400. *Pyrones. Part II.* Hispidin, a New Pigment and Precursor of a Fungus "Lignin."*

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The pigments of immature *Polyporus hispidus* fruits have as main component the styrylpyrone derivative (IV) (hispidin), which during ripening is oxidised enzymically to lignin-like material. The structure-determination and synthesis of trimethylhispidin are described, with syntheses of some related compounds.

In Britain, *Polyporus hispidus* (Bull.) Fr. is a common cause of white rot in hedgerow ash-trees; its conspicuous bracket-shaped fruits appear annually and when immature the red-brown flesh affords much ethanol-soluble pigment. The main constituent of this pigment we have named hispidin, and its structure was announced, without details, in 1959.[1] Edwards *et al.* recently described independent work on the same compound;[2] here we present details of our own work, leading to similar conclusions, with an assessment of the function of pigments of this type.

After somewhat tedious purification, hispidin was crystallised as the yellow hydrate, $C_{13}H_{10}O_5,H_2O$, which with diazomethane or dimethyl sulphate gives a high yield of tri-*O*-methylhispidin, $C_{13}H_7O_2(OMe)_3$. Since this derivative is conveniently obtained directly from the crude (methoxyl-free) pigment it formed the basis of our structural study.

Tri-*O*-methylhispidin is neutral, shows no quinonoid or ketone properties, and gives none of the colour reactions for the main types of natural pigment. The infrared spectrum suggested the presence of an ester-carbonyl group (probably in an enol-lactone) and a 1,2,4-substituted benzene ring, but no hydroxyl group; as the parent phenol gives a strong green ferric chloride reaction a 4-substituted catechol nucleus seemed probable. The ultraviolet absorption ($\lambda_{max.}$ at 250 and 367 mμ with only weak intervening absorption) resembles that of hispidin itself but differs markedly from those of any common natural pigments. Mild hydrogenation gives a colourless dihydro-derivative ($\lambda_{max.}$ 223 and 280 mμ) but further hydrogenation occurs easily. Oxidation with neutral permanganate gives veratric acid, and treatment with hot aqueous alkali gives 3,4-dimethoxycinnamic acid.

Consideration of biogenetically plausible structures for a C_{13} enol-lactone which would give a cinnamic acid hydrolytically led us to compare tri-*O*-methylhispidin with yangonin, a yellow styrylpyrone isolated by Borsche and Gerhardt[3] from "kawa," *Piper methysticum*. Dr. B. K. Blount very kindly supplied us with yangonin and we found the ultraviolet and infrared spectra strikingly similar to those of tri-*O*-methylhispidin. Having first shown[4] that yangonin is unambiguously 4-methoxy-6-4'-methoxystyryl-2-pyrone, we tested the analogous formulation of tri-*O*-methylhispidin, 4-methoxy-6-(3,4-dimethoxystyryl)-2-pyrone (I) by applying the degradation sequence used by Borsche for yangonin,[3] *viz.*, hydrolysis to the diketo-acid (II) and decarboxylation to the diketone (III), which we found identical with synthetic material. The formula (I) was then confirmed by synthesis from the α-pyrone isomer of "triacetic acid lactone methyl ether" (cf. ref. 4) and veratraldehyde.

The structure of the parent phenol, hispidin, is somewhat less certain, since for the "hydrate" open-chain diketo-acid structures analogous to (II; and tautomers) must be considered as well as the pyrone structure (IV). Potentiometric titration of hispidin

* Part I, Bu'Lock and Smith, *J.*, 1960, 502.

[1] Bu'Lock, Prague Symposium on Antibiotics, May, 1959; cf. *Folia Microbiologica*, 1960, **5**, 62; Bu'Lock and Smith, *Experientia*, 1961, **17**, 553.

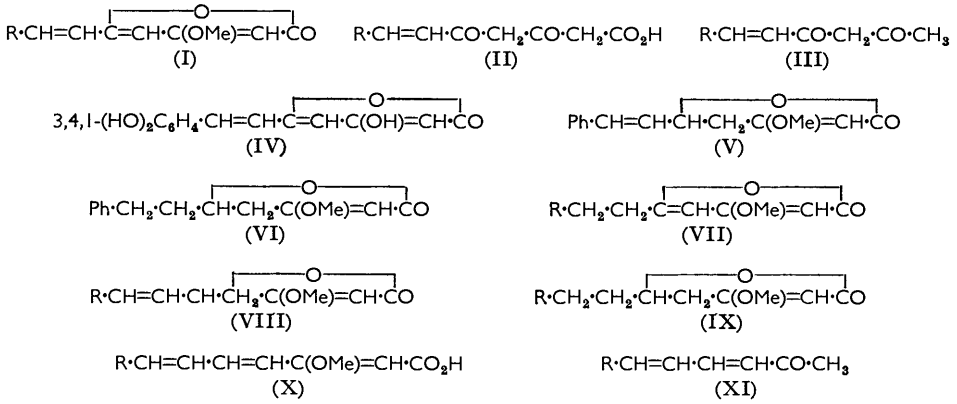
[2] Edwards, Lewis, and Wilson, *J.*, 1961, 4995; Edwards and Wilson, *J.*, 1961, 5003.

[3] Borsche and Gerhardt, *Ber.*, 1914, **47**, 2902.

[4] Bu'Lock and Smith, *J.*, 1960, 502.

does not resolve the problem, model compounds show that ultraviolet spectra will not distinguish reliably between structure (IV) and enolised forms of compounds such as (II), and decisive features of the infrared spectrum of the hydrate are obscured by hydrogen-bonding effects. Chemical evidence supports the cyclic structure (IV), in particular the smooth formation of the trimethyl derivative (I) with diazomethane in ether and the observation that carbon dioxide is not evolved under conditions in which the open-chain acid (II) is completely decarboxylated.

Because of reported pharmacological activities of yangonin and its congeners kawain (V) and marindinin (VI), some analogous derivatives of tri-*O*-methylhispidin were prepared. The dihydro-derivative already mentioned is formulated as (VII) by comparison with a synthetic isomer (VIII); the latter (an analogue of kawain) was obtained by Reformatsky reaction of 3,4-dimethoxycinnamaldehyde and methyl γ-bromo-α-methoxycrotonate, and shows ultraviolet absorption due mainly to the dimethoxystyryl group. Further hydrogenation of compound (VIII) gave the marindinin analogue (IX). An



R = 3,4-dimethoxyphenyl.

alternative product in the synthesis of compound (VIII) was the open-chain acid (X), which affords the arylhexadienone (XI) on hydrolysis and decarboxylation. The acid (IX) and its methyl ester have an open-chain form of the hispidin chromophore and show very similar absorption spectra.

When the crude ethanol-soluble pigment was treated with diazomethane or dimethyl sulphate and worked up to give tri-*O*-methylhispidin (I), very small amounts of a second, colourless, product (m. p. 198°) were consistently obtained, though they were separated from the trimethylhispidin only with some difficulty. Dr. Klyne informs us that this material, " substance A," shows some optical activity in the ultraviolet region and so is unlikely to have been formed from hispidin. What may be the same substance was encountered by Edwards *et al.* (m. p. 197°).[2] Analyses suggest that " substance A " is an isomer or oligomer of tri-*O*-methylhispidin (I), and the infrared spectra are very similar, but the ultraviolet spectrum of substance A (max. at 265 mμ, inflexion at 287 mμ) is that of the unextended styrene chromophore as in (VIII). Substance A forms a dihydro-derivative, m. p. 237—239°, but unlike tri-*O*-methylhispidin it is fairly stable to hot neutral permanganate and gives a deep violet solution in concentrated sulphuric acid. When a little alkali is added to substance A in ethanol at room temperature, a yellow solution (maxima at 235, 290, and 395 mμ, not shifted on acidification) is formed very rapidly; more slowly maxima at 255 and 345 mμ develop (shifted to 265 and 363 mμ on acidification), and ultimately veratraldehyde is produced. For comparison, the dihydropyrone (VIII) reacts with dilute alkali at 60° giving, apparently directly, the anion of acid (X), with maxima at 255 and 342 mμ shifting to 263 and 362 mμ on acidification. We conclude that substance A contains the 3,4-dimethoxystyryl group and

may give the acid (X) or a closely related substance with alkali, by way of a more highly conjugated intermediate; more precise formulation must await further material.

Whilst hispidin is the main component of the ethanol extracts from immature fruit-bodies, paper chromatography of these extracts and of acetone extracts from the residues reveals the presence of other phenolic pigments, as yet uncharacterised, some having similar spectra. The solubility and chromatographic behaviour show that some of the pigment mixture is polymeric. From more mature fruit-bodies, collected at successively later dates, which are increasingly tough and fibrous and show increasingly darker pigmentation, ethanol removes smaller yields of crude pigment, containing progressively less hispidin and more polymer. Acetone-soluble pigments show a similar decrease. We have observed that the immature fruits darken very rapidly on bruised or cut surfaces and yield an aqueous extract with a powerful oxidase action on catechol–glycine mixtures. This oxidase also reacts aerobically with crude pigment extracts and with purified hispidin, the ultimate products being yellow-brown precipitates; seen spectroscopically the oxidation is accompanied by the general broadening of absorption bands indicative of oxidation-polymerisation.[5] A similar, but controlled, process apparently takes place *in situ* during the ripening of the fruit-bodies and has the functional effect of toughening the cell-assembly. Since hispidin is biogenetically a clear example of a " C_6C_3 compound " (dihydroxycinnamoylacetoacetic acid), the toughening of the fruit-bodies in *P. hispidus* can fairly be regarded as a kind of lignification, unlike analogous processes in some other fungi effected by quinone-tanning reactions.[6]

In preliminary surveys we have found that the rather unusual absorption spectrum of the *P. hispidus* pigments can also be seen in crude extracts from other Polyporaceae, especially some with pigmented " woody " fruits, and therefore this type of phenolic pigment (with an extended cinnamoyl chromophore) is probably of wider distribution. This would accord with the general observation that so-called " fungus lignin " is often pigmented and frequently devoid of methoxyl groups.[7] Compounds based on the C_6C_3 unit (other than amino-acids) are not well-known as fungal products, save for the 4-methoxycinnamate derivatives formed by *Lentinus lepideus*.[8] However, from a fungus *Inonotus obliquus* (Pers.) growing on birch, Shevrina and her colleagues have extracted an orange-yellow phenolic mixture (" chagi ") which seems rather similar to crude *P. hispidus* extracts and contains both coniferyl and syringyl groups.[9]

Experimental

General.—Ultraviolet spectra are summarised in the Table; m. p.s are not corrected.

Extraction.—Immature *P. hispidus* fruits (gathered in late July to early August) were cut in pieces and steeped for 24 hr. at a time in successive lots of 95% ethanol. The combined extracts were filtered and evaporated under reduced pressure until the pigment was precipitated from the largely aqueous residues; the precipitate was defatted by extraction (Soxhlet) with light petroleum and benzene, to give the " crude pigment " as an orange-brown powder.

Hispidin (IV).—The crude pigment was extracted with ether (Soxhlet), and the soluble fraction purified by counter-current distribution in ether–acetone–water and recrystallisation from aqueous acetone, to give *hispidin hydrate*, yellow needles, m. p. 256—258° (Found: C, 58·9; H, 4·6. $C_{13}H_{10}O_5,H_2O$ requires C, 59·1; H, 4·6%), giving a green colour with ethanolic ferric chloride. On paper chromatography in butan-1-ol–acetic acid–water (4 : 1 : 5) it gave a yellow spot (R_F 0·80), reducing ammoniacal silver nitrate and becoming red-brown with diazotised sulphanilic acid; in the same solvent system the crude pigment gave an additional major spot (R_F 0·89) giving a lilac colour with the latter reagent.

Tri-O-methylhispidin (I).—(*a*) *From crude pigment.* The crude pigment (2·0 g.) in methanol

[5] Cf. Bu'Lock, *J.*, 1961, 52.
[6] Allport and Bu'Lock, *J.*, 1958, 4090.
[7] Freudenberg, in Paech and Tracey's " Moderne Methoden der Pflanzenanalyse," Springer, Berlin, 1955, Vol. III, p. 499.
[8] Birkinshaw and Findlay, *Biochem. J.*, 1940, **34**, 82.
[9] Lovyagina, Shevrina, and Platonova, *Biokhimiya*, 1960, **25**, 640.

Ultraviolet absorption spectra.

(In ethanol; $\lambda_{max.}$ in mμ, log ε in parentheses; —, data not recorded; * inflexion.)

Hispidin (hydrate) (IV)	223 (4·43)	248 (4·18)		367 (4·38)
Tri-*O*-methylhispidin (I)	221 (4·38)	250 (4·19)		367 (4·50)
Diketo-acid (II)	—	254 (4·1)		370 (4·42)
Diketone (III)	—	247 (4·2)		365 (4·53)
Triene acid (X)	—	263 (4·07)		355 (4·62)
Triene acid (X) (+ NaOH)	—	252 (4·38)		340 (4·69)
Triene acid (X) (Me ester)	—	264 (4·06)		359 (4·63)
Dienone (XI)	228 * (4·19)	250 (4·07)		357 (4·42)
Compound A	—	265 (4·2)	285 * (4·0)	
Styryldihydropyrone	223 (4·47)	265 (4·26)	292 (3·95)	
Pyrone (VII)	223 (4·09)		280 (3·99)	
Dihydro-compound A	228 (4·2)		281 (3·9)	
Dihydropyrone (IX)	230 (4·27)		279 (3·45)	

(200 ml.) was treated with an excess of diazomethane in ether; after 1 hr. the solution was evaporated and the residue extracted with hot benzene (leaving *ca.* 0·6 g. of polymeric material); the soluble fraction was purified by chromatography on neutral alumina and recrystallisation from benzene and ether, giving *tri*-O-*methylhispidin* (0·8 g.), m. p. 166—167° [Found: C, 66·6; H, 6·1; OMe, 32·0%; *M* (isothermal microdistillation in acetone), 286. $C_{16}H_{16}O_5$ requires C, 66·7; H, 5·6; 3OMe, 32·2%; *M*, 288]. From the mother-liquors, repeated recrystallisation from methanol afforded *Substance A* (*ca.* 50 mg.), m. p. 197—198° (Found: C, 66·5; H, 5·8; OMe, 31·2. $C_{16}H_{16}O_5$ requires C, 66·7; H, 5·6; 3OMe, 32·2%).

(*b*) *From hispidin hydrate.* Similar methylation of crystalline hispidin hydrate (0·5 g.) afforded the identical product (0·35 g.), m. p. 165—167°.

Over 2% palladium–calcium carbonate, previously saturated with hydrogen, the ether (I) (0·1 g.) took up 1·2 mol. of hydrogen in 3 hr., giving a colourless solution from which, after chromatography on magnesium silicate and recrystallisation from benzene, was obtained 4-*methoxy*-6-(3,4-*dimethoxyphenethyl*)-2-*pyrone* (VII), m. p. 74—75° (Found: C, 66·4; H, 6·4. $C_{16}H_{18}O_5$ requires C, 66·2; H, 6·3%).

When the ether (I) (16 mg.) was heated under reflux for 2 hr. with 10% aqueous sodium hydroxide (4 ml.), cooled, and filtered, the filtrate afforded 3,4-dimethoxycinnamic acid, m. p. and mixed m. p. 181—183° (from benzene).

Degradation of the Ether (I).—The experimental methods differ from those of Borsche *et al.*[3] The ether (0·1 g.) in ethanol (15 ml.) was heated under reflux with saturated aqueous barium hydroxide (5 ml.) and water (10 ml.) for 30 min.; on cooling, the precipitated salt was suspended in water (20 ml.) and carefully acidified with dilute hydrochloric acid. Extraction with ethyl acetate then afforded 7-(3,4-*dimethoxyphenyl*)-3,5-*dioxohept*-6-*enoic acid* (II), m. p. 110—112° (decomp.) (Found: C, 61·6; H, 5·5. $C_{15}H_{16}O_6$ requires C, 61·6; H, 5·5%). The crude acid (0·5 g.) was boiled in ethanol (50 ml.) in a stream of nitrogen; in 30 min. 1 mol. of carbon dioxide (collected as barium carbonate) was evolved, and removal of solvent and recrystallisation from benzene gave 6-(3,4-dimethoxyphenyl)hex-5-ene-2,4-dione (III), m. p. 90—92°.

Synthesis of the Diketone (III).—A solution of 3,4-dimethoxycinnamoyl chloride (obtained from the acid and thionyl chloride) (10 g.) in ether (200 ml.) and benzene (50 ml.) was stirred slowly into a suspension of methyl sodioacetoacetate (from 5·1 g. of ester) in ether (200 ml.), and the mixture stirred overnight. An excess of dilute sulphuric acid was added, and the ether layer was washed with water and shaken with saturated aqueous copper acetate (600 ml.). The precipitated copper complex was filtered off (m. p. after recrystallisation from ethyl acetate, 218—220°) and shaken with dilute sulphuric acid; extraction with ethyl acetate afforded methyl 2-(3,4-dimethoxycinnamoyl)acetoacetate (5·5 g.) (m. p. after recrystallisation from methanol, 105—106°). The crude product (2 g.) was heated for 6 hr. at 130° with water (10 ml.) in a sealed tube, and the product recrystallised from methanol, giving the diketone (III), m. p. and mixed m. p. 89—91° (Found: C, 67·7; H, 6·5. $C_{14}H_{16}O_4$ requires C, 67·7; H, 6·5%).

Synthesis of Tri-O-*methylhispidin* (I).—Authentic 4-methoxy-6-methyl-2-pyrone[4] (5 g.), m. p. 89°, was added to magnesium methoxide (from 2 g. of magnesium) in methanol (40 ml.), followed by veratraldehyde (6 g.) in methanol (30 ml.); after 4 hr. solvent was removed, dilute hydrochloric acid added, and the mixture extracted with ethyl acetate. Evaporation of the

extracts, trituration with warm benzene, and recrystallisation from methanol afforded tri-*O*-methylhispidin (I) (2·4 g.), m. p. 164—165°, identical in mixed m. p. and spectroscopically with naturally derived material.

Reformatsky Reaction.—A solution of methyl γ-bromo-α-methoxycrotonate,[10] b. p. 124—129°/23 mm., n_D^{20} 1·5062—1·5102 (2·1 g.), and 3,4-dimethoxycinnamaldehyde (1·9 g.), m. p. 84—85°, in tetrahydrofuran (25 ml.) was added to clean zinc (0·7 g.) with a trace of iodine, and the whole was stirred under reflux in nitrogen for 5 hr.; the mixture was cooled and poured into saturated ammonium chloride solution (200 ml.), and the product extracted with chloroform. Purification by chromatography on magnesium silicate and recrystallisation from benzene afforded 6-(3,4-*dimethoxystyryl*)-5,6-*dihydro*-4-*methoxy*-2-*pyrone* (VIII) (0·7 g.) (Found: C, 66·1; H, 6·3; OMe, 31·8. $C_{16}H_{18}O_5$ requires C, 66·2; H, 6·2; 3OMe, 32·0%). Hydrolysis as in the preparation of kawaic acid [11] gave 7-(3,4-*dimethoxyphenyl*)-3-*methoxyhepta*-2,4,6-*trienoic acid* (X), m. p. 166—169° [also obtained in earlier runs of the preparation of (VIII)] (Found: C, 62·3; H, 6·4; OMe, 30·0. $C_{16}H_{18}O_5,H_2O$ requires C, 62·3; H, 6·5; OMe, 30·1%); the *methyl ester* (prepared by use of diazomethane) had m. p. 98—100° (Found: C, 67·2; H, 6·6; OMe, 39·6. $C_{17}H_{20}O_5$ requires C, 67·1; H, 6·6; OMe, 40·1%), and decarboxylation of the acid (X) [as described for (II)] afforded 6-(3,4-*dimethoxyphenyl*)*hexa*-3,5-*dien*-2-*one* (XI), m. p. 102—105° (Found: C, 71·9; H, 7·0. $C_{14}H_{16}O_3$ requires C, 72·4; H, 6·9%) [2,4-*dinitrophenylhydrazone*, obtained directly from the acid (X), m. p. 228—229° (Found: C, 57·9; H, 4·9; N, 13·4. $C_{20}H_{20}N_4O_6$ requires C, 58·2; H, 4·9; N, 13·5%)]. Hydrogenation of the dihydropyrone (VIII) (100 mg.) over palladium–charcoal in tetrahydrofuran (uptake 1·1 mol.) afforded, after recrystallisation from benzene–light petroleum, 6-(3,4-*dimethoxyphenethyl*)-5,6-*dihydro*-4-*methoxy*-2-*pyrone* (IX), m. p. 107—109° (Found: C, 66·1; H, 7·0. $C_{16}H_{20}O_5$ requires C, 65·7; H, 6·9%).

Ultraviolet Absorptions.—Data for this series are tabulated.

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[10] Arndt, Loewe, Severge, and Türegün, *Ber.*, 1938, **71**, 1640; Kogl and de Bruin, *Rec. Trav. chim.*, 1950, **69**, 729.
[11] Kostermans, *Rec. Trav. chim.*, 1951, **70**, 79.