The Chemical Constitution of Rubrofusarin

Part II. Alkaline Degradation Studies

By Hiroshi Tanaka, Yasunori Ohne, Noboru Ogawa and Teiichi Tamura

Faculty of Agriculture, Nagoya University, Anjo, Aichi

Received September 6, 1962

Alkaline degradation of rubrofusarin and nor-rubrofusarin were studied; nor-rubrofusarin readily underwent hydrolysis to give a tetrahydroxynaphthalene, acetone, and acetic acid; whereas, rubrofusarin, after prolonged time of hydrolysis, yielded a \( \beta \)-methoxytrihydroxynaphthalene instead of the naphthol. Physical and chemical studies revealed that the naphthol is 1,3,6,8-tetrahydroxynaphthalene and it has been confirmed by the synthesis from chromotropic acid (disodium salt). Thus, evidently, rubrofusarin has a naphthalene nucleus to which a methoxyl group is attached at \( \beta \)-position. The formation, on the hydrolysis, of acetone and acetic acid, along with the naphthol, indicates the presence of 2-methyl-\( \gamma \)-pyrone structure in rubrofusarin.

In the former report\(^1\), on the evidence from zinc dust distillation, we proposed that the main nucleus of rubrofusarin, a pigment of \( \textit{Fusarium graminearum} \), is not the so far proposed xanthone but naphthalene. In this paper the results of alkaline degradations on the pigment and the nor-compound, that confirm our finding, are shown.

Alkali fusion is a generally accepted method for the structural elucidation of xanthone derivatives. Its repeated applications on our compounds, however, failed to give any definite products owing to the instability of the degradates in the air. Thus, the fusion on the nor-compound only gave an amorphous red compound as an acidic substance in poor yield. It has a violet ferric reaction; and reductive acetylation gave colorless compound, which shows a ultraviolet absorption spectrum resembling that of polyacetoxynaphthalene. On the fusion nor-rubrofusarin is supposed to be degraded to a naphthol sensitive to aerial oxidation. This assumption is in well accord with the fact that the main zinc dust distillate of rubrofusarin is naphthalene. In order to obtain the naphthol in better yield we preferred mild conditions, alkaline degradation in an atmosphere of nitrogen. By the refluxing of the alkaline solution of the nor-compound, acetone was gradually evolved and collected as the 2,4-dinitrophenylhydrazone. On acidification, the digested solution gave a nearly colorless precipitate (Ia), assumed to be identical with the naphthol-like compound obtained by the alkali fusion described above. Presence of acetic acid in the alkaline degradates was confirmed by paper chromatography of the hydroxamic acid. The precipitate (Ia) is unstable in the air and the attempts to purify by recrystallization were failed. The infrared spectrum shows no carbonyl band, which was shown in the original compound (\( \nu_{\text{C}=\text{O}} \sim 1660 \)), indicating that on hydrolysis the carbonyl group in nor-rubrofusarin is liberated from the

\(^1\) H. Tanaka and T. Tamura, \textit{This Journal}, \textbf{26}, 767 (1962).
The Chemical Constitution of Rubrofusarin 49

naphthalene nucleus. Ia gives a brown ferric reaction and was shown to increase the acidity of boric acid by 2.4 pH units. It is resistant to full methylation with diazomethane and gives a trimethoxynaphthol (Ib); acetylation of Ib with acetic anhydride-pyridine yields the monoacetate (Ic). Through these reactions the orientation of the hydroxyl groups in peri position was established. Acetylation of Ia with acetic anhydride and pyridine afforded a tetraacetate, $C_{18}H_{16}O_8$ (Id), which was purified by chromatography on calcium hydrogen phosphate. By degradation studies on this compound, further information about the orientation of the other two hydroxyl groups in the naphthol, Ia, were obtained. By chromic acid oxidation and subsequent methylation with dimethyl sulfate, Id gave rather unexpected 3,5-dimethoxybenzoic acid instead of an expected phthalic acid. Paper chromatography indicated the presence, in the oxidation products, of an o-hydroxyphthalic acid, but it was not isolated. Decarboxylation had probably occurred during oxidation process or by the procedure for methylation with dimethyl sulfate. 3,5-Dihydroxyphthalic acid was obtained by ozonolysis of the acetate, Id. The acid was verified by conversion with diazomethane to the dimethyl ether (Fig. 1).

FIG. 1. Infrared Spectra of 3,5-dimethoxyphthalic anhydride.
1, natural; 2, synthetic.

Aerial oxidation of the naphthol readily underwent with decrease of a bluish-green fluorescence; the alkaline solution immediately discolored to violet and in several hours the color reached to maximum. On acidification the colored solution gave a red quinone. Though attempts to crystallize this quinone were unsuccessful, it is assumed from its ultraviolet absorption spectrum to be identical with flaviolin$^2$) (3,6,8-trihydroxynaphthoquinone) (II), a pigment from Asp. citricus. From the above evidence, the naphthol, Ia, is assumed to be 1,3,6,8-tetrahydroxynaphthalene and this has been confirmed by synthesis: Alkali fusion of chromotropic acid (disodium salt) was reported to undergo ring fission to give 2,4-dihydroxybenzoic acid$^3$). We have succeeded in obtaining the naphthol by carrying out the fusion in an atmosphere of nitrogen using an alkali mixture (Ba(OH)$_2$·8H$_2$O, NaOH, and KOH). Without isolation, it was directly converted to the acetate, Id, by acetic anhydride and pyridine. It is identical with the natural acetate (Figs. 2, 3). Hydrolysis and subsequent methylation with alkaline dimethyl sulfate yielded 1,3,6,8-tetramethoxynaphthalene (Ie) (Chart I).

Now, the four oxygen atoms out of five in rubrofusarin can be disposed to be on the naphthalene nucleus.

Rubrofusarin has a methoxyl group and its

position was assigned to be $\beta$ as follows: alkaline degradation of rubrofusarin required prolonged time of refluxing and gave a methoxytrihydroxynaphthalene (II) with evolution of acetone. Acetylation of the methoxyl compound yielded a triacetate (Ig), the ultraviolet absorption of which (Fig. 4) shows a considerable bathochromic shift due to replacing of an acetoxyl group by a methoxyl group comparing with that of the tetraacetoxynaphthalene (Fig. 2). Fig. 4 shows that Ig has a $\beta$-naphthol type ultraviolet absorption spectrum due to the methoxyl group. Evidently the methoxyl group in rubrofusarin is attached on naphthalene nucleus in $\beta$-position. The above reactions are shown in Chart II.

Eleutherinol4) (III), isolated as a naturally occurring naphthopyrone from Eleutherine bulbosa, is known to undergo a retroaldol reaction when treated with alkali. This behaviour is similar to that of rubrofusarin, in-

---

The Chemical Constitution of Rubrofusarin

indicating the presence of 2-methyl-γ-pyrone structure. This is supported by the fact\(^5\) that rubrofusarin has an unreactive carbonyl group (ν\(_c=0\) 1660) and oxygen atom along with a C-methyl group.

Thus, the structure of rubrofusarin is assumed to be either IV or V.

Some other structures of 2-methylnaphtho-γ-pyrone may also satisfy the above reactions but can be left out of consideration because of the following reasons; (a) rubrofusarin has a comparatively unreactive hydroxyl group\(^5\), indicating the presence of hydrogen bond; (b) zinc dust distillation of rubrofusarin gives anthracene along with naphthalene\(^1\).

It is interesting that both the structure IV and V are in well accord with acetate theory and composed from head to tail linkage of seven acetic acid units.

---

EXPERIMENTAL

Paper chromatography (ascending) was performed on Toyo filter paper No. 50 with the following solvent systems (upper layer): (a) butanol-pyridine-sat. NaCl (1:1:2); (b) butanol-AcOH-water (4:1:5). Calcium hydrogen phosphate for column chromatography was activated by "dehydration" before use.

Infrared and ultraviolet absorption spectra and melting points are determined, otherwise stated, under the same conditions described in Part I.

Alkali Fusion of Nor-rubrofusarin. In a nickel crucible, protected free from air in an atmosphere of nitrogen, nor-rubrofusarin (220mg) was heated with KOH (5g) and 1ml of water to melt at 240—250°C (bath temperature) for half an hour. The sample reacted readily and developed a dark red color. The melt was cooled and dissolved in water (50ml). After acidification with dil. sulfuric acid (1:4), product was extracted with ether. The red ethereal extracts showed two spots; RF(a) 0.75 (red) and 0.62 (dark green) on paper chromatography. A part of this solution was evaporated to dryness. The residue having acidic odor contained red semicrystals and a black amorphous substance. The former, having no definite melting point, was soluble in benzene and ethanol. The ethanolic solution gave a violet ferric reaction and almost identical ultraviolet absorption spectrum (λmax 225, 270, 315, 375, and 440mμ) with that of flaviolin. The latter was insoluble in benzene but soluble in alcohol to give a dark green solution and showed a violet ferric reaction. It was probably derived from the former by further oxidation. To above ethereal extracts were added acetic anhydride (10ml), pyridine (10ml) and zinc dust (1g); the mixture was gently warmed to expel the ether and effect the reductive acetylation. The solution after overnight standing was poured onto ice-water to give precipitate. It was collected and chromatographed on calcium hydrogen phosphate in benzene. The eluted solution, on evaporation of the benzene and recrystallization from dil. alcohol, gave colorless needles, m.p. 123°C, in poor yield. λmax 228 mμ (E1% 1655) and 285 mμ (E1% 177). This acetate is supposed to be identical with 1,3,6,8-tetraacetoxynaphthalene.

Alkaline Degradation of Nor-rubrofusarin. In an apparatus for the micro acetyl determination, the sample (500mg) was gently refluxed with 20% sodium hydroxide sol. (20ml) at about 145°C (bath temperature) for 1.5 hrs. at the presence of zinc dust (1g). During this operation, aerial oxidation was avoided by passing a slow stream of nitrogen gas from a tube to the bottom of the flask serving to prevent the mixture from bumping. The effluent gases from the top of the condenser were passed through a 2,4-dinitrophenylhydrazine solution (1g in 150ml of 1:4-HCl). Emerged water accompanied with nitrogen gas was supplied from time to time. In ten minutes of refluxing, most of the compound was dissolved with week foaming and at the trap, precipitation of orange-yellow hydrazone began to occur. The most of the hydrazone produced was precipitated during the next 20 minutes. At the end of hydrolysis it was collected and purified through acid-washed alumina in benzene. Recrystallization from dil. alcohol gave orange-yellow plates, m.p. 123.5°C (135mg, 0.29M). Mixture melting point with the hydrazone of acetone showed no depression.

The alkaline solution, after hydrolysis, was cooled (in a stream of nitrogen) and decanted free from zinc dust into a mixture of ice (about 50g) and dil. sulfuric acid (1:4, 50ml). A pale yellow semicrystalline precipitate, Ia, formed was collected on a glass filter and washed twice with each 5ml of cold water and dried (yield 305mg). To detect any volatile acid in the filtrate, it was steam distilled; the distillate was neutralized by 0.1 N-NaOH (about 14ml, 0.7m) and evaporated to dryness. The residue was converted to a hydroxamic acid; on paper chromatography the acid showed RF(b) 0.58, corresponding to that of acetic
The Chemical Constitution of Rubrofusarin

acid. Thus, the presence of acetone and acetic acid in hydrolysates of nor-rubrofusarin is evident.

The Properties of the Precipitate (Ia). Freshly prepared compound was semicrystalline and did not melt even at 250°C (showed some decomposition). Attempts to purify it by recrystallization were unsuccessful. Thus, the above crude compound was used for the following reactions; it has a brown ferric reaction and showed an apparent acidity constant, 5.7, due to phenolic hydroxyl group. On paper chromatography it gave a red spot, \( R_F \) 0.77, showing an intense violet ferric reaction. The spot was identical with that of the aerial oxidized product of Ia. This means that Ia was oxidized instantly during the procedure for chromatography. A colorless alcoholic solution of Ia shows an intense greenish-blue fluorescence on addition of a drop of sulfuric acid or alkali. The alkaline solution is very unstable in air and turns violet undergoing aerial oxidation.

Zinc duct distillation of Ia (5mg) gave naphthalene only (0.2mg). Effect of Ia on acidity of boric acid was determined under the conditions described by Hochstein et al. for terranaphthol; Ia (19.2mg) was dissolved in 1 ml of alcohol and added with an aq. boric acid (0.5 ml, 2.5 ml). The solution gave pH 2.03 (pH value of a blank was 4.42). The depression was, therefore, 2.59 pH units (Hochstein et al. reported 2.8 pH units for 1,8-dihydroxynaphthalene and terranaphthol.) and this indicates that Ia has hydroxyl groups in peri position. The ultraviolet spectrum shows \( \lambda_{max} \) 242.5, 306, 318, 327, and 332 \( \mu \) (log \( \varepsilon \) 4.68, 3.73, 3.76, 3.75, and 3.78 resp.). The infrared spectrum shows no carbonyl band nor carboxyl band.

Derivatives of the Naphthol.

a) Acetate (Id): The precipitate, Ia, from 500 mg of nor-rubrofusarin was directly treated with acetic anhydride (5 ml) and pyridine (3 ml) and heated for 3 hrs. on the steam-bath. After overnight standing, it was treated with ice (200 g). Colorless crystals appeared after having been stood several hrs. were collected, washed with water, and dried. The crude acetate was purified by chromatography on calcium hydrogen phosphate in benzene. Eluted solution was evaporated to dryness to yield a crystalline acetate, which was crystallized from dil. alcohol; long prisms, m.p. 131°C (380 mg). Recrystallization from the same solvent gave colorless prisms, m.p. 134°C. Anal. Found: C, 59.90; H, 4.79; Ac, 49.2. Calcd. for C_{18}H_{16}O_{8}: C, 60.00; H, 4.48; Ac, 48.0%. From these data, the acetate is assumed to be 1,3,6,8-tetraacetoxynaphthalene and this has been confirmed by comparison with the synthetic sample (Fig. 3).

b) Trimethyl Ether (Ib). By the procedure above described, nor-rubrofusarin (500 mg) was hydrolyzed with alkali and the acidified solution containing a precipitate of the naphthol was extracted with ether (3×100 ml). The ether extracts were dried over anhydrous sodium sulfate, and treated with ethereal diazomethane (from 2 g of nitromethylurea). After overnight standing it was evaporated to dryness to yield a colored residue. The residue was purified by chromatography on alumina in benzene, and then recrystallized twice from methanol to give 60 mg of pure trimethyl ether, colorless prisms, m.p. 114~114.5°C. Anal. Found: C, 66.70; H, 6.30; methoxyl, 39.5. Calcd. for C_{13}H_{14}O_{4}: C, 66.65; H, 6.02; methoxyl, 39.7%. The ultraviolet spectrum (Fig. 2) shows \( \lambda_{max} \) 243, 289, 299, 312, and 326 \( \mu \) (log \( \varepsilon \) 4.83, 3.65, 3.66, 3.61, and 3.45 resp.).

Dissolved in sulfuric acid, it forms a yellow solution with an intense blue fluorescence. Its ethanolic solution bearing a blue fluorescence gave negative test on treatment with ferric chloride solution. On acetylation in acetic anhydride-pyridine (on the steam-bath, for 4 hrs.), this compound yielded a monoacetate (Ic), which was recrystallized from dil. ethanol; colorless prisms, m.p. 106~107°C. The ultraviolet spectrum shows \( \lambda_{max} \) 241, 248, 287, and 297 \( \mu \). The CrCl Oxidation of the Tetraacetate (Id). A mixture of the acetate (100 mg), acetic anhydride (6 ml), and acetic acid (3 ml) was maintained at 70~80°C, and then 3 ml of a chromic acid solution (chromic acid 2.5 g, water 2 ml, and acetic acid 25 ml) was gradually added and kept at 90°C for 3 hrs. After standing overnight the oxidised green solution was diluted with 40 ml of water and extracted repeatedly with ether. The green fluorescent ether extracts were evaporated to dryness under reduced pressure. The residue was hydrolyzed with 5% sodium hydroxide, acidified, and reextracted with ether. Paper chromatography indicated the presence, in the ether extracts, of an acid, \( R_F \) 0.62, which had a blue fluorescence and a violet ferric reaction, and this acid was assumed to be 3,5-dihydroxyphthalic acid. The ether extracts, after evaporation of the ether, were methylated with

---

dimethyl sulfate (3 ml) in 10% sodium hydroxide (20 ml) at 95°C for 4 hrs. The mixture was then acidified and extracted with ether. The acid thus obtained was recrystallized from dil. alcohol and benzene; light orange prisms, m.p. 180°C (10 mg).

Titration showed apparent pKa value of 4.4, equivalent weight 188 (calcld. 182). Mixture melting point with an authentic sample, kindly supplied by Dr. Y. Hatsuda, showed no depression. The infrared spectrum with an authentic sample, kindly supplied by Dr. Y. Hatsuda, showed no depression. 3,5-Dimethoxypythalic acid was not isolated; it is assumed that a yield of the phthalic acid was too low to isolate or methylation with alkylide dimethyl sulfate was accompanied by decarboxylation.

Ozonolysis of the Tetraacetate (Id). The acetate (180 mg) was dissolved in chloroform (10 ml) and ozone (3%, 50 ml/min.) was passed for 5 hrs. at 0°C. The solution was then removed at reduced pressure and the ozonide was decomposed by addition of 10 ml of water and standing of overnight. The mixture was further hydrolyzed by heating for 2 hrs. on the steam-bath with 2 n-sodium hydroxide. The solution gave, by the usual way of separation, a colored oil as acidic fraction; paper chromatography of this product showed an intense blue-fluorescent spot, Rf 0.63, which gave a reddish violet ferric chloride test. Thus, the presence of a o-hydroxyphthalic acid is evident. The acid was characterized by conversion to a methyl ether; the viscous oil, so obtained, was dried in desiccator with anhydrous sodium sulfate, and evaporated to dryness to yield 77 mg of methoxyl acid. This acid was too low to isolate or methylation with alkylide dimethyl sulfate was accompanied by decarboxylation.

Aerial Oxidation of the Naphthol (Ia). An almost colorless solution of Ia (50 mg) in 2 n-sodium hydroxide sol. immediately discolored to violet on aeration. Aerial oxidation advanced with increase in violet color and decrease in bluish-green fluorescence.

After two hours' oxidation the solution was acidified with dil. sulfuric acid, extracted with ether, and the deep red extracts were washed with water, dried over anhydrous sodium sulfate, and evaporated to dryness to give an amorphous red quinone. Attempts to crystallize from several solvents were unsuccessful. On paper chromatography the quinone gave a red spot, Rf 0.74, with an intense violet ferric reaction. The ultraviolet spectrum is similar to that of flavinol (II), λmax 268, 313, 384, and 465 mμ. It is readily soluble in alkali to form a violet solution and assumed to be identical with flavinol.

Alkaline Degradation of Rubrofusarin. Finely powdered pigment (500 mg) was heated with 20% sodium hydroxide sol. (30 ml) under reflux for 6 hrs. by the procedure described for the nor-compound. It gradually dissolved in alkali evolving acetone; 80 mg of pure 2,4-dinitrophenylhydrazone was obtained through the hydrolysis. The digested solution was cooled under nitrogen atmosphere and poured on a mixture of dil. HCl (1:1, 100 ml) and ice (100 g). After having been stood in ice-box for one hour yellow precipitate of methoxynaphthalene (If) was filtered promptly and washed with cold water. The ultraviolet spectrum of If is almost similar with that of Ia; λmax 242, 315, and 330 mμ. It is also unstable in air and it was directly converted to an acetate (Ig) by treating with acetic anhydride-pyridine (4 ml, 4 ml) for 5 hrs. on the steam-bath. The crude acetate, thus obtained, was purified by chromatography on calcium hydrogen phosphate in benzene. The eluate was collected and the benzene was removed to yield a crystalline compound. Recrystallization from ether-hexane gave 447 mg of pure acetate (Ig); colorless prisms, m.p. 128°C. Anal. Found: C, 61.34; H, 4.96; methoxyl 10.2. Calcd. for C₉H₈O₄: C, 61.44; H, 4.85; methoxyl, 9.3%.

This triacetoxy methoxynaphthalene has a β-naphthol type ultraviolet absorption spectrum (Fig. 4), indicating the methoxyl group in rubrofusarin is attached in β-position on naphthalene nucleus; λmax 235, 282, 317, and 331 mμ (log ε 4.80, 3.69, 3.19, and 3.24 resp.). The methoxynaphthol, If, by methylation with diazomethane, gave a dimethyl ether, light pink prisms, m.p. 141~142°C, which was identical with the trimethyl ether, Ib, derived from nor-rubrofusarin.
Preparation of 1,3,6,8-Tetraacetoxynaphthalene (Ic).

In a nickel crucible (250 ml) with a copper stirrer, chromotropic acid (disodium salt, 3x6 g), sodium hydroxide (3x24 g), potassium hydroxide (3x24 g), and barium hydroxide (3x36 g) were placed; the mixture was covered with a slow stream of nitrogen, and was then heated in an oil bath. At about 220°C (oil bath), the contents began to melt with violent evolution of the steam. The melt was then kept at 255~260°C (oil bath) for 7 hrs. with occasional stirring.

The cooled melt was solidified, crushed to small yellow pieces, and added gradually into a mixture of dil. sulfuric acid (1:1, 400 ml) and ice (200 g). The turbid solution was repeatedly extracted with ether (1x300 and 3x200 ml), and the combined ether solutions were washed with water, dried over anhydrous sodium sulfate, and filtered. The naphthol, thus prepared, gave on paper chromatography a spot, RF(a) 0.74, identical with that of natural naphthol, Ia, and was directly converted to the acetate; the deep red filtrate was added with acetic anhydride (10 ml), and the ether was removed. After most of ether was evaporated, the residual solution was further treated with each 10 ml of acetic anhydride and pyridine, and the mixture was warmed on the steam-bath for one hour, and allowed to stand overnight. It was then treated with 300 g of crushed ice to yield a crystalline precipitate of the acetate. The pale yellow acetate was filtered, washed well with water, and dried. This acetate is contaminated by a yellow by-product, which is bluish-green fluorescent and assumed to be a dinaphthyl. The compound expected was purified free from the yellow compound by chromatography on calcium hydrogen phosphate in benzene. Evaporation of the solvent from the eluate and recrystallization from dil. alcohol gave 2.2 g (yield 12%) of pure 1,3,6,8-tetraacetoxynaphthalene in colorless prisms, m.p. 137°C, undepressed on admixture with the natural acetate. Anal. Found: C, 60.26; H, 4.64. for C₉H₆O₈: C, 60.00; H, 4.48%. The alkali fusion at a slightly higher temperature improved the yield but the rate of the yellow by-product was also increased and difficulties on separation were accompanied. The tetraacetate is readily soluble in most organic solvents but insoluble in petr. ether and water.

Methylation of the acetate with dimethyl sulfate and 10% sodium hydroxide, in a slow stream of nitrogen, on the steam-bath for 2 hrs. gave 1,3,6,8-tetramethoxynaphthalene (Ie), colorless prisms, m.p. 107°C, after purification through alumina in benzene and recrystallization from benzene-hexane (containing a trace of methanol). Anal. Found: C, 67.98; H, 6.95. Calcd. for C₁₄H₁₄O₄: C, 67.74; H, 6.50%.

The ultraviolet spectrum is practically identical with that of the trimethyl ether (Ib): λmax 242, 290, 299, 312, and 326 mμ (log ε 4.79, 3.69, 3.70, 3.58, and 3.42 resp.).