983. *Constituents of the Higher Fungi. Part I. Hispidin, A New 4-Hydroxy-6-styryl-2-pyrone from* Polyporus hispidus *(Bull.) Fr.*

By R. L. Edwards, D. G. Lewis, and D. V. Wilson.

The isolation of a new 6-(3,4-dihydroxystyryl)-4-hydroxypyrone from *Polyporus hispidus* is described. Properties and reactions of this compound leading to assignment of its structure (I; R = OH) are described. The structure has been verified by synthesis of the derived trimethyl ether.

The basidiomycete *Polyporus hispidus* (Bull.) Fr., parasitic chiefly on ash (*Fraxinus excelsior*), produces considerable quantities of yellow-brown colouring matters in its bracket-shaped sporophore. Zopf [1] isolated several yellow amorphous materials from the fungus but only described colour reactions. Zellner [2] reported similar amorphous materials. Robertson and his co-workers [3] obtained eburicoic acid from the mycelium of the fungus grown in culture, but did not investigate any pigments.

In this laboratory *Polyporus hispidus* collected from ash has yielded a yellow crystalline, optically inactive metabolite, which we name hispidin. The yield varied from different batches of fungi; actively growing, small fruiting bodies provide a cleaner product in greater quantity than do the dark mature specimens.

The pigment, when purified by crystallisation from ethanol or through one of the acetates, has the formula $C_{13}H_{12}O_6$, contains no methoxyl or *C*-Me group, is not reduced by sodium dithionite or sulphur dioxide, and does not give Craven's reaction [4] or colour tests with indole or ethylenediamine (for benzoquinones and naphthaquinones [5]). It yields an olive-green colour with ferric chloride, and reduces silver nitrate and Fehling's solution, but gives no precipitate with Brady's reagent. It yields no colour with magnesium and hydrochloric acid (flavones and flavanols), gives a negative Gibbs test and is insoluble in dilute acids, but dissolves in concentrated sulphuric acid to a yellow and in 2N-sodium hydroxide to an orange-yellow solution.

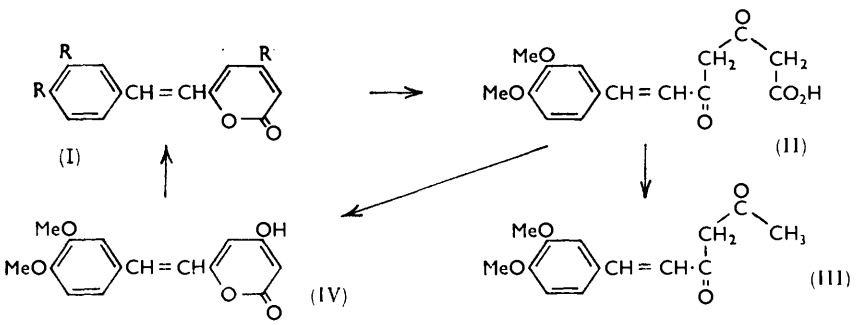
With acetic anhydride hispidin yields (*a*) di-*O*-acetylhispidin, $C_{17}H_{14}O_7$, slowly dissolving in cold sodium hydrogen carbonate to a yellow solution, (*b*) tri-*O*-acetylhispidin, $C_{19}H_{16}O_8$, insoluble in sodium bicarbonate solution, readily formed from the diacetate, and hydrolysed

[1] Zopf, *Bot. Z.*, 1889, **47**, 53.
[2] Zellner, *Monatsh.*, 1920, **41**, 443.
[3] Cort, Gascoigne, Holker, Ralph, Robertson, and Simes, *J.*, 1954, 3721.
[4] Craven, *J.*, 1931, 1605.
[5] Karius and Mapstone, *Chem. and Ind.*, 1956, 266; Bu'Lock and Harley-Mason, *J.*, 1951, 703.

to the diacetate by boiling alcohol, and (*c*) a pale yellow compound $C_{21}H_{18}O_9$ containing one *C*- and three *O*-acetyl groups insoluble in sodium hydrogen carbonate solution but dissolving slowly in 2N-sodium hydroxide to an orange solution, and giving a precipitate with Brady's reagent. With dimethyl sulphate, hispidin yields a yellow and a colourless trimethyl ether, both $C_{16}H_{16}O_5$; diazomethane gives only the yellow ether (the relationship between these two ethers has not been established; they give similar infrared spectra). Di-*O*-acetylhispidin reacts readily with diazomethane, to yield a compound, $C_{18}H_{16}O_7$, which is readily hydrolysed to *O*-methylhispidin which with diazomethane yields the yellow tri-*O*-methylhispidin. The formulæ of these derivatives suggest that the pigment is hydrated.

Chromic acid oxidises di-*O*-acetylhispidin to protocatechuic acid diacetate; protocatechualdehyde diacetate is produced by ozonolysis of tri-*O*-acetylhispidin, indicating that a catechol nucleus is present but not included in a fused ring system.

The ultraviolet spectrum of hispidin is similar to the spectra of certain unsaturated ketones.[6] The high-intensity band at 3730 Å suggests the presence of a carbonyl group conjugated *via* several double bonds to a benzene ring, and since hispidin does not show ketonic properties the carbonyl group is presumably present in a cyclic structure (*e.g.*, a lactone or a pyrone). Hispidin has a strong infrared band at 3392 cm.$^{-1}$, with broad but less intense absorption at 2564 cm.$^{-1}$ characteristic of strongly chelated phenols.[7] Intense absorption also occurs at 1684 and 1661 cm.$^{-1}$, at first identified with the carbonyl stretching vibration of a strongly chelated aromatic acid; however, no chemical evidence for the existence of such a group could be obtained. Strong absorption by di-*O*-acetyl-



hispidin at 2914 cm.$^{-1}$ confirms the presence of a free hydroxyl group, but the absorption at 1684 cm.$^{-1}$ in the spectrum of hispidin has been replaced by bands at 1757 and 1706 cm.$^{-1}$. The higher-frequency band is in the position appropriate for a phenolic acetate group. Tri-*O*-acetylhispidin, $C_{19}H_{16}O_8$, shows no absorption at 2941 cm.$^{-1}$ but intense absorption at 1757 and 1715 cm.$^{-1}$. The carbonyl absorption band in the derivatives of hispidin is at a higher wavelength than that normally associated with β-, γ-, or δ-lactones. Randall *et al.*[8] quote 1721 cm.$^{-1}$ for the carbonyl absorption of dehydroacetic acid, but Bellamy[9] notes complex interactions for lactones with two carbonyl groups in the same six-membered ring.

Naturally occurring 6-substituted 2-pyrones include yangonin[10] [(I; R = OMe) but without the 3′-methoxy-group], anibine,[11] and 5,6-dehydrokawain;[11] methysticin,[10]

[6] Wilds, Beck, Close, Djerassi, Johnson, and Shunk, *J. Amer. Chem. Soc.*, 1947, **69**, 1984.
[7] Bellamy, " The Infra-Red Spectra of Complex Molecules," Methuen, London, 2nd edn. 1958, pp. 99—105.
[8] Randall, Fowler, Fuson, and Dangle, " Infra-red Determination of Organic Structures," Van Norstrand, Princeton, 1949, p. 231.
[9] Bellamy, ref. 7, p. 186.
[10] Borsche and Lewinsohn, *Ber.*, 1933, **66**, 1792 and references therein.
[11] Gottlieb and Mors, *J. Org. Chem.*, 1959, **24**, 17; Gottlieb, Mors, and Djerassi, *J. Amer. Chem. Soc.*, 1957, **79**, 4507.

marindinin,[12] and kawain [10] are similar 5,6-dihydro-2-pyrones. Several of them have been differentiated spectroscopically [13] from the 4-pyrone isomers: the carbonyl absorption of methoxy-2-pyrones occurs at 1724—1709 cm.$^{-1}$ and of 4-pyrones at ~1667 cm.$^{-1}$.

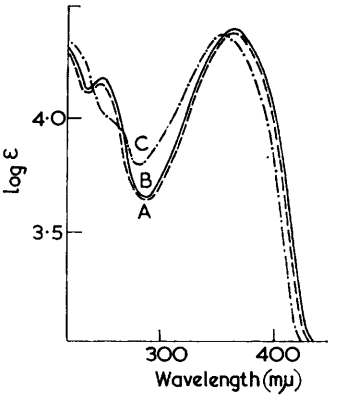


FIG. 1. Ultraviolet absorption spectra of tri-*O*-methylhispidin [(A) from hispidin, (B) synthetic] and (C) yangonin.

The infrared similarities were re-inforced when it was found that yangonin has an ultraviolet absorption peak at 3600 Å and tri-*O*-methylhispidin one at 3660 Å (cf. Fig. 1). The position of this maximum suggests that this latter is 6-(3,4-dimethoxystyryl)-4-methoxy-2-pyrone (I; R= OMe): the isomeric 4-pyrone would be expected to absorb at about ~3500 Å (cf. pseudoyangonin 3450 Å). Presumably the bathochromic shift of 60 Å is due to the additional methoxy-group in the benzene ring. This formulation is supported

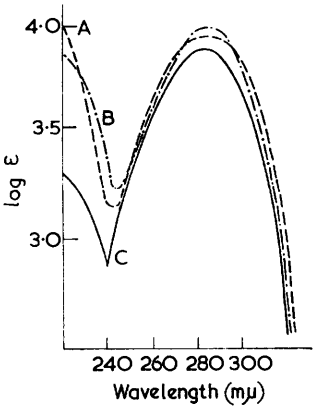


FIG. 2. Ultraviolet absorption spectra of (A) di-*O*-acetyldihydrohispidin, (B) dihydrohispidin, and (C) compound (V; R = H).

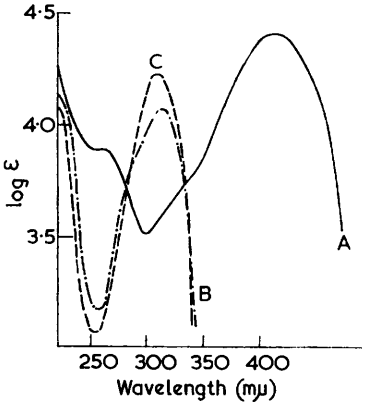


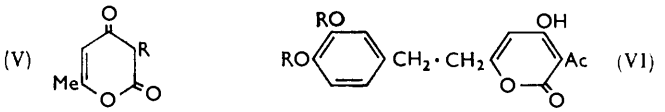
FIG. 3. Ultraviolet absorption spectra of (A) 3-acetylhispidin, (B) 3-acetyldihydrohispidin, and (C) dehydroacetic acid (V; R = Ac).

by the results of alkaline degradation. Tri-*O*-methylhispidin resembles yangonin and the constituents of the rosewoods (*Aniba* species) in that the ring is opened with simultaneous removal of the 4-methoxy-group. The unsaturated keto-acid (II) readily lost carbon dioxide on warming to produce the diketone (III) and was recyclised with acetic anhydride to the 4-hydroxy-2-pyrone (IV). The identity of the last compound was confirmed by methylation to tri-*O*-methylhispidin with diazomethane.

[12] Van Veen, *Rec. Trav. chim.*, 1939, **58**, 521.
[13] Chmielewska, Cieslak, Gorezynska, Kontnik, and Pitakowska, *Tetrahedron*, 1958, **4**, 36; Herbst, Mors, Gottlieb, and Djerassi, *J. Amer. Chem. Soc.*, 1959, **81**, 2427; Bu'Lock and Smith, *J.*, 1960, 502.

On the above evidence we formulate hispidin as 4-hydroxy-6-(3,4-dihydroxystyryl)-2-pyrone (I; R = OH). Di-*O*-acetylhispidin is the 3′,4′-diacetate, the yellow triacetate is the 4,3′,4′-derivative, and *O*-methylhispidin is the 4-methyl ether.

3,4-Dihydro-6-methylpyran-2,4-dione (triacetic lactone) (V; R = H) is readily *C*-acetylated, yielding dehydroacetic acid [14] (V; R = Ac). The fourth acetyl group introduced into hispidin or into tri-*O*-acetylhispidin by use of the boron trifluoride–acetic acid complex in acetic anhydride is similarly considered to be a 3-*C*-acetyl group. The ultraviolet absorption maximum at 4100 Å supports this formulation. 3-Acetylhispidin,



obtained from its triacetate, is bright red and sparingly soluble in polar solvents in which it yields intensely yellow solutions showing a faint green fluorescence. Benzoylation yielded a dibenzoate, and the triacetate was re-formed by acetylation. With dimethyl sulphate it gave a trimethyl ether; with methyl iodide it gave the same trimethyl ether and the 3′,4′-dimethyl ether, the latter soluble in warm aqueous sodium hydroxide but not amenable to acetylation. The infrared spectra of 3-acetylhispidin and its derivatives show a new band, above 1666 cm.$^{-1}$, corresponding to the new reactive carbonyl group (see Table).

TABLE 1.

Infrared absorption bands (cm.$^{-1}$) of hispidin and its derivatives.

Hispidin derivative	C=O of OAc	C=O of 3-Ac	C=O of pyrone
(Hispidin)			1684, 1661
Di-*O*-acetyl	1757		1706
Tri-*O*-acetyl	1757		1715
Tri-*O*-methyl			1701
O-Methyl			1664
Di-*O*-acetyl-*O*-methyl	1752		1715
3-Acetyl		1712	1686
3-Acetyl, triacetate	1776	1736	1729
3-Acetyl, trimethyl ether		1724	1701
3-Acetyl, dimethyl ether		1733	1718
3-Acetyl dihydro, diacetate	1758i	1733	1715
3-Acetyl dihydro		1724i	1704

i = Inflexion.

Difficulty was experienced in hydrogenating hispidin and its derivatives in the presence of Adams catalyst. The products were in most cases oils which could not be crystallised. However, 3-acetylhispidin triacetate and dimethyl ether gave crystalline dihydro-compounds (VI; R = Ac and Me respectively). The removal of an *O*-acetyl group from the former is comparable with the removal of an *O*-acetyl group from acetylyanganolactone on hydrogenation.[15] The identity of these products was established by their ultraviolet absorption spectra (cf. Fig. 2); both show an intense peak at 3100 Å, similar to that [16] of dehydroacetic acid (V; R = Ac) and in accord with the observations by Borsche and his co-workers [10] that the double bond between the two rings in yangonin is preferentially reduced on catalytic hydrogenation. Di-*O*-acetylhispidin was hydrogenated over palladium–charcoal to the above-mentioned crystalline dihydro-derivative which was readily hydrolysed to dihydrohispidin. Both these compounds have an ultraviolet absorption maximum at 2850 Å [cf. (V; R = H) [16] at 2830 Å (see Fig. 3)].

[14] Collie, *J.*, 1900, **77**, 976.
[15] Borsche and Bodenstein, *Ber.*, 1929, **62**, 2513.
[16] Berson, *J. Amer. Chem. Soc.*, 1952, **74**, 5172.

From the position of the pyrone carbonyl band in the infrared spectra of hispidin, *O*-methylhispidin, and 3-acetylhispidin it appears at first sight that these compounds exist preferentially as the 4-pyrone isomers in the solid state. In alcohol, as revealed by the ultraviolet spectra, these compounds exist as the 2-pyrones. The acetates seem to exist as 2-pyrones both in the solid state and in solution. Similar observations have been made by Herbst *et al.*;[13] hydroxy-6-phenylpyrone has been observed to exist as the 4-pyrone in the solid state and as the 2-pyrone in alcoholic solution. An explanation based on tautomerism is, however, difficult to reconcile with the infrared absorption of *O*-methylhispidin, which appears to exist as the 4-pyrone in the solid state and the 2-pyrone in alcohol ($\lambda_{max.}$ 3780 Å); this compound is prepared by the acid hydrolysis of di-*O*-acetyl-*O*-methylhispidin, which occurs as the 2-pyrone both in the solid state and in solution. Tautomerism is impossible for *O*-methylhispidin and we must assume that both it and its diacetate are 2-pyrones, the infrared pyrone carbonyl absorption being modified and moved to higher wavelength by intermolecular bonding. It is significant that this shift of absorption occurs only in those compounds in which the hydroxyl groups in the benzene ring are unsubstituted.

Bu'Lock and Smith[13] recently synthesised yangonin by an unambiguous method involving the condensation of *p*-methoxybenzaldehyde with the 4-methyl ether of compound (V). We carried out a similar synthesis with veratraldehyde in place of *p*-methoxybenzaldehyde: tri-*O*-methylhispidin, identical with that from hispidin, was obtained.

Experimental

Ultraviolet spectra were determined for alcohol solutions and infrared spectra for potassium bromide discs.

Isolation of Hispidin.—The fresh brownish-yellow sporophore was sliced and extracted (1 l. Soxhlet) with alcohol until the syphoning extract was pale yellow. 1 Litre of alcohol was used for six batches of fresh fungus. The extract (1 l.) was poured into water (5 l.) and set aside overnight. The solid was separated, dried, and extracted with benzene (Soxhlet) until the extract was colourless. The residue was extracted with acetone and the reddish-brown solution (450 ml.) concentrated to 100 ml. Ether (300 ml.) was added, and the yellowish-brown solid (A) (13 g.) which separated was filtered off and washed with ethyl acetate (3 × 150 ml.). The ethyl acetate washings were combined with the main filtrate; this led to the deposition of a further quantity of solid (B) (5 g.). The red filtrate was evaporated to 150 ml., then cooled for 2 hr. at 0° and the reddish-yellow precipitate (C) (6 g.) collected and washed with ethyl acetate (50 ml.).

Evaporation of the ethyl acetate solution to 75 ml. gave more yellow solid (D) (11 g.) when kept at 0° overnight. Each fraction was acetylated.

Fractions C and D (5 g.) were left overnight with acetic anhydride (50 ml.) and pyridine (10 ml.), then poured into water (500 ml.). The precipitate (7·6 g.) was extracted with benzene (2 × 50 ml.), and the yellow residue (2·9 g.) filtered off and crystallised three times from ethyl methyl ketone, to yield *di-O-acetylhispidin* as pale yellow needles, m. p. 227° [Found: C, 61·75; H, 4·2%; *M* (ebullioscopic in acetone), 311. $C_{17}H_{14}O_7$ requires C, 61·8; H, 4·2%; *M*, 330], $\lambda_{max.}$ 351, 255, and 234 mμ (log ε 4·40, 4·14, and 4·20).

The benzene mother-liquor was evaporated to 5 ml. and set aside for 12 hr. The yellow-brown solid (0·2 g.), crystallised once from benzene and twice from alcohol, gave pale yellow needles and plates of *tri-O-acetylhispidin*, m. p. 142—143° [Found: C, 61·5; H, 4·2%; *M* (*X*-ray), 368. $C_{19}H_{16}O_8$ requires C, 61·3; H, 4·3%); *M*, 372] $\lambda_{max.}$ 360, 269, 262, and 237 mμ (log ε 4·40, 4·10, 4·10, and 4·06).

Fractions A and B gave oils which yielded no crystalline material.

Tri-*O*-acetylhispidin (or di-*O*-acetylhispidin) (0·25 g.), ethanol (20 ml.) and 2N-sulphuric acid (20 ml.) were refluxed for 2 hr., then set aside at room temperature for 12 hr., and the yellow precipitate filtered off. Recrystallisation from aqueous alcohol yielded *hispidin* as yellow needles (0·7 g.), m. p. 259° (decomp.) (preheated block) [Found: C, 58·7; H, 4·7%; *M* (potentiometric titration), 260. $C_{13}H_{10}O_5,H_2O$ requires C, 59·1; H, 4·5%; *M*, 264], $\lambda_{max.}$

373 and 257 mμ (log ε 4·28 and 4·07), insoluble in light petroleum, sparingly soluble in hot chloroform or benzene, and very soluble in hot alcohol or acetone.

Occasionally, sporophores particularly rich in hispidin were encountered; material obtained from them on evaporation of the acetone solution crystallised from alcohol and aqueous alcohol to yield pure hispidin with much loss.

Acetyl Derivatives of Hispidin.—Hispidin (0·1 g.), acetic anhydride (2 ml.), and pyridine (1 drop) were set aside at room temperature for 8 hr., then poured into cold water (25 ml.). The benzene-soluble portion of the precipitate, when crystallised three times from methyl alcohol, yielded tri-*O*-acetylhispidin (0·08 g.) as golden-yellow needles, m. p. and mixed m. p. 142—143° (Found: C, 61·0; H, 4·3%).

Use of 1 ml. of pyridine led to a solid which, after extraction with cold benzene (10 ml.), recrystallised from ethyl methyl ketone to yield di-*O*-acetylhispidin (0·07 g.), m. p. and mixed m. p. 227° (Found: C, 61·8; H, 4·5%).

Tri-*O*-acetylhispidin (0·12 g.) was refluxed for 7 hr. with 95% ethanol (10 ml.), then evaporated; the residual solid recrystallised from ethyl methyl ketone to yield di-*O*-acetylhispidin (0·05 g.), m. p. and mixed m. p. 227°.

A suspension of di-*O*-acetylhispidin (0·5 g.) in ether (10 ml.) was treated with diazomethane (1·1 g. in 75 ml. of ether). After 5 hr. at room temperature the excess of diazomethane was destroyed by acetic acid. The solid (0·47 g.) was collected and recrystallised three times from methanol, to yield *di*-O-*acetyl*-O-*methylhispidin* as pale yellow needles, m. p. 205° (Found: C, 62·7; H, 4·8. $C_{18}H_{16}O_7$ requires C, 62·8; H, 4·65%), $\lambda_{max.}$ 346 and 255 mμ (log ε 4·40 and 4·12).

O-*Methylhispidin.*—Di-*O*-acetyl-*O*-methylhispidin (0·21 g.) was refluxed for 30 min. with 2N-sulphuric acid (6 ml.) and ethanol (6 ml.). The yellow solution deposited O-*methylhispidin* (0·14 g.) when left overnight. Crystallisation from glacial acetic acid yielded needles, m. p. 257° (decomp.) (preheated block) (Found: C, 64·7; H, 4·8. $C_{14}H_{12}O_5$ requires C, 64·6; H, 4·6%), $\lambda_{max.}$ 378, 255 mμ (log ε 4·26, 4·10). Acetylation of this product with acetic anhydride and a trace of pyridine for 15 hr. at 20° regenerated di-*O*-acetyl-*O*-methylhispidin, m. p. 205°, in almost quantitative yield.

Tri-O-*methylhispidin.*—(i) A mixture of hispidin (0·3 g.), acetone (15 ml.), potassium carbonate (6 g.), and dimethyl sulphate (3 ml.) was refluxed for 5 hr., then filtered and evaporated *in vacuo* to a red oil which rapidly solidified. This was shaken with 2N-sodium hydroxide (25 ml.) and filtered, yielding *tri*-O-*methylhispidin* (0·19 g.), m. p. 153—158°. After crystallising four times from methanol the compound was obtained as pale yellow needles, m. p. 164° [Found: C, 66·6; H, 5·4; *M* (ebullioscopic in acetone) 287, (mass spectroscopy) 288. $C_{16}H_{16}O_5$ requires C, 66·7; H, 5·6%); *M*, 288], $\lambda_{max.}$ 366 and 251 mμ (log ε 4·39 and 4·17). Evaporation of the mother-liquors from the recrystallisation gave needles (25 mg.) of an *isomer*, m. p. 197° (from ethyl acetate) (Found: C, 66·4; H, 5·8%), $\lambda_{max.}$ 266 mμ (log ε 4·21).

(ii) Hispidin (0·1 g.) was left with diazomethane (0·2 g.) in methanol (3 ml.) and ether (25 ml.) at room temperature for 1·5 hr., then treated with acetic acid and filtered [to remove the trimethyl ether (0·02 g.), m. p. 162°]. Evaporation gave an oil which solidified on trituration with water. Recrystallisation from aqueous alcohol and then from methanol yielded a further 0·03 g. of pure trimethyl ether.

(iii) *O*-Methylhispidin (0·05 g.) in methanol (2 ml.) and ether (10 ml.) with diazomethane (0·2 g.) (16 hr.) gave an oil which was dissolved in chloroform (10 ml.), washed with sodium hydrogen carbonate solution (10 ml.), dried (Na_2SO_4), and chromatographed on magnesium oxide (B.D.H. chromatographic grade). Elution with chloroform gave a fast-moving yellow band yielding tri-*O*-methylhispidin, m. p. and mixed m. p. 164°.

3-*Acetylhispidin Triacetate.*—Di-*O*-acetylhispidin (0·5 g.) in acetic anhydride (10 ml.) and boron trifluoride–acetic acid complex (5 drops) was heated on the water bath for 30 min., then poured into water. The solid was collected and recrystallised three times from alcohol, to yield 3-*acetylhispidin triacetate* as yellow needles (0·15 g.), m. p. 193—194° (decomp.) [Found: C, 61·1; H, 4·45%; *M* (ebullioscopic in acetone), 368. $C_{21}H_{18}O_9$ requires C, 60·9; H, 4·3%; *M*, 414], $\lambda_{max.}$ 365 and 231 mμ (log ε 4·38 and 4·26), dissolving slowly in 2N-sodium hydroxide to a red solution.

3-*Acetylhispidin.*—3-Acetylhispidin triacetate (0·25 g.) was refluxed with ethanol (20 ml.) and 2N-sulphuric acid (20 ml.) for 45 min.; this gave orange-red plates of 3-*acetylhispidin* (0·16 g.), m. p. 306° (decomp.) (from acetic acid) (Found: C, 62·7; H, 4·2. $C_{15}H_{12}O_6$ requires

C, 62·5; H, 4·2%), $\lambda_{max.}$ 410 and 260 mμ (log ε 4·40 and 3·99). This afforded a 2,4-*dinitrophenylhydrazone*, red rhombs (from dioxan), m. p. 279° (Found: N, 12·0. $C_{21}H_{16}N_4O_9$ requires N, 12·0%).

3-*Acetylhispidin Trimethyl Ether*.—3-Acetylhispidin (0·9 g.), acetone (45 ml.), potassium carbonate (18 g.), and dimethyl sulphate (9 ml.) were refluxed for 6 hr., cooled, filtered, and evaporated under a vacuum. The orange residue, recrystallised three times from alcohol, gave the *trimethyl ether* as golden-yellow needles (0·27 g.), m. p. 182° (Found: C, 65·4; H, 5·4. $C_{18}H_{18}O_6$ requires C, 65·4; H, 5·4%), $\lambda_{max.}$ 404 and 255 mμ (log ε 4·44 and 4·17). This gave a 2,4-*dinitrophenylhydrazone*, red needles (from acetic acid), m. p. 209° (Found: N, 11·2. $C_{24}H_{22}N_4O_9$ requires N, 11·0%).

3-*Acetylhispidin Dimethyl Ether*.—3-Acetylhispidin (5 g.), acetone (25 ml.), methyl iodide (7·0 g.), and potassium carbonate (4·0 g.) were refluxed for 18 hr., then evaporated. Water (25 ml.) was added and the mixture was cooled in ice and shaken. The red oil, which rapidly solidified, was filtered off and recrystallised from alcohol (2·5 ml.) to yield the trimethyl ether (0·17 g.). The mother-liquor was acidified with dilute hydrochloric acid, and the precipitate recrystallised three times from acetic acid to give the *dimethyl ether* as orange-yellow needles (0·2 g.), m. p. 213° (Found: C, 64·5; H, 5·1. $C_{17}H_{16}O_6$ requires C, 64·4; H, 5·1%), $\lambda_{max.}$ 399 and 258i mμ (log ε 4·36 and 4·02). The latter gave a 2,4-*dinitrophenylhydrazone*, orange-red needles (from acetic acid), m. p. 299° (Found: N, 11·6. $C_{23}H_{20}N_4O_9$ requires N, 11·3%).

3-*Acetylhispidin Dibenzoate*.—Benzoylation of 3-acetylhispidin in pyridine at room temperature gave yellow needles of the *dibenzoate*, m. p. 186° (from acetic acid) (Found: C, 70·1; H, 4·0. $C_{29}H_{20}O_8$ requires C, 70·2; H, 4·0%), dissolving slowly in cold sodium hydroxide to a yellow solution. This product was not formed if the reaction mixture was heated.

3-*Acetyldihydrohispidin Diacetate*.—3-Acetylhispidin triacetate (2·0 g.) in ethyl acetate (100 ml.) was shaken with hydrogen at atmospheric pressure in the presence of Adams catalyst (0·1 g.) (absorption 180 ml.) for 12 hr. The solution was filtered and evaporated *in vacuo*. The residual oil was extracted with hot light petroleum (150 ml.; b. p. 80—100°). Colourless plates of 3-*acetyldihydrohispidin diacetate* (0·6 g.) were deposited on cooling and after recrystallising twice from alcohol and once from light petroleum had m. p. 121° (Found: C, 60·9; H, 4·9. $C_{18}H_{18}O_8$ requires C, 60·9; H, 4·9%), $\lambda_{max.}$ 311 mμ (log ε 4·10).

Similarly 3-acetylhispidin dimethyl ether (0·5 g.) yielded 3-*acetyldihydrohispidin dimethyl ether* (0·34 g.) as pale yellow plates, m. p. 141° (from alcohol) (Found: C, 64·1; H, 5·7. $C_{17}H_{18}O_6$ requires C, 64·1; H, 5·7%), $\lambda_{max.}$ 308 mμ (log ε 3·98).

3-*Acetyldihydrohispidin*.—3-Acetyldihydrohispidin diacetate (0·4 g.), on acid hydrolysis, gave yellow needles of 3-*acetyldihydrohispidin* (0·28 g.) which, after crystallising from dilute alcohol and then from toluene, formed yellow needles, m. p. 188° (Found: C, 61·9; H, 4·8. $C_{15}H_{14}O_6$ requires C, 62·1; H, 4·9%), $\lambda_{max.}$ 312 mμ (log ε 4·09) [2,4-*dinitrophenylhydrazone*, orange red plates (from acetic acid), m. p. 241° (Found: N, 11·7. $C_{21}H_{18}N_4O_9$ requires N, 11·9%)].

Di-O-*acetyldihydrohispidin*.—Di-*O*-acetylhispidin (0·5 g.) was hydrogenated at atmospheric pressure over 5% palladium–charcoal (0·15 g.). The reaction was stopped after absorption of 1·2 mol. of hydrogen (45 min.). Filtration and evaporation, followed by recrystallisation from toluene, gave colourless plates (0·2 g.) of *di*-O-*acetyldihydrohispidin*, m. p. 155° (Found: C, 61·6; H, 5·1. $C_{17}H_{16}O_7$ requires C, 61·4; H, 4·8), $\lambda_{max.}$ 285 mμ (log ε 3·92). An alcohol solution of this product gives a characteristic purple-brown colour, which changes to red and then becomes colourless on addition of sodium hydroxide solution.

Acid hydrolysis of this diacetate gave needles of *dihydrohispidin monohydrate*, m. p. 104—108° (from hot water) (Found: C, 58·5; H, 5·6. $C_{13}H_{12}O_5,H_2O$ requires C, 58·6; H, 5·3%), $\lambda_{max.}$ 285 mμ (log ε 3·97).

6-(3,4-*Dimethoxyphenyl*)*hex*-5-*ene*-2,4-*dione* (III).—Tri-*O*-methylhispidin (0·5 g.) in N-ethanolic potassium hydroxide (200 ml.) was set aside at room temperature in a stream of nitrogen for 36 hr. Yellow plates of the potassium salt of the acid (II) slowly separated. These (0·57 g.) were collected, washed with absolute alcohol, and dried in air. Cautious acidification of an aqueous solution of this salt gave the free acid as an amorphous yellow solid. The potassium salt (0·2 g.) was refluxed for 1 hr. with 2N-sulphuric acid (5 ml.) and ethanol (3 ml.) Pale yellow plates of the *diketone* (0·04 g.) were deposited on cooling; recrystallised from light petroleum they had m. p. 92° (Found: C, 67·5; H, 6·5. $C_{14}H_{16}O_4$ requires C, 67·7; H, 6·45%).

Di-O-*methylhispidin*.—The acid (II) (0·3 g.) was refluxed with acetic anhydride (5 ml.) for

1 hr., then poured into water (50 ml.). The resulting yellow oil slowly solidified, to yield di-*O*-methylhispidin, m. p. 184° (decomp.) (from alcohol) (Found: C, 61·6; H, 5·8%), $\lambda_{max.}$ 366, 251i mμ (log ε 4·15, 4·07). Tri-*O*-methylhispidin, identified by its infrared spectrum and mixed m. p., was obtained from this compound by treatment with diazomethane.

Oxidation of 3-Acetylhispidin Triacetate.—This compound (1·2 g.), dissolved in acetic acid (36 ml.) and acetic anhydride (36 ml.), was heated at 55—60° with stirring while chromium trioxide (2·4 g.) in water (1·8 ml.) and glacial acetic acid (25 ml.) was added. The temperature was kept at 65—70° for 4 hr., then the solution was poured into water (500 ml.). After filtration the filtrate was extracted with ether for 3 hr.; the extract was evaporated to 180 ml. and washed with saturated sodium hydrogen carbonate solution (1 × 200 ml., 2 × 100 ml.). The sodium hydrogen carbonate extract was acidified with 30% hydrochloric acid and extracted with ether (2 × 200 ml., 2 × 100 ml.). The ethereal solution was washed, dried, and evaporated, to yield a waxy yellow residue which, after recrystallisation from benzene, provided 0·2 g. of colourless needles, m. p. 146—148°. Repeated recrystallisation from water raised the m. p. to 157°. The product was identical with protocatechuic acid diacetate (m. p., mixed m. p., and infrared spectrum) (Found: C, 55·4; H, 4·3. Calc. for $C_{11}H_{10}O_6$: C, 55·5; H, 4·2%).

Oxidation of di-*O*-acetylhispidin yielded the same acetate, m. p. 157°.

Ozonolysis of Tri-O-acetylhispidin.—Ozonised oxygen was passed for 20 min. through a solution of tri-*O*-acetylhispidin (0·66 g.) in chloroform (15 ml.) cooled in isopropyl alcohol and solid carbon dioxide. Shaking the mixture with water (30 ml.) for 2·5 hr., followed by separation of the organic layer, drying, and removal of the solvent gave a residue. The 2,5-dinitrophenylhydrazone of this residue was purified by chromatography on acid-washed alumina from chloroform, and identified as the 2,4-dinitrophenylhydrazone (18 mg.) of protocatechualdehyde diacetate by m. p. and mixed m. p. (212°) (Found: C, 51·1; H, 3·5; N, 13·9. Calc. for $C_{17}H_{14}N_4O_8$: C, 50·75; H, 3·5; N, 13·9%).

Oxidation of 3-Acetylhispidin Trimethyl Ether.—A refluxing acetone solution of this ether (0·5 g. in 30 ml.) was treated portion-wise with solid potassium permanganate until a faint permanent pink colour developed. The solvent was removed, water was added, and sulphur dioxide passed into the solution until all the manganese dioxide had dissolved. The solution was steam-distilled and the distillate (200 ml.) acidified and extracted with ether. Evaporation of the dried extract yielded only 5 mg. of an oil which was not identified. The residual aqueous solution was acidified and extracted with ether (3 × 20 ml.); evaporation of the dried ether extract yielded veratric acid (80 mg.) which, after sublimation *in vacuo* and recrystallisation from light petroleum ether, had m. p. and mixed m. p. 181° (Found: C, 59·6; H, 5·7. Calc. for $C_9H_{10}O_4$: C, 59·3; H, 5·9%).

Synthesis of Tri-O-methylhispidin.—Veratraldehyde (5·0 g.) and 4-methoxy-6-methyl-2-pyrone (4·2 g.) (from triacetic lactone and dimethyl sulphate) were refluxed for 4 hr. with magnesium methoxide (from 2·0 g. of magnesium and 100 ml. of methanol). After 4 hr., more methanol (100 ml.) was added and the solution filtered. The deposited yellow tri-*O*-methylhispidin (1·2 g.) was filtered off and the filtrate evaporated to dryness. The gummy residue was treated with 2N-sulphuric acid (10 ml.), and the residue dissolved in chloroform, which, after being washed with water and dried (Na_2SO_4), was chromatographed on magnesium oxide (B.D.H. chromatographic grade). The eluted yellow band yielded an oil which on trituration with methanol yielded *tri-O-methylhispidin* (0·75 g.) as yellow needles, m. p. and mixed m. p. 164° (Found: C, 66·6; H, 5·5; OMe, 29·3. $C_{16}H_{16}O_5$ requires C, 66·7; H, 5·6; OMe, 32·3%), $\lambda_{max.}$ 366 and 251 mμ (log ε 4·39 and 4·17) (cf. Fig. 1).

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Department of Chemical Technology,
Bradford Institute of Technology, Bradford, 7. [*Received, April 12th*, 1961.]