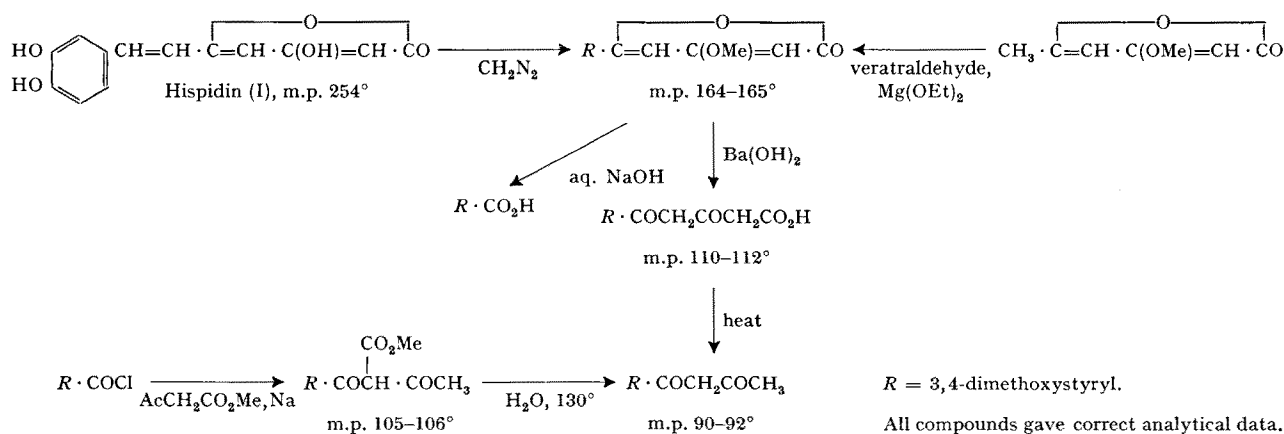
The case is different with many Basidiomycetes which form superficially 'woody' sporophores. We have found that several pigmented species of this kind produce, even in vegetative mycelia, substances with the absorption spectra of oxygenated cinnamoyl derivatives. A simple example of such C_6C_3 metabolites is the methyl *p*-methoxy-cinnamate of *Lentinus lepideus*[4]. The more complex

[1] J. W. FOSTER, *Chemical Activities of Fungi* (New York 1949), p. 99. – K. FREUDENBERG, in K. PAECH and M. V. TRACEY, *Moderne Methoden der Pflanzenanalyse* (Berlin 1955), vol. 3, p. 499.
[2] J. D. BU'LOCK and D. C. ALLPORT, J. chem. Soc. *1958*, 4090; *1960*, 654.
[3] P. E. BARBESGAARD and S. WAGNER, Hereditas *45*, 564 (1959).
[4] G. EBERHARDT, J. Amer. chem. Soc. *78*, 2832 (1956).

$$HO\text{-}C_6H_3(HO)\text{-}CH=CH\cdot C=CH\cdot C(OH)=CH\cdot CO \xrightarrow{CH_2N_2} R\cdot C=CH\cdot C(OMe)=CH\cdot CO \xleftarrow[Mg(OEt)_2]{veratraldehyde,} CH_3\cdot C=CH\cdot C(OMe)=CH\cdot CO$$

Hispidin (I), m.p. 254°   m.p. 164–165°

aq. NaOH $R\cdot CO_2H$

$\Big\downarrow Ba(OH)_2$

$R\cdot COCH_2COCH_2CO_2H$

m.p. 110–112°

$\Big\downarrow$ heat

$$R\cdot COCl \xrightarrow[AcCH_2CO_2Me,Na]{CO_2Me} R\cdot COCH\cdot COCH_3 \xrightarrow{H_2O,\ 130°} R\cdot COCH_2COCH_3$$

m.p. 105–106°   m.p. 90–92°

$R = 3,4$-dimethoxystyryl.

All compounds gave correct analytical data.

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.

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[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

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[2] T. S. Sadasivan and L. Saraswathi-Devi, Curr. Sci. 26, 74 (1957).