

## Fungus Pigments. XXIX.\* The Pigments of *Hydnellum ferrugineum* (Fr.) Karsten and *H. zonatum* (Batsch) Karsten

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From the sporophores of *Hydnellum ferrugineum* (Fr.) Karsten and *H. zonatum* (Batsch) Karsten two pigments, hydnuferugin and hydnuferuginin have been isolated. Based mainly on spectral analyses of the pigments and their derivatives the structures 2,3-dihydro-2,8,9-trihydroxySpiro[4*H*-benzofuro-[2,3-*g*]-1-benzopyran-4,2'(5'*H*)-furan]-5,5',11-trione (3) and 2',3',7',8'-tetrahydro-2',7'-dihydroxydispiro[furan-2(5*H*),4'(9'*H*)-benzo[1,2-*b*:4,5-*b'*]dipyran-9',2''(5''*H*)-furan]-5,5',5'',10'-tetraone (12) are proposed for hydnuferugin and hydnuferuginin, respectively. The structure of hydnuferuginin has been confirmed by a single crystal X-ray analysis which also defines the stereostructure as 2'R\*,4'R\*,7'S\*,9'S\* (13).

*Hydnellum ferrugineum* (Fr.) Karsten and *H. zonatum* (Batsch) Karsten are fungi which grow on sandy soil. The rather similar-looking sporophores, which are of a dark brown-violet colour, appear in the autumn. Although they usually can be found in the same localities, their amount varies greatly from year to year. The material used in the present investigation has been collected in the parishes of Pojo (*H. ferrugineum*) and Tenala (*H. zonatum*) in south-western Finland.

Extraction of the sporophores gave in both cases a mixture of the same two pigments. One is dark violet, almost black, and has been termed hydnuferugin. The amount of hydnuferugin is about 0.8 % in *H. ferrugineum* and 1.8 % in *H. zonatum*. A preliminary report on this compound has appeared.<sup>1</sup> The present paper gives a full account of this work and describes the isolation and chemistry of the second, yellow pigment for which the name hydnuferuginin is now proposed. The amount is

about 1.2 % in both species. The pigments can be separated by taking advantage of the usually higher solubility of hydnuferugin. A small amount of atromentin (2,5-dihydroxy-3,6-bis(*p*-hydroxyphenyl)-1,4-benzoquinone) was also isolated from *H. ferrugineum*.

### RESULTS AND DISCUSSION

#### Hydnuferugin

Crude hydnuferugin, obtained as described in the experimental part, was purified by recrystallisation from dioxane. It consists of dark violet, almost black, microcrystals which decompose without melting above 200 °C. Analyses and mass spectra of hydnuferugin and its derivatives (see below) leads to the composition C<sub>18</sub>H<sub>10</sub>O<sub>9</sub> for the pigment. Some difficulties were encountered in reaching this conclusion, because the analyses did not clearly distinguish between this formula and the one with two more hydrogen atoms, and the apparent molecular mass peaks pointed mainly to the latter formula. Only the molecular mass peak of the leucoacetate was in agreement with the formula C<sub>18</sub>H<sub>10</sub>O<sub>9</sub> for the parent compound. Since the <sup>1</sup>H NMR spectra of none of the unreduced compounds contained any signals that could be attributed to the two "extra" hydrogens, it was concluded that the composition of hydnuferugin has to be C<sub>18</sub>H<sub>10</sub>O<sub>9</sub>. The apparent molecular mass peaks of the unreduced derivatives of hydnuferugin are thus to be regarded as (M+2)-peaks.

Since it has been shown<sup>2</sup> that quinones in many cases give no or only very weak M-peaks, but stronger (M+2)-peaks, the presence of a quinone grouping in hydnuferugin is strongly indicated.

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Upon acetylation hydnuferugin gave a yellow triacetate and reductive acetylation produced, in agreement with its quinonoid nature, a colourless dihydropentaacetate. When a methanolic solution of hydnuferugin was treated with a small amount of conc. hydrochloric acid a monomethyl derivative was formed, slowly even at room temperature. This could be acetylated to a diacetate and further methylation with dimethyl sulfate gave a trimethyl ether of hydnuferugin.

Hydnuferugin has thus three hydroxyl groups. The ease with which hydnuferugin reacts with methanol in acidic solution indicates that one of these is hemiacetalic. The two others are evidently phenolic.

The ultraviolet and visible spectrum of hydnuferugin is very characteristic and close to that of 8-hydroxy-3,7-dimethoxydibenzofuran-1,4-dione (1). (These spectra are shown in Fig. 1 of Ref. 1, where the reference compound is erroneously designated as 8-hydroxy-2,7-dimethoxydibenzofuran-1,4-dione.) The ultraviolet spectrum of the leucoacetate is again similar to that of dibenzofuran (see Fig. 2 in Ref. 1). Hydnuferugin is clearly a derivative of dibenzofuran-1,4-dione.

The  $^1\text{H}$  NMR spectra of all the derivatives of hydnuferugin contain two singlets due to aromatic protons. They are evidently flanked by hydroxyl groups, since the signals are shifted to lower field in the acetylated derivatives.

The partial structure A is thus indicated for hydnuferugin. This is further supported by the formation of 5,6-dimethoxybenzofuran-2,3-dicarboxylic acid (isolated as its dimethyl ester) upon oxidation of hydnuferugin trimethyl ether.

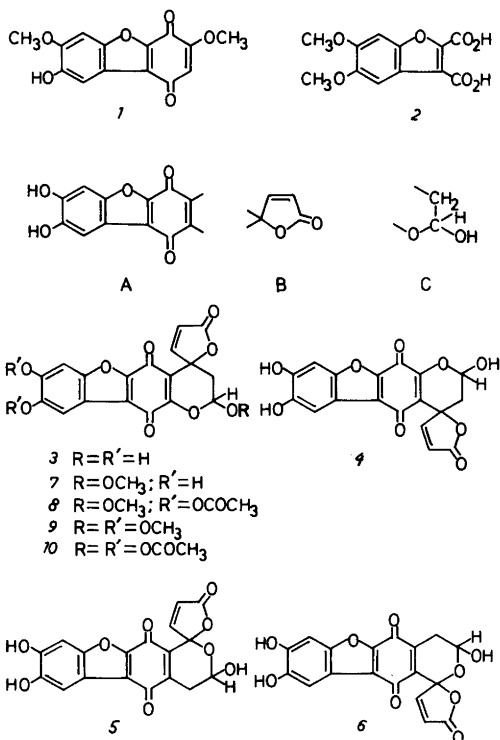
In addition to the signals from the aromatic protons, an AB-system ( $\delta$  7.57–7.95 and  $\delta$  6.20–6.46 with  $J=5-5.5$  Hz) is apparent in all the spectra. These values are in good agreement with those found for the vinylic protons in  $\alpha,\beta$ -unsaturated  $\gamma$ -lactones.<sup>3-8</sup> Since there is no further splitting of the signal at lower field the  $\gamma$ -atom is fully substituted. The strong absorption around  $1750\text{ cm}^{-1}$  also supports the presence of an  $\alpha,\beta$ -unsaturated  $\gamma$ -lactone in hydnuferugin. The partial structure B is thus indicated.

There are two further signals in the  $^1\text{H}$  NMR spectra (in addition to the signals from the methyl and acetyl groups as appropriate). One is a one-proton signal that occurs in the methylated derivatives around  $\delta$  5.50, but is shifted to  $\delta$  6.74 in the triacetate and to  $\delta$  6.68 in the leucoacetate. This

signal represents evidently a proton on a hemiacetalic carbon. This signal is coupled to a two-proton signal around  $\delta$  2.5. This latter signal is unfortunately in most cases rather diffuse and in the acetyl derivatives partly hidden behind the signals from the acetyl groups. However, double resonance experiments on the leucoacetate (as described in the experimental part) reveals that these protons form the AB-part of an ABX-system, the X-proton being the hemiacetal proton ( $J_{AB}=14$  Hz;  $J_{AX}=J_{BX}=3$  Hz). Thus the third partial structure C is indicated.

The partial structures A, B and C contain all the atoms of hydnuferugin and, since there is no possibility of any atoms overlapping, the structure of hydnuferugin has to be a combination of A, B and C. They can be combined in four different ways giving the structures 3–6.

The structures 3 and 4 are derivatives of 3,4-dihydro-2H-1-benzopyran-2-ol, whereas 5 and 6 are derivatives of 3,4-dihydro-1H-2-benzopyran-3-ol. In 3,4-dihydro-2H-1-benzopyran-2-ol derivatives the signal for the 2-H occurs at  $\delta$  5.3–5.7 (shifted to  $\delta$  6.4–6.9 upon acetylation) and for the 3-H's at  $\delta$  1.7–2.1.<sup>9-17</sup> In 3,4-dihydro-1H-2-benzopyran-3-ol derivatives the signal for the 3-H occurs at  $\delta$



5.0–5.25 (no information seems to be available on the influence of acetylation) and for the 4-H:s at  $\delta$  2.9–3.9.<sup>18–20</sup> The values found for the derivatives of hydnuferugin are clearly in better agreement with the former system. The structures 5 and 6 can therefore be dismissed as possible structures for hydnuferugin.

However, a definite decision between 3 and 4 is not possible on the basis of the available data. From a biogenetic point of view (see below) the structure 3 is to be preferred and is hence proposed for hydnuferugin. (The structures 5 and 6 are also from this point of view highly improbable). The mono-methyl ether, its diacetate, the trimethyl ether and the triacetate are thus represented by the structures 7, 8, 9 and 10, respectively.

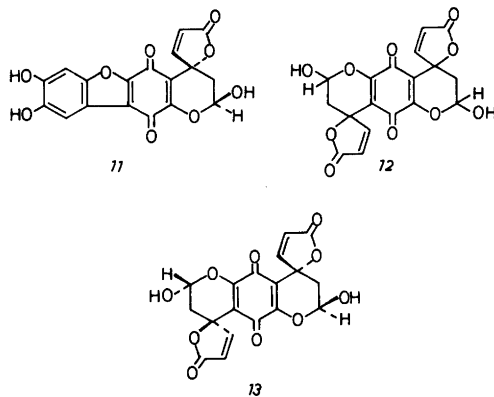
The structure 3 has two chiral atoms (as do of course also the structures 4–6) and should thus be able to occur in optically active forms. No optical rotation has been observed, but because of the low solubility of the compounds the determinations had to be done on rather dilute solutions and the result cannot be regarded as completely conclusive.

No information is available about the relative configuration of the two chiral atoms, but it can of course be speculated that the stereochemistry corresponds to that of hydnuferuginin (see below). Hydnuferugin should thus be represented by the stereostructure 11 and/or its enantiomer.

### Hydnuferuginin

Hydnuferuginin was purified by recrystallisation from methanol and obtained as yellow crystals without m.p. Like hydnuferugin it reacted readily with methanol in acidic solution giving a methyl derivative. However, this could not be further methylated nor acetylated. Hydnuferuginin could also be acetylated and reductive acetylation gave a colourless leucoacetate. Analyses and mass spectra of these compounds lead to the formula  $C_{18}H_{12}O_{10}$  for hydnuferuginin, the methyl derivative being a dimethyl ether, the acetate a diacetate and the leucoacetate a dihydrotetraacetate. Even in this case the mass spectra of the unreduced compounds show no M-peak, only the corresponding (M+2)-peaks.

The  $^1H$  NMR spectra of these compounds are very similar to the spectra of the corresponding derivatives of hydnuferugin, except that no signals for aromatic protons are present. A further important fact is that the signals account for only half



of the protons required by the formulae. This points to a symmetrical structure for hydnuferuginin.

The structure 12 fulfills these requirements. It is also supported by the IR spectra with absorptions at  $\sim 1750$  and  $\sim 1650\text{ cm}^{-1}$  (in the leucoacetate the latter one is absent) and the UV and visible spectra with maxima around 285 nm ( $\log \epsilon$  4.2) and 365 nm ( $\log \epsilon$  2.6) in the unreduced compounds. These values are in good agreement with the values reported for 2,5-dimethoxy-*p*-benzoquinone (277 nm ( $\log \epsilon$  4.24) and 360 nm ( $\log \epsilon$  2.51)).<sup>21</sup>

In order to confirm this proposal a single crystal X-ray analysis was carried out in the Laboratory of Inorganic Chemistry of this department. Suitable

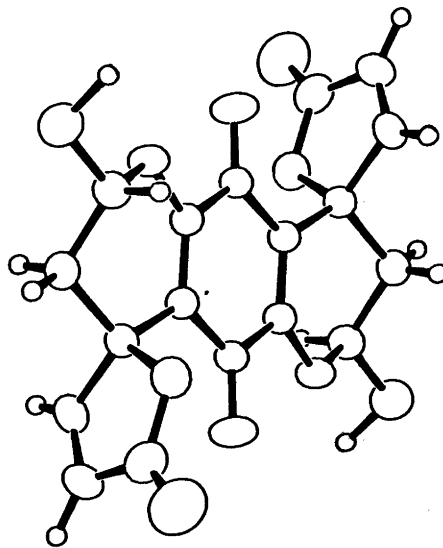
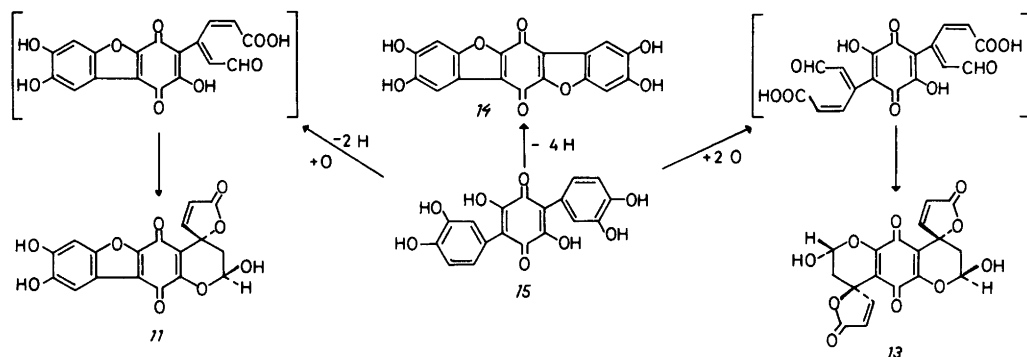


Fig. 1. Perspective drawing of the molecule of hydnuferuginin.



crystals for X-ray work were obtained by adding ethyl acetate to a solution of hydnuferuginin in a minimum amount of dimethyl sulphoxide. This analysis, which will be published in a separate paper,<sup>22</sup> confirms indeed the proposed structure and leads to the complete stereostructure 13 for hydnuferuginin (see Fig. 1). Because of the center of symmetry present in the molecule, hydnuferuginin is optically inactive.

The structure of hydnuferuginin and, to a lesser degree, that of hydnuferuginin bear a close relationship to that of thelephoric acid (14), which has been found in several species of *Hydnum* and *Hydnellum*.<sup>23</sup> One can visualize the terphenylquinone 15 (not yet found in nature) as a common precursor to all three compounds. Thelephoric acid (14) is formed by dehydrogenation, hydnuferuginin (11) by dehydrogenation in one half and oxidation in the other half of the molecule and hydnuferuginin (13) by oxidation on both sides.

## EXPERIMENTAL

Spectra were obtained using the following instruments: UV and visible spectra on a Beckmann DK-2, IR spectra on PE 125 and PE 700, <sup>1</sup>H NMR spectra (unless stated otherwise) on Varian A60 and Jeol PMX60 and mass spectra on a PE 270B. Elemental analyses were carried out by Ilse Beetz, Microanalytisches Laboratorium, Kronach, German Federal Republic.

### Isolation of the pigments

From *H. ferrugineum*. Powdered, dried fungus material (360 g) was extracted in a Soxhlet apparatus with light petroleum (b.p. 60–95 °C) overnight. This light yellow extract has not been investigated. The material was then extracted suc-

cessively with four portions of acetone, the first for 24 h, the second for 4 days and the third and fourth for 5 days each. The first acetone extract was filtered through a short column of silica gel impregnated with NaH<sub>2</sub>PO<sub>4</sub> and evaporated under vacuum. The residue was triturated with light petroleum and the insoluble sticky mass treated with acetone, giving an insoluble residue (930 mg) of a mixture of pigments. The acetone solution was then treated twice in the same manner giving an additional amount (980 mg) of the pigment mixture. The combined mixtures were boiled with a small amount of dioxane and filtered hot. Upon cooling crude hydnuferuginin crystallised. This was repeated as long as any significant amount of material crystallised. Altogether 1 g of crude hydnuferuginin was thus obtained. The light brown material (650 mg) which was insoluble in boiling dioxane was crude hydnuferuginin. The crude hydnuferuginin was further purified by a repetition of the treatment with boiling dioxane. From the two first extracts crystallised yellow needles (65 mg) which were identified as atromentin by a comparison with authentic material (IR and TLC). From the following extracts almost pure hydnuferuginin was obtained as a dark violet-brown microcrystalline powder.

The second of the original acetone extracts was evaporated under vacuum and the residue (6180 mg) treated with portions of cold dioxane and filtered, until the dioxane solution which at first was very dark, almost black, was only weakly coloured. The dioxane solution which apparently contains mostly polymeric material has not been investigated. The insoluble residue was then treated with boiling dioxane as described above, giving crude hydnuferuginin (1050 mg). The material, insoluble in boiling dioxane, was crude hydnuferuginin (1290 mg). The third portion of the acetone extracts gave in the same way 650 mg of crude hydnuferuginin and 1025 mg of crude hydnuferuginin. The fourth portion, after evaporation under vacuum and treatment with a small amount of acetone, gave almost pure hydnuferuginin (1200 mg).

From *H. zonatum*. The fresh fungus material (weight after extraction 31 g) was allowed to stand overnight with acetone and filtered. This treatment was repeated five times whereafter the acetone solution was only weakly coloured. The combined acetone solutions were partly evaporated under vacuum and ethyl acetate was added. Two layers were formed and between them a brown amorphous precipitate. The ethyl acetate solution was washed with water and most of the solvent removed. The crystalline precipitate which was formed was treated with boiling dioxane as described above, giving 550 mg of hydnuferugin and 380 mg of hydnuferuginin.

#### Characterisation of the compounds

*Hydnuferugin* (2,3-dihydro-2,8,9-trihydroxy-spiro[4*H*-benzofuro[2,3-*g*]-1-benzopyran-4,2'(5'*H*)-furan]-5,5',11-trione) (3). The crude material was further purified by recrystallisation from dioxane, and obtained as dark violet, almost black microcrystals, which contain one molecule of dioxane. It decomposes above 200 °C without melting. It is insoluble in nonpolar solvents, slightly soluble in dioxane and the lower alcohols and readily soluble in pyridine. Anal. C<sub>18</sub>H<sub>10</sub>O<sub>9</sub>·C<sub>4</sub>H<sub>8</sub>O: C, H. UV (dioxane) λ<sub>max</sub>(log ε): 255 (4.35), 293 (4.06), 322 (3.71), 410 sh (3.37), 485 (3.47) nm; λ<sub>min</sub>(log ε): 235 (4.05), 276 (3.92), 312 (3.67), 361 (3.18) nm. IR (KBr): 3420, 3310, 3090, 1730, 1680, 1650, 1590, 1550, 1460, 1295, 1270, 1220, 1150, 1070, 1005, 925, 915, 850, 800 cm<sup>-1</sup>.

*Hydnuferugin monomethyl ether* (2,3-dihydro-8,9-dihydroxy-2-methoxyspiro[4*H*-benzofuro[2,3-*g*]-1-benzopyran-4,2'(5'*H*)-furan]-5,5',11-trione) (7). Hydnuferugin (150 mg) was dissolved in methanol (300 ml) and conc. HCl (1 ml) was added. The mixture was kept at room temperature for 10 days. Most of the solvent was evaporated under vacuum and ethyl acetate was added and the solution washed with water. The product was purified by TLC on silica gel impregnated with NaH<sub>2</sub>PO<sub>4</sub> using chloroform–acetone (4:1) as solvent. The main violet band gave hydnuferugin monomethyl ether as dark violet crystals. It decomposes without melting. Anal. C<sub>19</sub>H<sub>12</sub>O<sub>9</sub>: C, H. UV (dioxane) λ<sub>max</sub>(log ε): 255 (4.40), 295 (4.10), 321 (3.75), 425 sh (3.38), 488 (3.52) nm; λ<sub>min</sub>(log ε): 235 (4.07), 277 (3.95), 312 (3.73), 360 (3.17) nm. IR (KBr): 3400, 1730–1750, 1640, 1550, 1270, 1020, 1005, 805 cm<sup>-1</sup>. <sup>1</sup>H NMR (CF<sub>3</sub>COOH): δ 2.57 (2 H, br), 3.80 (3 H, s), 5.67 (1 H, br t; irr. at 2.57→s), 6.46 (1 H, d; *J* 5 Hz), 7.13 (1 H, s), 7.50 (1 H, s), 7.94 (1 H, d; *J* 5 Hz).

*Hydnuferugin monomethyl ether diacetate* (8,9-diacetoxy-2,3-dihydro-2-methoxyspiro[4*H*-benzofuro[2,3-*g*]-1-benzopyran-4,2'(5'*H*)-furan]-5,5',11-

trione) (8) was obtained by acetylation of hydnuferugin monomethyl ether with acetic anhydride and a drop of pyridine at room temperature. Treatment of the reaction mixture with water gave a yellow precipitate of the diacetate which was purified by TLC with chloroform as eluent. Anal. C<sub>23</sub>H<sub>16</sub>O<sub>11</sub>: C, H. MS [70 eV; *m/e* (% rel. int.)]: 470 (4, *M*+2), 468 (2.5), 426 (31), 384 (100), 356 (12), 352 (14), 324 (22), 311 (15), 309 (17), 298 (26), 296 (14), 258 (14), 85 (21), 83 (31), 43 (63). UV (dioxane) λ<sub>max</sub>(log ε): 251 (4.54), 275 sh (3.91), 310 (3.99), 420 sh (3.20) nm; λ<sub>min</sub>(log ε): 229 (4.18), 287 (3.74) nm. IR (KBr): 1760, 1680, 1660, 1570, 1270, 1205, 1050, 1005 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 2.34 (8 H, s+m), 3.68 (3 H, s), 5.51 (1 H, m), 6.27 (1 H, d; *J* 6 Hz), 7.56 (1 H, s), 7.63 (1 H, d; *J* 6 Hz), 7.91 (1 H, s).

*Hydnuferugin trimethyl ether* (2,3-dihydro-2,8,9-trimethoxyspiro[4*H*-benzofuro[2,3-*g*]-1-benzopyran-4,2'(5'*H*)-furan]-5,5',11-trione) (9). This was obtained by methylation of the monomethyl ether with dimethyl sulphate and K<sub>2</sub>CO<sub>3</sub> in acetone and purified by TLC with chloroform as eluent. Dark violet crystals without m.p. UV (dioxane) λ<sub>max</sub>(log ε): 255 (4.36), 288 (4.11), 297 sh (4.08), 319 (3.70), 420 sh (3.30), 485 (3.49) nm; λ<sub>min</sub>(log ε): 237 (4.13), 275 (4.01), 308 (3.65), 357 (3.14) nm. IR (KBr): 1760, 1675, 1645, 1550, 1470, 1270, 1085, 1020 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 2.32 (2 H, m), 3.67 (3 H, s), 3.97 (6 H, s), 5.50 (1 H, m), 6.26 (1 H, d; *J* 5.5 Hz), 7.08 (1 H, s), 7.39 (1 H, s), 7.62 (1 H, d; *J* 5.5 Hz).

*Hydnuferugin triacetate* (2,8,9-triacetoxy-2,3-dihydrospiro[4*H*-benzofuro[2,3-*g*]-1-benzopyran-4,2'(5'*H*)-furan]-5,5',11-trione) (10). This was prepared by treating hydnuferugin with acetic anhydride and a drop of pyridine at room temperature. Yellow needles, m.p. 178–183 °C (dec.). Anal. C<sub>24</sub>H<sub>16</sub>O<sub>12</sub>: C, H. UV (dioxane) λ<sub>max</sub>(log ε): 250 (4.53), 275 sh (3.90), 309 (3.97), 410 sh (3.23) nm; λ<sub>min</sub>(log ε): 228 (4.15), 286 (3.72) nm. IR (KBr): 1770, 1685, 1670, 1565, 1370, 1270, 1205, 1190, 1150, 1090, 1075, 1030, 1015, 950, 915, 815 cm<sup>-1</sup>. <sup>1</sup>H NMR (CDCl<sub>3</sub>): δ 2.22 (3 H, s), 2.34 (8 H, s+m), 6.40 (1 H, d; *J* 5.5 Hz), 6.74 (1 H, m), 7.57 (2 H, s+d; *J* 5.5 Hz), 7.90 (1 H, s).

*Hydnuferugin leucoacetate* (2,5,8,9,11-pentaacetoxy-2,3-dihydrospiro[4*H*-benzofuro[2,3-*g*]-1-benzopyran-4,2'(5'*H*)-furan]-5'-one). Hydnuferugin was reduced by zinc in acetic anhydride and a drop of pyridine. The reaction was complete at room temperature in a few minutes. The product was purified by TLC with ethyl acetate as eluent and recrystallised from chloroform–ethanol. M.p. 275–277 °C. MS [70 eV; (% rel. int.)]: 582 (0.4, *M*), 540 (3), 498 (19), 456 (9), 438 (8), 414 (3), 411 (5), 396 (10), 393 (10), 354 (9), 351 (9), 106 (12), 91 (14), 43 (100). UV (dioxane) λ<sub>max</sub>(log ε): 251 (4.24), 261 (4.32), 294 (4.52), 310 sh (3.98), 323 sh (3.76) nm; λ<sub>min</sub>(log ε): 248 (4.22), 256 (4.19), 270 (3.77) nm. IR (KBr):

1760–1780, 1455, 1365, 1270, 1190, 1170, 1150, 1115, 1010, 985  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  (100 MHz) ( $\text{CDCl}_3$ ):  $\delta$  2.09 (3 H, s), 2.21 (1 H, dd;  $J$  14 and 3 Hz; irr. at 6.68  $\rightarrow$  d;  $J$  14 Hz; irr. at 6.68 and 2.95  $\rightarrow$  s), 2.29 (3 H, s), 2.31 (3 H, s), 2.33 (3 H, s), 2.41 (3 H, s), 2.95 (1 H, dd;  $J$  14 and 3 Hz; irr. at 6.68  $\rightarrow$  d;  $J$  14 Hz; irr. at 6.68 and 2.21  $\rightarrow$  s), 6.20 (1 H, d;  $J$  5.5 Hz), 6.68 (1 H, t;  $J$  3 Hz; irr. at 2.95 and 2.21  $\rightarrow$  s), 7.42 (1 H, s), 7.65 (1 H, s), 7.76 (1 H, d;  $J$  5.5 Hz).

*Oxidation of hydnuferrugin trimethyl ether.* To hydnuferrugin trimethyl ether (40 mg) dissolved in acetone (100 ml) an acetone solution of  $\text{KMnO}_4$  was added dropwise until the violet colour persisted for some time. Working up gave a  $\text{NaHCO}_3$ -soluble fraction which was methylated with diazomethane. The product was purified by TLC with chloroform as eluent and the band with a strong blue fluorescence in UV-light was eluted, giving dimethyl 5,6-dimethoxybenzofuran-2,3-dicarboxylate (3 mg) identified by comparison with an authentic sample<sup>24</sup> (m.p., IR, TLC).

*8-Hydroxy-3,7-dimethoxydibenzofuran-1,4-dione (I).* This was prepared according to Erdtman<sup>25</sup> with the slight modification that the ring closure of the intermediate diquinone was accomplished by heating an anisole solution in the presence of a small amount of propionic acid. Upon cooling, the product crystallised as red needles. M.P. 253 °C (Lit.<sup>25</sup> 250 °C). UV (dioxane)  $\lambda_{\text{max}}$ (log  $\epsilon$ ): 249 (4.28), 281 (4.01), 320 (3.93), 400 sh (3.51), 470 (3.66) nm;  $\lambda_{\text{min}}$ (log  $\epsilon$ ): 232 (4.09), 262 (3.96), 299 (3.74), 353 (3.19) nm. IR (KBr): 3390, 1670, 1650, 1625, 1595, 1550, 1470, 1440, 1270, 1240, 1115, 1060, 1030, 855, 850, 810  $\text{cm}^{-1}$ .

*Hydnuferruginin (2'R\*,4'R\*,7'S\*,9'S\*-2',3',7',8'-tetrahydro-2',7'-dihydroxydispiro[furan-2(5H),4'-(9'H)-benzo[1,2-b;4,5-b']dipyran-9',2''(5''H)-furan]-5,5',5'',10'-tetraone) (13).* The crude hydnuferruginin was purified by recrystallisation from methanol. Yellow plates without m.p. Anal.  $\text{C}_{18}\text{H}_{12}\text{O}_{10}$ : C, H. UV (ethanol)  $\lambda_{\text{max}}$ (log  $\epsilon$ ): 288 (4.21), 372 (2.72) nm;  $\lambda_{\text{min}}$ (log  $\epsilon$ ): 254 (3.74), 344 (2.67) nm. IR (KBr): 3520, 3095, 1750 br, 1665, 1605, 1380, 1320, 1295, 1255, 1195, 1140, 1115, 1085, 990, 975, 940, 830, 815  $\text{cm}^{-1}$ .

*Hydnuferruginin dimethyl ether (2',3',7',8'-tetrahydro-2',7'-dimethoxydispiro[furan-2(5H),4'-(9'H)-benzo[1,2-b;4,5-b']dipyran-9',2''(5''H)-furan]-5,5',5'',10'-tetraone).* Hydnuferruginin (200 mg) was dissolved in conc.  $\text{H}_2\text{SO}_4$  (4 ml), to which solution methanol (100 ml) was added. After a few minutes, precipitation of yellow crystals began. The crystals (140 mg) of hydnuferruginin dimethyl ether were collected after 4 days. It decomposes without melting at about 220 °C. Anal.  $\text{C}_{20}\text{H}_{16}\text{O}_{10}$ : C, H. MS [70 eV; m/e (% rel. int.):] 418 (3, M+2), 388 (7), 373 (3), 356 (4), 330 (6), 300 (9), 283 (4), 218 (14), 82 (16), 73 (40), 44 (100). UV (dioxane)  $\lambda_{\text{max}}$ (log  $\epsilon$ ): 285 (4.18), 364 sh (2.52) nm;  $\lambda_{\text{min}}$ (log  $\epsilon$ ): 249 (3.49)

nm. IR (KBr): 1750, 1655, 1495, 1320, 1300, 1210, 1100, 1030, 990, 960, 925, 905, 815  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  ( $\text{CF}_3\text{COOH}$ ):  $\delta$  2.50 (2 H, m), 3.74 (3 H, s), 5.60 (1 H, m), 6.44 (1 H, d;  $J$  5 Hz), 7.85 (1 H, d;  $J$  5 Hz).

*Hydnuferruginin diacetate (2',7'-diacetoxy-2',3',7',8'-tetrahydrodispiro[furan-2(5H),4'-(9'H)-benzo[1,2-b;4,5-b']dipyran-9',2''(5''H)-furan]-5,5',5'',10'-tetraone).* Hydnuferruginin (90 mg) was suspended in acetic anhydride (2 ml). A drop of conc.  $\text{H}_2\text{SO}_4$  was added, whereby a clear solution resulted. After a few minutes crystallisation of the acetate set in. Treatment of the mother liquor with water gave a further crop of the acetate. Yield 90 mg of pale yellow crystals without m.p. Anal.  $\text{C}_{22}\text{H}_{16}\text{O}_{12}$ : C, H. MS [40 eV; m/e (% rel. int.):] 474 (4, M+2), 432 (6), 430 (3), 414 (3), 386 (6), 372 (6), 370 (11), 369 (6), 355 (4), 345 (13), 327 (20), 299 (9), 245 (7), 149 (20), 121 (9), 97 (10), 78 (41), 63 (49), 60 (44), 57 (20), 45 (61), 44 (100), 43 (91). UV (dioxane)  $\lambda_{\text{max}}$ (log  $\epsilon$ ): 282 (4.22), 360 sh (2.58) nm;  $\lambda_{\text{min}}$ (log  $\epsilon$ ): 241 (3.53) nm. IR (KBr): 1755, 1670, 1610, 1220, 1195, 1140, 1095, 995, 970, 950  $\text{cm}^{-1}$ .  $^1\text{H NMR}$  ( $\text{CF}_3\text{COOH}$ ):  $\delta$  2.30 (3 H, s), 2.53 (2 H, d;  $J$  5 Hz; irr. at 6.75  $\rightarrow$  s), 6.50 (1 H, d;  $J$  5 Hz; irr. at 7.73  $\rightarrow$  s), 6.75 (1 H, t;  $J$  5 Hz), 7.73 (1 H, d;  $J$  5 Hz).

*Hydnuferruginin leucoacetate (2',5',7',10'-tetraacetoxy-2',3',7',8'-tetrahydrodispiro[furan-2(5H),4'-(9'H)-benzo[1,2-b;4,5-b']dipyran-9',2''(5''H)-furan]-5,5',5'',10'-dione).* Hydnuferruginin (30 mg) was suspended in acetic anhydride (5 ml) and a small amount of zinc powder and a drop of conc.  $\text{H}_2\text{SO}_4$  were added. After stirring for a few minutes the solution was colourless. The precipitate was filtered off and washed with water. Addition of water to the filtrate gave a further amount of precipitate. The combined precipitates were dissolved in chloroform and filtered from unreacted zinc. Addition of light petroleum to the chloroform solution gave colourless crystals of hydnuferruginin leucoacetate which decomposes without melting. MS [70 eV; m/e (% rel. int.):] 558 (0.17, M), 474 (15), 432 (4.5), 387 (3), 369 (5), 327 (5), 207 (8), 68 (40), 66 (59), 64 (100), 44 (76), 43 (46). UV (dioxane)  $\lambda_{\text{max}}$ (log  $\epsilon$ ): 296 sh (3.65), 304 (3.68) nm;  $\lambda_{\text{min}}$ (log  $\epsilon$ ): 265 (2.86) nm. IR (KBr): 1760–1780, 1455, 1375, 1285, 1225, 1200, 1185, 1120, 1100, 1050, 1000, 970, 940  $\text{cm}^{-1}$ .

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