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Studies on the Chemistry of Lichens

X.* The Structure of Porphyrilic Acid **

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Porphyrilic acid from *Haematomma coccineum* (Dicks.) has been ascribed the structure (III) consistent with the results of oxidative degradations described earlier [1,2] and in harmony with the negative Gibbs' reaction of methyl porphyrilate as well as the negative ferric reaction of O-monomethylporphyrilic acid (IV). The position of the nuclear methyl group has been confirmed by oxidation of O,O-dimethylporphyrilic acid to a tricarboxylic acid (XIII), further degraded to 1,7-dihydroxy-3-methyldibenzofuran (X), obtained synthetically. The acidity of the phenolic groups of porphyrilic acid and its derivatives before and after hydrolysis is discussed.

As described in part VII of this series [2], porphyrilic acid, a lichen acid of the composition $C_{16}H_{10}O_7$ from *Haematomma coccineum* (Dicks.), has been subjected to oxidative degradation, the result of which established the acid as a derivative of 1,7-dihydroxydibenzofuran. A consideration of the properties and colour reactions of the acid together with derivatives and degradation products led to three alternative formulae, (I)—(III), as the most probable structures for the acid itself. Of these, alternative (III) with the carboxyl group in the *para*-position to a phenolic hydroxyl group was rejected since chromatography of porphyrilic acid on buffered paper gave evidence for the formation of a borate complex, characteristic for aromatic *ortho*-hydroxy carboxylic acids [3]. However, porphyrilin [2], the decarboxylation product of porphyrilic acid, has now been found to be retarded on a borate paper as compared with a phosphate paper, provided the pH-values of the buffer solutions are chosen above 9, and hence the borate method is of little value in the present case. Furthermore, methyl porphyrilate, prepared by methanolysis of methyl O,O-diacetylporphyrilate, gave no colour with 2,6-dichloroquinone monochloroimide (Gibbs' reagent) [4] whereas the acid itself gave a clear blue colour with this reagent, as characteristic for phenolic compounds devoid

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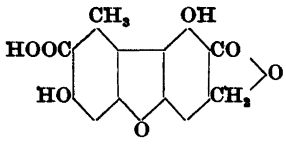
of ordinary carbonyl functions and containing a free position or an easily removable grouping, *e. g.* a carboxyl group, in the *para*-position to a free hydroxyl group [5,6]. It was therefore necessary to reconsider structure (III), the only formula which accounts for a negative Gibbs' reaction after esterification of the carboxyl group.

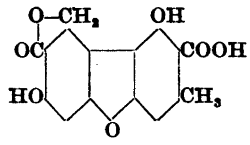
Further evidence in favour of structure (III) has now been obtained by partial methylation of porphyrilic acid. As stated in the experimental part of part VII [2], the acid titrates in the cold approximately as a dibasic acid with thymolphthalein as indicator. The titration is conveniently carried out in aqueous acetone with thymol blue as indicator. Hence, one of the two phenolic hydroxyl groups should be fairly acidic and the acid when treated for a short time with an excess of diazomethane in aqueous acetone at —10° gave a good yield of a methyl O-monomethylporphyrilate, hydrolysed by alkali to O-monomethylporphyrilic acid. These derivatives were only very sparingly soluble in the common solvents, the methyl ether being conveniently purified *via* its acetate, crystallising as a mixed anhydride from acetic anhydride. The methyl ether as well as its methyl ester gave no or at most a faintly yellowish colour with ferric chloride in ethanol, whereas an ethanolic solution of porphyrilic acid of a similar, very low concentration (0.01 %) gave a quite distinct, reddish-blue colour. Since, of the six possible monomethyl ethers derivable from the structures (I)—(III), only the methyl ether (IV) should give a negative ferric reaction, these results point definitely to this structure for the methyl ether and structure (V) for the corresponding methyl ester. The methyl ether (IV), in contrast to its ester (V), gave a clear blue colour with Gibbs' reagent, which constitutes a further confirmation of the proposed structures.

Porphyrilin, according to the above results formulated as (VI), also possesses a phenolic hydroxyl group of a high acidity, as revealed in aqueous acetone or aqueous pyridine when the lactone titrates in the cold approximately as a monobasic acid with thymol blue as indicator. Furthermore, treatment of porphyrilin in aqueous acetone containing a trace of methanol with an excess of ethereal diazomethane for 5—10 minutes at —10° afforded a quantitative yield of a monomethyl ether which gave no ferric reaction but a strong blue colour with Gibbs' reagent and hence must be ascribed the structure (VII). It melted with decomposition at 315—320° which is higher than the melting point of porphyrilin and was conveniently characterized by paper chromatography and by its acetate (VIII), m.p. 254—257°. The same monomethyl ether (VII) of porphyrilin was obtained from O-monomethylporphyrilic acid (III) by sublimation *in vacuo* or more conveniently by prolonged boiling with formic acid.

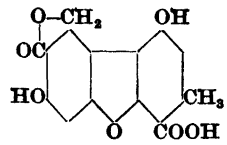
A derivative of porphyrilin containing a free acidic hydroxyl group could be prepared by treatment of its diacetate [2] with aniline at room temperature. The resulting monoacetate, which crystallised directly from the reaction mixture, gave an intense blue ferric reaction but no blue colour with Gibbs' reagent and hence must be ascribed the structure (IX). It titrated in the cold as a monobasic acid with thymol blue as indicator and on methylation with diazomethane yielded the O-monomethylporphyrilin monoacetate (VIII), m. p. 254—257°, mentioned above.



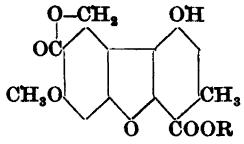
I



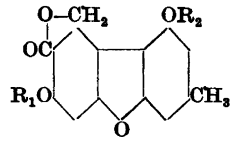
II



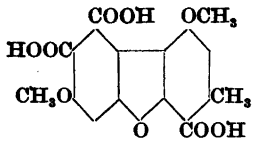
III



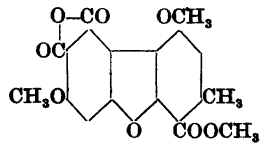
IV : R = H
V : R = CH_3



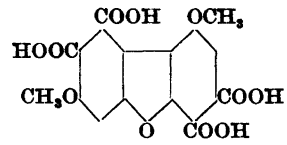
VI : $R_1 = R_2 = H$
VII : $R_1 = CH_3$ $R_2 = H$
VIII : $R_1 = CH_3$ $R_2 = CH_3CO$
IX : $R_1 = H$ $R_2 = CH_3CO$



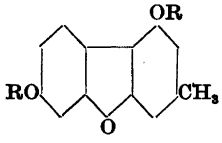
XIII



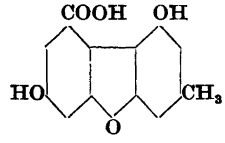
XIV



XV



X : R = H
XI : R = CH_3CO



XII

The colour reactions given by these monosubstituted derivatives of porphyrilin provide strong evidence for formulating this compound as (VI) and hence establish porphyrilic acid as derived from 1,7-dihydroxy-3-methyldibenzofuran (alternatives (II) or (III)), and not from 1,7-dihydroxy-9-methyldibenzofuran (alternative (I)).

The position of the nuclear methyl group has been confirmed by partial oxidation of O,O-dimethylporphyrilic acid [2], carried out in a dilute alkaline solution at 80—90° with an excess of permanganate. The desired tricarboxylic acid (A) could be isolated in a pure state as the monomethyl ester of its anhydride (B) which on prolonged boiling with hydrobromic acid lost two molecules of carbon dioxide with simultaneous demethylation to a dihydroxy-monocarboxylic acid (C), characterised by paper chromatography and by its methyl ester. The methyl ester, like the free acid, gave no distinct ferric reaction.

On decarboxylation with quinoline and a copper chromite catalyst, the acid (C) yielded a phenol $C_{13}H_{10}O_3$, (D), m. p. 165—166°, shown to be identical with 1,7-dihydroxy-3-methyldibenzofuran (X), synthesised by a method analogous to that used for 1,7-dihydroxydibenzofuran [2].

The mixed Ullmann-coupling, carried out with 4-iodo-3,5-dimethoxytoluene and an excess of 4-iodoresorcinol dimethyl ether, yielded a mixture of diphenyls which could not be easily separated into its components. The crude mixture on treatment with boiling hydrobromic acid furnished a mixture of dihydroxydibenzofurans partly freed from 3,7-dihydroxydibenzofuran [7] by crystallisation from benzene. The desired phenol (X) was isolated from the mother liquor by chromatography on thick filter paper or on aluminium oxide. Its identity with the phenol (D), indicated by paper chromatography, was definitely established by mixed melting points of the phenols as well as of their acetates.

Hence the acid (C) must have structure (XII), the carboxyl group of which is not in the *ortho*- or *para*-position to any hydroxyl group. The structure of the tricarboxylic acid (A) and the corresponding ester-anhydride (B) however can be established as (XIII) and (XIV), respectively, only with due regard to the results obtained concerning the position of the carboxyl group of porphyrilic acid. Taking into account these results, the tetracarboxylic acid $C_{18}H_{12}O_{11}$ (tetramethyl ester, m. p. 221.5—223°) obtained earlier [2] must be ascribed the structure (XV), previously considered improbable.

The proposed structure (III) for porphyrilic acid can be substantiated by a more detailed examination of the acidic functions of the acid and its derivatives. It is a well known fact that intramolecular hydrogen bonds in aromatic *ortho*-hydroxy carboxylic acids strikingly affect the dissociation constants of the acidic groups in these acids as compared with their *meta*- or *para*-isomers. Thus salicylic acid and *p*-hydroxybenzoic acid show $pK_1 = 3.0$ and 4.5, respectively [8,9]. The former acid can be titrated with some accuracy even with an indicator such as bromocresol green (pH-range 3.8—5.4) whereas the latter requires at least bromothymol blue (pH-range 6.0—7.6). Porphyrilic acid, when titrated in aqueous acetone, behaves like *p*-hydroxybenzoic acid.

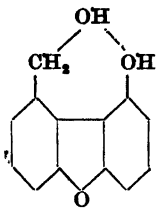
Similarly, the phenolic hydroxyl group in salicylic acid or its esters is of extremely low acidity [9,10], also revealed by the slow reaction of methyl salicylate with diazomethane (compare, *e. g.*, Schönberg and Mustafa [11]). On the other hand, the hydroxyl group of *p*-hydroxybenzoic acid ($pK_2 = 9.3$) [9] is acidic enough to interfere in the titration of this acid using thymol blue and its acidity is still further enhanced in methyl *p*-hydroxybenzoate [10], which can be titrated in aqueous acetone or pyridine with thymolphthalein as indicator. It is therefore in harmony with structure (III) that methyl porphyrilate in cold aqueous pyridine can be titrated fairly sharply as a dibasic acid using the indicator mentioned. Furthermore, porphyrilic acid contains no hydroxyl group showing great resistance towards methylation with diazomethane.

The high acidity of that phenolic hydroxyl group which accounts for the blue ferric reaction of porphyrilic acid and porphyrilin, also found in, *e. g.*, mycophenolic acid [12] or in 7-hydroxy-3-methylphthalide ($pK = 8.5$) [13], is evidently characteristic for *ortho*-hydroxyphthalides containing the hydroxy-group in the *ortho*-position to the lactonised carboxyl group and points to a

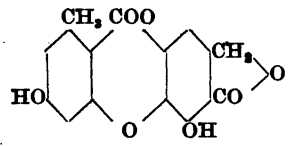
reduced chelation in these compounds as compared with, *e. g.*, methyl salicylate. The rapid methylation with diazomethane in aqueous acetone of the hydroxyl group in question is then explained as is also the very low solubility of porphyrilin even in boiling chloroform. It is of interest that recent infra-red measurements by Duncanson, Grove and Zealley [14] on simple compounds of the types discussed show strongly reduced though still distinct chelation in the 7-hydroxyphthalides.

While the above considerations account for the results obtained by titration in the cold of porphyrilic acid and its derivatives, the hydrolytic titrations require further discussion. Unexpectedly, O-monomethylporphyrilin (VII) titrated fairly sharply as a dibasic acid after hydrolysis with excess of alkali in aqueous ethanol and back titration with thymolphthalein as indicator. The titration could not be performed in an aqueous solution since the lactone, being of the phthalide type, precipitated at high pH-values. Porphyrilin (VI) was still more easily precipitated even from alkaline solutions containing ethanol, but on hydrolytic titration in aqueous pyridine this compound also titrated approximately as a dibasic acid. The result previously reported of the hydrolytic titration of porphyrilin in an aqueous solution with thymol blue as indicator could not be reproduced. Furthermore, porphyrilic acid (III) as well as its monomethyl ether (IV) proved approximately tribasic on hydrolytic titrations using the indicator mentioned.

A possible explanation of the observed low equivalent weights involves the assumption of an intramolecular hydrogen bond between the methylol group and the phenolic hydroxyl group of the partial structure (XVI), resulting in an increased acidity of the phenolic hydroxyl group. It seems quite possible that the postulated hydrogen bond, perhaps weak in an unionized phenol of the type (XVI), becomes fairly strong only after ionization of the phenolic group, but this question clearly requires further investigations on simple model compounds. A somewhat analogous case is given by 1,8-dihydroxynaphthalene [15]. Also 1,9-dihydroxydibenzofuran which titrates sharply as a monobasic acid with thymol blue as indicator offers an interesting example of a similar intramolecular hydrogen bond, which in this case seems to be of a considerable strength in the unionized phenol also [16]. The hydroxyl group in the *ortho*-position to the lactonised carboxyl group is not titrated after hydrolysis of the lactone ring, when the hydroxyphthalide structure changes to a salicylic acid structure (*cf.* [12]).



XVI



XVII

The indigo-blue colour reaction with ferric chloride given by porphyrilic acid and some of its derivatives seems to be of diagnostic value as it is reported also for 7-hydroxyphthalide [14] itself as well as for related compounds such as strepsilin [17] or mycophenolic acid [12]. Salicylic acid and related compounds generally give a violet ferric reaction.

The possibilities for formation of borate complexes of porphyrilic acid and its derivatives seem to be greater than previously supposed. As not only porphyrilin and methyl porphyrilate but also O-monomethylporphyrilic acid is slowed down on a borate paper, no conclusions concerning their structures can be drawn from these results without further studies on model compounds.

The unsymmetrical dihydroxydibenzofuran structure of porphyrilic acid (III) is unique among the lichen acids although there is paper chromatographic evidence for the occurrence of another closely related acid in very small amounts in *H. coccineum*. The crustose lichen *Ochrolechia parella* (L.) Mass. contains an isomeric compound of the depsidone type, variolaric acid, ascribed the structure (XVII) by Murphy, Keane and Nolan [18]. Both porphyrilic acid and variolaric acid are composed of orsellic acid units and contain the hydroxyphthalide structure and they may both be considered as having been formed from the same two phenolic acids by different mechanisms of dehydrogenative coupling. It is possible that in *O. parella* a preformed depside is dehydrogenated with formation of the depsidone, whereas in *H. coccineum* the dehydrogenase system attacks the simple phenolic compounds resulting in the formation of a diphenyl derivative further stabilised by ring closure to the dibenzofuran derivative, porphyrilic acid. The positions involved in the presumed dehydrogenative couplings to variolaric acid and porphyrilic acid are unusual, being *ortho* to both hydroxyl groups of the orcinol skeleton of one of the components, whilst as a rule in the lichen series the coupling involves the *o*-, *p*-positions. The chemical relations discussed may be of special interest in view of the presumed close relations between the genera *Haematomma* and *Ochrolechia*. Thus, in both lichens identical dehydrogenase systems may operate, *H. coccineum*, however, largely lacking the enzymatic requirements for the formation of depsides of the orcinol type.

EXPERIMENTAL *

Unless otherwise stated the paper chromatography was carried out at 25° using the solvent system *n*-butanol-water. Whatman No. 1 paper was used after impregnation with buffer solutions as previously described [2,3,19].

Methyl porphyrilate. Methyl O,O-diacetyl porphyrilate [2] (1 g) was boiled for 30 min. with 1.5 N methanolic hydrogen chloride (50 ml), yielding methyl porphyrilate (0.8 g) which crystallised from the boiling solution. The analytical sample crystallised as needles from a mixture of pyridine and glacial acetic acid (1:1) and was dried *in vacuo* at 150° C. It showed no melting point but decomposed gradually above 320°. The ester gave no colour with Gibbs' reagent [4] when tested in a borate buffer of pH 9 but a light brown colour with *bis*-diazotised benzidine [20] (on buffered filter paper) and a distinct blue colour with ferric chloride in ethanol. R_F-values on phosphate-impregnated papers: 0.65 (0.1 M Na_2HPO_4, pH 8.9); 0.10 (0.1 M Na_3PO_4, pH 12.1) and on borate-impregnated paper: 0.35 (0.1 M borate buffer of pH 9.1).

The equivalent weight was determined by titration of the ester in a cold pyridine solution with 0.05 N aqueous sodium hydroxide using thymolphthalein as indicator. (Found: C 61.9; H 3.60; OCH_3 9.6; equiv. weight, 165. $C_{17}H_{12}O_7$ requires C 62.2; H 3.68; OCH_3 9.5; equiv.weight (as a dibasic acid) 164.1.)

Methyl O-monomethylporphyrilate (V). To a solution of porphyrilic acid (1 g, *ca.* 3 mmole) in acetone (800 ml) and water (75 ml), cooled to −10 – −15°, diazomethane

* All melting points uncorrected.

(20 mmole) in ether (60 ml) was added in a few portions with stirring during 5 min. After another 5 min., the excess of diazomethane was destroyed by the addition of acetic acid. The reaction product, which rapidly crystallised from the cold solution, was collected after a few hours and proved to be fairly pure methyl O-monomethylporphyrilate (0.65 g). It gave no colour with Gibbs' reagent and no or only a faintly yellowish colour with ferric chloride in ethanol. On buffered paper (0.1 M Na_3PO_4) a spot, $R_F = 0.45$, of greenish-white colour in ultra-violet light was obtained, which gave a faint yellowish-brown colour with *bis*-diazotised benzidine. In addition a faint spot of methyl porphyrilate ($R_F = 0.10$) was observed on the chromatogram. The mother liquor on evaporation yielded a second fraction (0.35 g), only partly soluble in cold alkali and evidently containing methyl O,O-dimethylporphyrilate [2]. This compound was obtained as the main reaction product when the methylation was performed at higher temperature or when too large an excess of diazomethane was used.

The analytical sample was crystallised from pyridine and from pyridine-ethanol. Needles, dried *in vacuo* at 150°. M. p. 340–345° (decomp.) after darkening from 300°. (Found: C 63.6; H 4.51; OCH_3 17.7. $C_{18}H_{14}O_7$ requires C 63.2; H 4.12; OCH_3 18.1.)

O-Monomethylporphyrilic acid (IV). Crude methyl O-monomethylporphyrilate (V) (0.3 g) in 0.2 N sodium hydroxide solution (200 ml) was warmed to 50° overnight and the solution then diluted with acetone (200 ml) and acidified with hydrochloric acid, whereupon the monomethyl-derivative (IV) crystallised as microscopic needles containing solvent of crystallisation. It was almost insoluble in dry acetone but could be crystallised from 80 % aqueous acetone (150 ml to 100 mg of the acid). The analytical sample was obtained by hydrolysis of the mixed anhydride of its monoacetate (see below) with equal volumes of 0.1 N sodium hydroxide solution and acetone. It was dried *in vacuo* at 150°. M. p. 305–310° (decomp.). The equivalent weight was determined before hydrolysis by back titration of a solution of the acid in an excess of 0.05 N sodium hydroxide using phenol red as well as after hydrolysis by treatment of the acid with excess alkali for 1 h at 90° and back titration with thymolphthalein as indicator. (Found: C 62.0; H 3.51; OCH_3 8.8; equiv. weight 335 (before hydrolysis) and 114 (after hydrolysis). $C_{17}H_{12}O_7$ requires C 62.2; H 3.68; OCH_3 9.5; equiv. weight 328.3 (as a monobasic acid) and 109.4 (as a tribasic acid).)

O-Monomethylporphyrilic acid (0.01 % in ethanol) gave only a faintly yellowish colour with ferric chloride but a strong blue colour with Gibbs' reagent and a dark wine-red colour when treated with the benzidine reagent on buffered paper. The R_F-values of the acid on buffered paper were 0.20 (Na_2HPO_4), 0.05 (Na_3PO_4) and 0.10 (borate buffer, pH 9.1), respectively. The spots showed a greenish-white fluorescence in ultra-violet light.

Acetylation of O-monomethylporphyrilic acid. Crude monomethyl ether (IV) (0.5 g) was warmed with acetic anhydride (15 ml) and a few drops of pyridine until complete solution had been effected. The acetate crystallised on cooling and was obtained chromatographically pure as needles, m. p. 212–215°, from acetic anhydride. For chromatography, the acetate was dissolved in pyridine and the chromatogram run on buffered paper (Na_3PO_4) when a single spot of the monomethyl ether (IV) was obtained due to hydrolysis of the acetoxyl groups. The acetate which in the crystalline state exhibited an extraordinarily intense white fluorescence in ultra-violet light proved insoluble in cold aqueous sodium hydroxide and was established as a mixed anhydride by the hydroxamate test as well as by analysis. (Found: OCH_3 7.5; CH_3CO 20.7. $C_{21}H_{16}O_9$ requires OCH_3 7.5; CH_3CO 20.9.)

When warmed with glacial acetic acid the above acetate dissolved but acetolysis of the mixed anhydride occurred and the sparingly soluble monoacetate of O-monomethylporphyrilic acid soon crystallised from the hot solution.

The same monoacetate was obtained by acetylation of the monomethyl ether (50 mg) with acetic anhydride (0.5 ml) and pyridine (1 ml). Needles, m. p. 260–270° (decomp.), dried *in vacuo* at 150°. The equivalent weight was determined by hydrolytic titration (thymolphthalein). (Found: CH_3CO 11.0; equiv. weight 95. $C_{19}H_{14}O_8$ requires CH_3CO 11.6; equiv. weight 93.3 (after complete hydrolysis).)

O-Monomethylporphyrilin (VII). a) *Partial methylation of porphyrilin* (VI). Porphyrilin (360 mg, 1.2 mmole) in acetone containing a trace of methanol (200 ml) and water (20 ml) was methylated at −10° with diazomethane (5 mmole) in ether (25 ml), added in portions during 10 min. Acetic acid was then added and evaporation of the solution yielded a crystallisate practically free from porphyrilin as found by paper chromato-

graphy. The substance gave no ferric reaction but an immediate blue colour with Gibbs' reagent. R_F-value on buffered paper (Na_3PO_4): 0.85. It crystallised as colourless needles from acetone and was finally sublimed *in vacuo*, m. p. 315—320° (decomp., darkening from 300°). The equivalent weight was determined by hydrolysis of 50 mg with 0.1 N aqueous sodium hydroxide (6 ml) and ethanol (10 ml) for 1 hour at 90°, followed by back titration of the cooled solution with 0.1 N hydrochloric acid, using thymolphthalein as indicator. (Found: C 67.5; H 4.54; OCH_3 10.8; equiv. weight 140. $C_{16}H_{12}O_5$ requires C 67.6; H 4.25; OCH_3 10.9; equiv. weight (as a dibasic acid) 142.1.)

Acetylation with acetic anhydride and a trace of pyridine yielded O-monomethylporphyrilin monoacetate (VIII), needles from acetone, m. p. 254—257° after sintering from 245°. (Found: CH_3CO 13.1. $C_{18}H_{14}O_6$ requires CH_3CO 13.2.)

b) *Decarboxylation of O-monomethylporphyrilic acid (IV).* The methyl ether (IV) (50 mg) in formic acid (5 ml; $d = 1.22$) was boiled for two days and the solution was then filtered hot and evaporated to dryness. The product obtained, m.p. 305—310° after sublimation *in vacuo*, proved to be identical with the monomethyl ether (VII), obtained from porphyrilin, by its colour reactions, by paper chromatography and finally by conversion to its acetate, m.p. 250—255° (after sintering), undepressed on admixture with the acetate (VIII) described above. The decarboxylation could also be effected by sublimation of the acid (IV) *in vacuo*, although some decomposition then occurred.

Partial deacetylation of porphyrilin diacetate [2]. The diacetate (350 mg) in aniline (10 ml) was kept at room temperature with occasional stirring. The acetate soon dissolved and the crude monoacetate (IX) (120 mg) which gradually crystallised from the reaction mixture was filtered off after 24 h. It crystallised from acetone as needles, m.p. 227—229°, dried *in vacuo* at 150°. The monoacetate gave an intense blue reaction with ferric chloride in ethanol but no blue colour when tested with Gibbs' reagent in a borate buffer of pH 9. The equivalent weight was determined by titration in cold acetone with thymol blue as indicator. (Found: equiv. weight 310. $C_{17}H_{12}O_6$ requires equiv. weight 312.3.)

A small sample of the monoacetate was methylated with ethereal diazomethane yielding a product of m.p. 255—258° which by mixed melting point determination proved identical with the derivative (VIII) described above.

Partial oxidation of O,O-dimethylporphyrilic acid [2]. The dimethyl ether (1.5 g, 4.5 mmole) in 0.2 N potassium hydroxide solution (100 ml) was boiled for 10 min. to hydrolyse the lactone ring. After dilution with water (700 ml) the solution was warmed on the boiling water bath and 0.06 M potassium permanganate solution (130 ml, a 30 % excess of the amount calculated for oxidation of the carbinol group) added dropwise with rapid stirring during 10 h. After filtration, the solution was acidified with conc. hydrochloric acid (10 ml) and heated to boiling, when a yellow crystalline material separated. It was filtered off (1.0 g) and dried *in vacuo* at 180° to complete the formation of the anhydride. The desired anhydride of the tricarboxylic acid (A) proved difficult to isolate in a pure state but after short treatment of the reaction product in acetone suspension with an excess of ethereal diazomethane, the monomethyl ester of the anhydride (B) could be isolated from the reaction product by extraction with hot methyl ethyl ketone. It was obtained in a pure state as yellow needles, (0.3 g), m. p. 286—292°, by recrystallisation from the same solvent. The equivalent weight was determined by titration in aqueous pyridine with thymol blue as indicator. (Found: C 61.3; H 3.80; OCH_3 24.5 equiv. weight 187. $C_{19}H_{14}O_8$ requires C 61.6; H 3.81; OCH_3 25.1; equiv. weight (as a dibasic acid) 185.1.)

Demethylation and decarboxylation of acid (A). The methyl ester monoanhydride (B) of acid (A) (0.24 g) was boiled in a nitrogen atmosphere with hydrobromic acid (25 ml, $d = 1.5$) for two days. After cooling, the crude monocarboxylic acid (C) (0.16 g) was collected by filtration and purified by precipitation with hydrochloric acid from a bicarbonate solution. The acid did not crystallise well but was obtained as a microcrystalline brownish powder, reasonably pure as shown by chromatography on buffered paper (Na_3PO_4; $R_F = 0.45$). The spot which showed a greenish white fluorescence in ultraviolet light gave a red colour with *bis*-diazotised benzidine and an intense blue colour with Gibbs' reagent. A small sample of the acid was purified *via* the methyl ester (see below) and obtained as yellowish needles, m. p. 265—270°, by sublimation *in vacuo* (1 mm, bath-temperature 250—260°). It gave no distinct colour with ferric chloride. (Found: equiv. weight 263. $C_{14}H_{10}O_5$ requires equiv. weight 258.2.)

The methyl ester, obtained by short treatment of the crude acid in ice-cold acetone solution with ethereal diazomethane, crystallised as yellowish prisms from ethanol and

was finally sublimed *in vacuo*. It gave no colour with ferric chloride and showed m. p. 232–235°. (Found: OCH_3 12.1. $C_{15}H_{12}O_5$ requires OCH_3 11.4.)

Decarboxylation of the acid (C). The crude acid (C) (0.13 g) in quinoline (2.5 ml) was boiled for 2 h with a copper chromite catalyst (25 mg). The reaction mixture was diluted with ether, filtered and the insoluble material washed with ether. The combined ether solutions were washed with 4 N sulphuric acid, bicarbonate solution and water, dried with sodium sulphate and evaporated to dryness, yielding the crude phenol (D) as an oil (0.11 g) which crystallised after distillation *in vacuo*. It was crystallised from methanol-water and finally from benzene, yielding prisms, m. p. 165–166°, undepressed on admixture with synthetic 1,7-dihydroxy-3-methyldibenzofuran (X), described below. The two phenols also showed the same R_F-values (0.20; solvent system benzene-water) and gave identical colour reactions: *bis*-diazotised benzidine, red, Gibbs' reagent, blue, and ferric chloride, no colour. (Found: C 72.3; H 4.45. $C_{13}H_{10}O_3$ requires C 72.9; H 4.70.)

The phenol was further characterised by its diacetate which crystallised from methanol in needles, m. p. 129–130°, undepressed on admixture with synthetic 1,7-diacetoxy-3-methyldibenzofuran (XI), m. p. 129–130°. (Found: C 68.6; H 4.70. $C_{17}H_{14}O_5$ requires C 68.5; H 4.73.)

1,7-Dihydroxy-3-methyldibenzofuran. 4-Iodo-3,5-dimethoxytoluene [21] was prepared *via* 4-nitro-orcinol obtained in 5–6 % yield by nitration of orcinol in ether solution and subsequent steam distillation as described by Henrich and Meyer [22]. Methylation of the crude nitro-orcinol with dimethyl sulphate and alkali yielded 4-nitro-3,5-dimethoxytoluene, m. p. 147–148° after crystallisation from ethanol (Posternak *et al.*[21] report m. p. 147–147.5°). It was hydrogenated in ethanol with Adams platinum oxide catalyst to 4-amino-3,5-dimethoxytoluene and the crude product (m. p. 60–62°; Posternak *et al.*[21] give m. p. 64–65°) was diazotised and transformed into 4-iodo-3,5-dimethoxytoluene as described by Posternak *et al.*[21]. After crystallisation from ethanol it showed m. p. 96–97°, in agreement with the value given by Posternak *et al.*[21].

4-Iodo-3,5-dimethoxytoluene (1 g), 4-iodoresorcinol dimethyl ether [23] (9 g) and copper bronze (30 g) were thoroughly mixed and the reaction carried out in an analogous manner to that described in an earlier paper [2]. It proved convenient to extract the reaction product in a Soxhlet apparatus with light petroleum (b. p. 40–60°) for 12 h. In contrast to acetone, this solvent did not extract much resinous by-product, nor the symmetrical 4,4′-dimethyl-2,6,2′,6′-tetramethoxydiphenyl[21] (m. p. 145–146°) which should be formed in minute amounts. The petroleum extract deposited an oil which soon crystallised (3.4 g; m. p. 80–105°). All attempts to isolate the desired unsymmetrical diphenyl derivative in the pure state were unsuccessful.

The mixture of diphenyls (3.0 g) was boiled for 7 h under reflux with hydrobromic acid (60 ml; $d = 1.5$) in a carbon dioxide atmosphere. After cooling overnight in the refrigerator, the deposited crystalline material was collected, washed with water and dried *in vacuo* (yield, 1.9 g). Paper chromatography of the crude product (solvent system: benzene-water) showed one spot (grey colour with Gibbs' reagent) of 3,7-dihydroxydibenzofuran [7], $R_F = 0.07$, and a second spot, $R_F = 0.20$, which gave a blue colour with Gibbs' reagent, as expected for 1,7-dihydroxy-3-methyldibenzofuran. This second spot was in all respects identical with the spot from the phenol (D) described above. On extraction of the crude product with benzene in a Soxhlet apparatus a considerable enrichment of 3,7-dihydroxydibenzofuran in the crystallisate obtained was achieved. The unsymmetrical compound could be isolated from the mother liquor by chromatography on thick filter paper or more conveniently on alumina. The column was constructed in benzene using aluminium oxide (10 g) deactivated with nitric acid [24]. The phenol mixture (200 mg) in benzene (150 ml) was adsorbed on the column and eluted with benzene (750 ml). The fractions (25 ml) were tested with Gibbs' reagent and if necessary analysed by paper chromatography. Ca. 50 mg of chromatographically pure 1,7-dihydroxy-3-methyldibenzofuran (X) was obtained from the fractions Nos. 10–25. It was crystallised from methanol-water, and finally obtained from benzene as prisms, m. p. 166–167°. (Found: C 73.0; H 4.74. $C_{13}H_{10}O_3$ requires C 72.9; H 4.70.)

1,7-Diacetoxy-3-methyldibenzofuran (XI) was obtained from the above phenol by the action of acetic anhydride and pyridine and crystallised from methanol in needles, m. p. 129–130°. (Found: C 68.5; H 4.67. $C_{17}H_{14}O_5$ requires C 68.5; H 4.78.)

Paper chromatography of porphyrilin. The R_F-values of porphyrilin obtained on buffered papers were as follows: With 0.1 M phosphate buffer, pH 8.9: 0.78; pH 10.0: 0.70; pH 10.6: 0.60, and with 0.1 M borate buffer, pH 9.1: 0.55; pH 9.8: 0.40.

Titration of porphyrilin (VI). a) *Before hydrolysis:* The lactone, dissolved in 90 % aqueous acetone or in 90 % aqueous pyridine, was titrated in the cold with 0.05 N aqueous sodium hydroxide, using thymol blue as indicator. (Found: equiv. weight 275 ± 10. $C_{15}H_{10}O_5$ requires equiv. weight 270.2.)

b) *After hydrolysis:* Porphyrilin (40 mg) in 0.1 N sodium hydroxide solution (6 ml) and pyridine (10 ml) was hydrolysed on the boiling water bath for 1 h and back titrated with 0.1 N hydrochloric acid (thymolphthalein). The sample of porphyrilin used gave a yellow solution with alkali (*cf.* [2]) and the end point was somewhat indefinite. (Found: equiv. weight 150 ± 20. $C_{15}H_{10}O_5$ requires equiv. weight 135.1 (as a dibasic acid).)

Titration of porphyrilic acid (III) after hydrolysis. The acid was hydrolysed for 1 h at 90° with an excess of 0.05 N sodium hydroxide solution and back titrated with 0.02 N acid (thymolphthalein). (Found: equiv. weight 109. $C_{16}H_{10}O_7$ requires equiv. weight 104.7 (as a tribasic acid).)

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