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Studies of Mono-C-Methylquinalizarins in Relation to a Phenolic Metabolite of *Penicillium islandicum*

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A polyhydroxyanthraquinone obtained from a metabolite of *Penicillium islandicum* is shown to be a methylquinalizarin. Three isomeric methylquinalizarins have been synthesized. The isolated polyhydroxyanthraquinone has been shown to be identical with one of them, 1,4,7,8-tetrahydroxy-2-methylanthraquinone.

In an earlier paper [1] a new phenolic metabolite, called compound A, was isolated from the culture medium of *Penicillium islandicum*. As this compound is easily transformed to a polyhydroxyanthraquinone by treatment with concentrated sulfuric acid, the structure of compound A may have an interest in the view of the biogenesis of polyhydroxyanthraquinones.

Since compound A is available only in very small amounts, it has not been possible to make any derivatives or degradation experiments on it, other than the transformation to the hydroxyanthraquinone. A structural analysis of the polyhydroxyanthraquinone is apt to give some information about the unknown structure of compound A. The investigation of the structure of the anthraquinone obtained from compound A will be the subject of this paper.

The elementary analysis of the unknown anthraquinone indicates a tetrahydroxymethylanthraquinone (Found: C 61.8; H 3.9. $C_{15}H_{10}O_6$ requires: C 62.9; H 3.5).

The anthraquinone shows a weak fluorescence when dissolved in acetic acid. According to Raistrick *et al.*[2] this is a characteristic property of polyhydroxyanthraquinones with two hydroxyl groups in the *para* position to each other. The occurrence of hydroxyl groups in the *para* position is further established by the strong absorption in the region 490—520 mμ which the anthraquinone shows in ethanolic solution.

When zirconium nitrate in diluted hydrochloric acid is added to an acetone solution of the anthraquinone, a blue colouration is obtained which is the usual behaviour of a polyhydroxyanthraquinone with two hydroxyl groups in the *ortho* position [3].

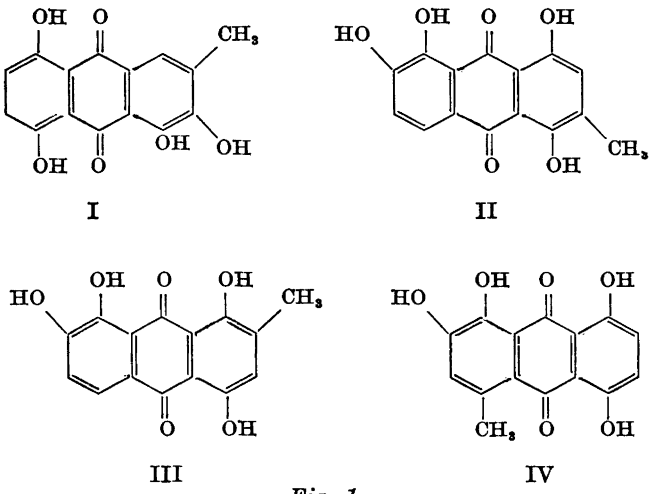

Fig. 1.

It is well known that a polyhydroxyanthraquinone with one hydroxyl group in β-position is soluble in 1 N Na_2CO_3 and there are several examples that polyhydroxyanthraquinones with more than one β-hydroxyl group is soluble in 0.5 N $NaHCO_3$. As reported in an earlier paper [1] the unknown anthraquinone is soluble in 1 N Na_2CO_3 but insoluble in 0.5 N $NaHCO_3$ which would then suggest one β-hydroxyl group in the molecule.

If the conclusions from the reactions above are true the unknown anthraquinone has to contain two hydroxyl groups in the *para* position and two hydroxyl groups in the *ortho* position. Of the latter one occupies an α-position and the other a β-position. These positions of the hydroxyl groups leave two possible alternatives. The pairs of *ortho* and *para* hydroxyl groups are either located in different rings (quinalizarin type) or the arrangement may be that of one hydroxyl group in one ring and three in the other (purpurin type). That the former alternative is the most probable is shown by two further properties of the unknown anthraquinone.

Polyhydroxyanthraquinones with hydroxyl groups in *para* position are oxidized by lead tetraacetate in glacial acetic acid to coloured diquinoid compounds. All polyhydroxyanthraquinones with *para* hydroxyl groups tested form a rather stable colouration with this reagent except for purpurin whose colour disappears rapidly since it is very easily oxidized. The unknown anthraquinone gives a stable colouration indistinguishable in appearence from that of quinalizarin. The relation of the unknown anthraquinone to quinalizarin rather than to purpurin is further indicated by the similarity of the absorption spectra of the former two compounds (Fig. 4).

As a working hypothesis the unknown anthraquinone is supposed to be a C-methylquinalizarin. Its structure is further proven by synthesis as described below.

There are theoretically four different isomers of methylquinalizarin (Fig. 1).

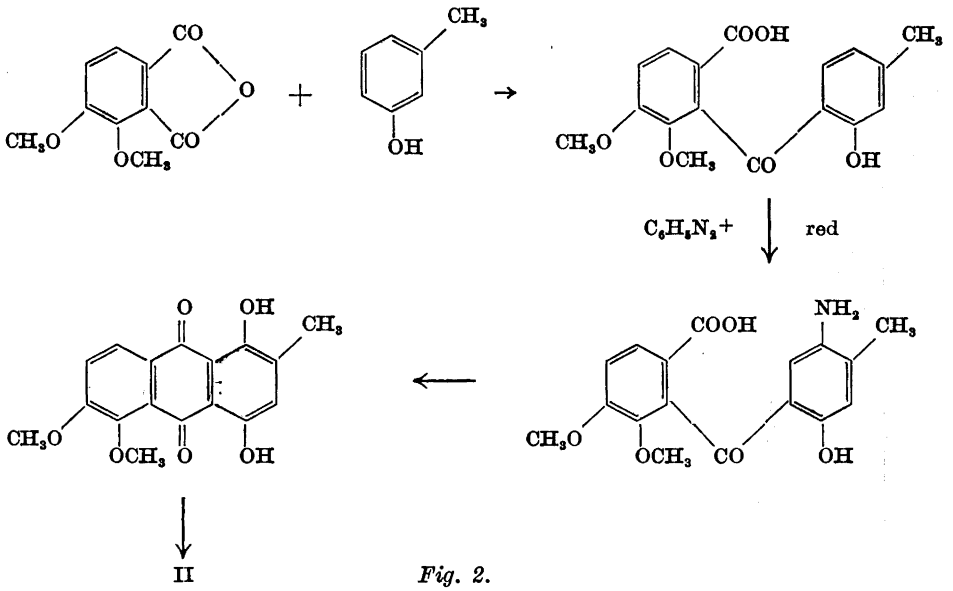

Fig. 2.

Of these four possibilities, structure IV is considered as less probable since a hydroxyanthraquinone with a C-substituent in an α-position has never been found among the mold pigments. The other three methylquinalizarins have been synthesized and their properties compared with those of the unknown anthraquinone.

I was synthesized by condensing 3,6-dihydroxyphthalic anhydride and 3-methylpyrocatechol with $AlCl_3$. I has m. p. 280—282°C and forms wool-like crystals from toluene.

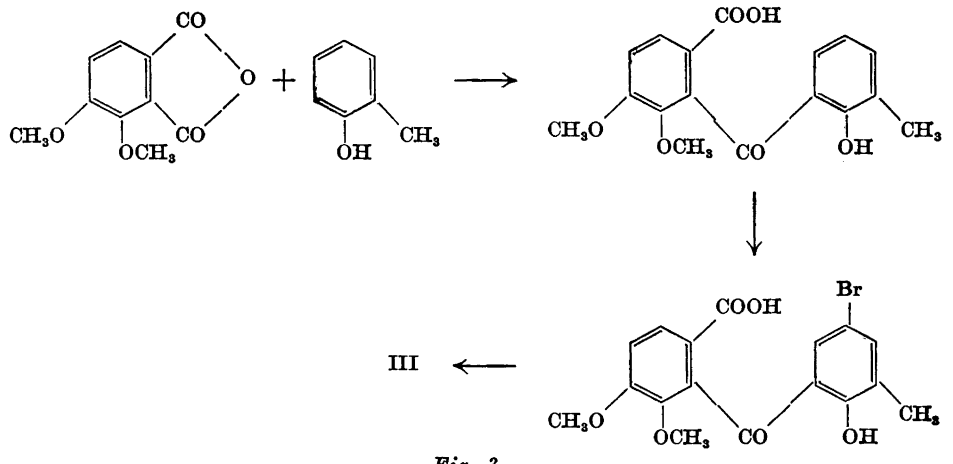

Fig. 3.

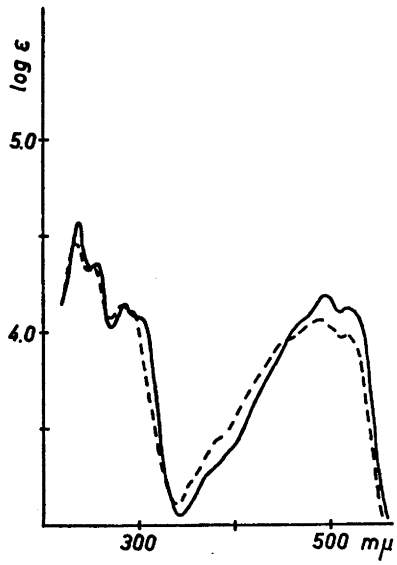

Fig. 4. Molecular extinction curves of quinalizarin — — — — — and II, III, the unknown anthraquinone ————.

II was prepared according to the reaction series in Fig. 2. It has m. p. 230°C and crystallizes in small plates from toluene.

III has been synthesized as indicated in Fig. 3 in very poor yield. It was easily obtained by a direct condensation of hemipinic anhydride and toluhydroquinone with $AlCl_3$, followed by demethylation with hydrobromic acid. III has m. p. 255°C and gives red needles when crystallized from toluene.

The unknown anthraquinone melts at 255°C and forms red needles when crystallized from toluene. The melting point of the unknown anthraquinone was not depressed on admixture with III.

II, III and the unknown anthraquinone have the same R_F value (0.75) on paper chromatogram developed in benzene-formic acid (2 %) (10:1). They have identical color reactions with methanolic magnesium acetate (blue), ethanolic ferric chloride (blue violet) and zirconium nitrate in diluted hydrochloric acid (light blue). They have practically identical absorption in UV and visible light (Fig. 4). The colors produced by I in the reactions mentioned had a tinge of red compared with those produced by the other compounds. The absorption curve of I shows small deviations from the others.

The data presented indicate the structure of the unknown anthraquinone to be identical with III.

The difference in structure of the identified anthraquinone and compound A should apparently be one molecule of water. Addition of one molecule of water to the anthraquinone gives theoretically rise to several possibilities of the structure of compound A, *e. g.* benzoylbenzoic acids, hydratized anthraquinones and naphthoquinone derivatives. These possibilities will be further investigated.

EXPERIMENTAL

Preparation of the unknown anthraquinone. Compound A was treated with concentrated H_2SO_4 (*d*, 1.84) for half an hour at room temperature. The violet sulfuric acid solution was poured onto crushed ice. The anthraquinone formed a red precipitate. After extraction with ether and evaporating the ether extract to dryness the anthraquinone was sublimed in vacuum at 150—160°C (bath temperature). The sublimate was recrystallized from acetic acid and toluene until a constant melting point (255°C) was obtained.

Synthesis of I. An intimate mixture of 0.6 g of 3,6-di-hydroxyphthalic anhydride and 0.5 g of 3-methylpyrocatechol was added in portions to 10 g of anhydrous $AlCl_3$ and 3 g of NaCl and fused together at 160°C. The temperature was kept at 160°C for 10 min after the last portion had been added. After cooling, the melt was boiled with diluted HCl for 15 min and the anthraquinone extracted with ether. The residue obtained after evaporation of the ether extract was sublimed in vacuum at 170—180°C (bath temperature). The anthraquinone was recrystallized from acetic acid and toluene (m. p. 280—282°C). (Found C 62.0; H 3.9. Calc. C 62.9; H 3.5.) At the condensation described no isomer anthraquinone was formed which was tested by paper chromatography.

The tetraacetyl derivative was prepared by boiling the anthraquinone for 2 min with acetic anhydride and a few drops of concentrated H_2SO_4. The acidic solution was poured onto crushed ice and the tetraacetylanthraquinone isolated by filtration and recrystallization from acetic acid, m. p. 208°C.

Synthesis of II. 3,4-dimethoxy-2(2-hydroxy-4-methylbenzoyl)-benzoic acid was synthesized as described by Simonsen [4].

Keeping the temperature below +5°C, a diazonium solution, prepared in the usual way from 0.852 g of aniline, was added with stirring to 2 888 g of the benzoylbenzoic acid dissolved in 50 ml of 1 N NaOH. The temperature and stirring were maintained for one hour, after which the solution was acidified with concentrated HCl. The precipitated yellow brown azocompound was filtered off, washed with water and dried at 100°C. It was recrystallized from benzene. Yield 2.4 g, m. p. 239°C.

To 2.4 g of the azocompound dissolved in 100 ml of ethanol, 8 g of solid sodium hydrosulphite was added. After heating the suspension to boiling, 300 ml of water was slowly added. The colour of the red solution faded rapidly and a lemon yellow solution was obtained. The amine precipitated in light yellow crystals on cooling the solution. (Found: N 3.9. Calc. N 4.2.) Yield 1.7 g, m. p. 148—250°C.

In a mixture of 4 ml of concentrated H_2SO_4 and 0.5 ml of water, 0.632 g of the amine was dissolved at 0°C. An equivalent amount of solid $NaNO_2$ was added in portions to the sulfuric acid solution. The reaction mixture was kept cold for half an hour at which time the solution became almost solid. Further 15 ml of concentrated H_2SO_4 were added and the temperature was raised to 150°C during half an hour and kept constant until the nitrogen evolution had ceased. After cooling to room temperature, the solution was poured onto crushed ice. The anthraquinone was filtered off and washed with water. The yield was almost quantitative. The air-dried anthraquinone was demethylated by refluxing for 12 h with a mixture of 20 ml of aqueous HBr (48 %) and 60 ml of acetic acid. The acetic acid was driven off in vacuum and the anthraquinone filtered off and washed with water. After repeated recrystallization from acetic acid and toluene it had m. p. 230°C. (Found: C 63.1; H 3.7. Calc. C 62.9; H 3.5.)

The tetraacetyl derivative was prepared in the same way as described for I, m.p. 210—212°C.

Synthesis of III. III was synthesized in two ways.

3,4-Dimethoxy-2(2-hydroxy-3-methylbenzoyl)-benzoic acid was prepared according to Jacobson and Adams [5].

In 15 ml of acetic acid, 0.709 g of the benzoylbenzoic acid (m.p. 251°C) was dissolved and then 0.115 ml of Br_2 in 5 ml of acetic acid added in small portions at 60°C. The reaction mixture was kept at 60°C for two hours and then at room temperature over night. The acetic acid was distilled off in vacuum and the residue recrystallized twice from methanol-water, m.p. 238°C.

Treatment of the bromocompound with a mixture of boric acid and concentrated H_2SO_4 at 150°C for 3 h resulted in a brown solution. III could be isolated, in very poor yield, by pouring the sulfuric acid solution on ice and extracting the water solution with ether. The ether extract was developed on a thick paper chromatogram (Whatman 3

MM) in benzene-formic acid. The anthraquinone was extracted from the paper with acetone. The acetone solution was evaporated to dryness and the anthraquinone recrystallized from acetic acid, m. p. 255°C. The amount of pure anthraquinone obtained in this way was too small for characterization.

A more convenient method for the preparation of III is to condense 0.5 g of hemipinic anhydride and 0.5 g of toluhydroquinone using the same procedure as described for the preparation of I. The demethylated product was recrystallized several times from acetic acid and toluene. Yield 0.09 g, m. p. 255°C. (Found: C 62.8; H 3.7. Calc. C 62.9; H 3.5.)

The tetraacetyl derivative was synthesized as described above, m. p. 208–210°C.

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