225. Shoji Shibata, Eisaku Morishita, and Yasuo Arima*1:
Metabolic Products of Fungi. XXV,*2 Synthesis of
Rubrofusarin and Its Derivatives.*3

(Faculty of Pharmaceutical Sciences, University of Tokyo*1)

Rubrofusarin, a metabolic product of *Fusarium culmorum* Sacc, and its methyl ethers were synthesized by the Claisen condensation of 2-acetylnaphthalene derivatives (XII, XV and XVIII), which were prepared starting from 2α-resorcylic acid. The structures of rubrofusarin monomethyl ether A and nor-rubrofusarin diacetate were established spectrometrically and synthetically.

(Received March 2, 1967)

In 1937, Raistrick, et al.,*3 isolated rubrofusarin, m.p. 210–211°, an orange red pigment from a plant pathogenic fungus, *Fusarium culmorum* (W.G. Smith) Sacc. The structure of this pigment was established as being 5,6-dihydroxy-8-methoxy-2-methyl-4H-naphtho[2,3-b]pyran-4-one (I) by Stout, et al.,*2 by the X-ray crystallographical method, and at almost the same time by Tamura, et al.*3 by the chemical degradation.

Afterwards Roberts, et al.*4 proposed a structure (II) for rubrofusarin monomethyl ether A, m.p. 203–204°, which was prepared by methylation of rubrofusarin with diazomethane. This has been deduced by the analogy of methylation of musizin (III) with diazomethane,*5 which contrary to usual expectation, methylates preferentially the hydrogen bonded hydroxyl adjacent to methylketone.

\[ \text{Chart 1.} \]

\[ \text{Chart 2.} \]

In the present paper we report the synthesis of rubrofusarin and its methyl ethers. An attempt to condense ethyl 1,3-dihydroxy-6,8-dimethoxy-2-naphthoate (V)*6 and its derivatives, VI, VII and VIII, with acetone by the Claisen reaction*7 was unsuccessful recovering the starting materials.

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The following process which involves 2-acetyl-naphthalene as an intermediate for the synthesis of rubrofusarin has been studied. By the Arndt-Eistert reaction, 3,5-dimethoxybenzoic acid chloride (\(V\)) was converted into 3,5-dimethoxyphenylacetic acid (\(X\)) whose chloride (\(X\)) was reacted with ethyl acetocetate by the Spassow\(^8\) or Claisen condensation to afford ethyl 2-(3,5-dimethoxyphenylacetoxyacetic acid (\(\Xi\)), which was characterized as the copper salt (m.p. 178~179\(^\circ\)). On vacuum distillation,\(^9\) \(\Xi\) was cyclized to afford 2-acetyl-6,8-dimethoxy-1,3-naphthalenediol (\(\Xi\I\)), m.p. 193~194\(^\circ\), whereas by the action of polyphosphoric acid at 100\(^\circ\), for 5 min., \(\Xi\) or its copper salt yielded ethyl 3-hydroxy-6,8-dimethoxy-1-methyl-2-naphthoate (\(\Xi\IV\)), m.p. 130~131\(^\circ\).

![Chemical diagram](image)

Chart 3.

The structures of \(\Xi\) and \(\Xi\IV\) were proved by the ultraviolet (UV), infrared (IR) nuclear magnetic resonance (NMR) spectra whose data are given in the following table.

**Table I.**

<table>
<thead>
<tr>
<th></th>
<th>(\Xi)</th>
<th>(\Xi\IV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV</td>
<td>(\lambda_{\text{max}}) ((\mu)) (log (\varepsilon))</td>
<td>(\lambda_{\text{max}}) ((\mu)) (log (\varepsilon))</td>
</tr>
<tr>
<td></td>
<td>231 (4.40), 278 (4.66), 314 (4.00), 245 (4.64), 250 (4.65), 295 (3.67)</td>
<td>326 (3.98), 385 (3.60)</td>
</tr>
<tr>
<td>IR</td>
<td>(\nu_{\text{max}}) cm(^{-1})</td>
<td>(\nu_{\text{max}}) cm(^{-1})</td>
</tr>
<tr>
<td></td>
<td>3330 (OH), 1645 (C=O)</td>
<td>3260, 3560 (OH), 1665, 1725 (C=O)</td>
</tr>
<tr>
<td>NMR</td>
<td>(\tau_{\text{CH3}})</td>
<td>(\tau_{\text{CH3}})</td>
</tr>
<tr>
<td>arom. H</td>
<td>-0.73 (H), -2.80 (H)</td>
<td>-0.55 (H)</td>
</tr>
<tr>
<td>-OCH(_3)CH(_3)</td>
<td>3.46(H), 3.55 (H, d, J=2 c.p.s.), 3.77 (H, d, J=2 c.p.s.)</td>
<td>2.97 (H), 3.44 (H, d, J=2 c.p.s.), 3.62 (H, d, J=2 c.p.s.)</td>
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<tr>
<td>arom. OCH(_3)</td>
<td>5.95 (3H), 6.14 (3H)</td>
<td>5.49 (2H, q, J=5.6 c.p.s.)</td>
</tr>
<tr>
<td>arom. CH(_3)</td>
<td>6.09 (6H)</td>
<td>7.06 (3H)</td>
</tr>
<tr>
<td>COCH(_3)</td>
<td>7.25 (3H)</td>
<td>8.57 (3H, t, J=5.6 c.p.s.)</td>
</tr>
<tr>
<td>-OCH(_3)CH(_3)</td>
<td>8.57 (3H, t, J=5.6 c.p.s.)</td>
<td></td>
</tr>
</tbody>
</table>

Partial acetylation of \(\Xi\) with acetic anhydride and anhydrous sodium acetate gave 3-acetoxy-2-acetyl-6,8-dimethoxy-1-naphthol (\(\Xi\VI\)) which was converted into \(\Xi\VII\) on meth-

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ylation, and XVIII by subsequent deacetylation. By the Claisen reaction, 2-acetyl-naphthalene derivatives (XIII), (XV) and (XVIII) with ethyl acetate afforded the corresponding 2-acetoacetylnaphthalene derivatives which were cyclized by the action of conc. HCl in methanol or HIO in acetic anhydride to form XX, XX, and XXI, respectively. The compound (XX) was proved to be identical with nor-rubrofusarin, m.p. 298-299° (decomp.) and XXI was identified as rubrofusarin dimethyl ether, m.p. 186-187°, by the comparison with the authentic specimens. By the comparison of the IR spectra and thin-layer chromatograms as well as by the mixed fusion, the product (XX) was proved to be identical with rubrofusarin monomethyl ether B, m.p. 213°, which was yielded by the partial methylation of rubrofusarin (I) with dimethyl sulfate in acetone. This result showed the correctness of the structure of rubrofusarin monomethyl ether A (II) proposed by Roberts.10

An attempt for preparing rubrofusarin (I) by the partial methylation of nor-rubrofusarin (XX) was unsuccessful, but it has been performed by the partial demethylation of rubrofusarin monomethyl ether A (XVII) prepared from XX with 5 N-hydrochloric acid. Thus the final product, 5,6-dihydroxy-8-methoxy-2-methyl-4H-naphtho[2,3-b]pyran-4-one (XXIII), m.p. 216°, has been established to be identical with the naturally occurring rubrofusarin (I) by the mixed fusion and the comparison of IR spectra and thin-layer chromatograms.

The results of this study and the synthesis of hydroxynaphthopyrone derivatives of angular and linear types carried out by Fukushima, et al.11 have shown that the Wessely-Moser rule12 which was generally adopted to flavonoid, chromones, and xanthones has also been applied to the naphthopyrone. Thus naphthopyrones having a free hydroxyl at the 10 position is stable in angular type, and others are stabilized to form linear type. The structure of nor-rubrofusarin diacetate13 which was remained ambiguous has now been established to be 6,8-diacetoxy-5-hydroxy-2-methyl-4H-naphtho[2,3-b]pyran-4-

one (XXIV) by the comparison of chemical and spectroscopical properties with rubrofusarin monomethyl ethers A (XXII) and B (XX). The result showed the presence of an enolic hydroxyl at C₁₀ which is strongly hydrogen bonded with the carbonyl of pyrone ring (Table II).

<table>
<thead>
<tr>
<th></th>
<th>XXIV</th>
<th>XXII</th>
<th>XIX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluorescence</td>
<td></td>
<td>yellow</td>
<td>—</td>
</tr>
<tr>
<td>FeCl₃ in EtOH</td>
<td></td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>5% NaOH</td>
<td>insoluble</td>
<td>soluble</td>
<td>insoluble</td>
</tr>
<tr>
<td></td>
<td>(orange red)</td>
<td>3770</td>
<td></td>
</tr>
<tr>
<td>IR νₓ max cm⁻¹</td>
<td>1775, 1650</td>
<td>1642</td>
<td>1655</td>
</tr>
<tr>
<td>(OH)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(C=O)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NMR νₓ max cm⁻¹</td>
<td>-4.70</td>
<td>0.05</td>
<td>-4.95</td>
</tr>
<tr>
<td>(OH)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

6-Monomethyl ether of nor-rubrofusarin (XXV) had been suggested by Stodola as the structure of fonsecin, m.p. 198° (decomp.), a pigment of *Aspergillus fumigatus*. At that time when we prepared the compound XX, it was considered to be identical with monomethyl ether of fonsecin, m.p. 176°, but XX showed remarkably different melting point (m.p. 213°).

Having doubt the Stodola's formula, we prepared an isomer of rubrofusarin, 5-monomethyl ether of nor-rubrofusarin (XXVII), m.p. 235°-237° (decomp.), as a possible formula of fonsecin by the methylation of XXIV followed by the decacylation with 10% H₂SO₄. Afterwards Stodola amended the formula of fonsecin as being 6-methyl ether of hydrated nor-rubrofusarin (XXVIII) by the NMR spectral analysis.

![Chemical structures](chart.png)

**Chart 5.**

### Experimental

**Ethyl 1,3-Dihydroxy-6,8-dimethoxy-2-naphthoate (IV) Derivatives** — 1) Diacetate: On acetylation with Ac₂O and pyridine, N afforded diacetate. Colorless needles (from EtOH), m.p. 137°-137.5°. Yield: 88%. *Anal.* Calcd. for C₁₇H₁₀O₄: C, 60.64; H, 5.32. Found: C, 60.88; H, 5.31. IR νₓ max cm⁻¹: 1770, 1720 (C=O).


3) Ethyl 3-hydroxy-1,6,8-trimethoxy-2-naphthoate (V): A mixture of N (0.7 g), Ac₂O (12 ml) and Ac₂O (0.1 g) was allowed to stand overnight at room temperature to form 3-monoacetate which was decrystallized from EtOH to pale yellow needles, m.p. 123.5°-124°. Yield: 0.72 g, 90%. *Anal.* Calcd. for C₁₇H₁₂O₇: C, 61.07; H, 5.39. Found: C, 61.29; H, 5.62. IR νₓ max cm⁻¹: 1760, 1640 (C=O). Ethyl 3-acetoxy-1,6,8-trimethoxy-2-naphthoate (V), which was obtained by methylation of the above 3-monoacetate (0.8 g) with etheral CH₂O, was warmed with 5% Ba(OH)₂ solution (30 ml) for 30 min. on a water bath. After

deacetylation, the solution was extracted with ether to remove dimethyl ether (V). The aqueous layer was acidified with 5% H$_2$SO$_4$ and extracted with ether. The ether extract was shaken with 5% NaHCO$_3$ to remove a little amount of free acid (3-hydroxy-1,6,8-trimethyl-2-naphthoic acid). It is pale yellow needles, m.p. 172–173° (decomp.) (from MeOH). Anal. Calcd. for C$_{18}$H$_{19}$O$_4$: C, 60.43; H, 5.04. Found: C, 60.51; H, 5.13. IR $\nu_{\text{max}}$ cm$^{-1}$: 2930 (OH), 1700 (C=O). Its methyl ether was yielded as pale yellow needles, m.p. 98–99° (from 50% MeOH) by usual method using an ethereal CH$_2$N$_2$. Anal. Calcd. for C$_{18}$H$_{21}$O$_4$: C, 61.64; H, 5.48. Found: C, 61.73; H, 5.65. IR $\nu_{\text{max}}$ cm$^{-1}$: 1673 (C=O). The solvent was evaporated and the residue was recrystallized from 50% EtOH to pale yellow needles, m.p. 95–96°. Yield, 0.42 g., 58%. Anal. Calcd. for C$_{18}$H$_{19}$O$_5$: C, 62.75; H, 5.88. Found: C, 62.66; H, 5.84. IR $\nu_{\text{max}}$ cm$^{-1}$: 1670 (non-chelated C=O).

Methyl 1,3,6,8-Tetramethoxy-2-naphthoate (VII)—The compound (VII) suspended in 20% NaOH and EtOH was refluxed at 120–130° for 3 hr. in a sealed tube. After hydrolysis, the solution was acidified to separate precipitates which were recrystallized from MeOH to colorless needles, m.p. 161° (decomp.). Yield: 89%. Anal. Calcd. for C$_{18}$H$_{21}$O$_4$: C, 61.64; H, 5.48. Found: C, 61.42; H, 5.46. IR $\nu_{\text{max}}$ cm$^{-1}$: 1700 (C=O).

Its methyl ester was obtained as colorless needles, m.p. 137–137.5° (from MeOH) by usual method using an ethereal CH$_2$N$_2$. Yield: 95%. Anal. Calcd. for C$_{18}$H$_{21}$O$_4$: C, 62.74; H, 5.88. Found: C, 62.78; H, 5.88. IR $\nu_{\text{max}}$ cm$^{-1}$: 1735 (C=O).

Attempted Preparations of 2-Acetoacetyl-3-hydroxy-1,6,8-trimethoxynaphthalene and Its Derivatives—The Caisen condensation of V or VII with acetonitrile was failed to react in the presence of NaH or Na, and also VII did not react by the method of Wawonek.}

3.5-Dimethoxybenzoyl Chloride (VIII)—3,5-Dimethoxybenzoic acid (10 g.)$^{14}$ was refluxed with SOCl$_2$ (14 g.) for 1 hr. Evaporation of the excess SOCl$_2$ and the residue was distilled in vacuo to give a pale yellow oil, b.p. 135°. Yield: 10 g. (91%), which was solidified under ice cooling to colorless needles, m.p. 30–32° (from ligroin). It was characterized as an amide, colorless needles, m.p. 141–142° (from EtOH).

3.5-Dimethoxynaphzyloiodoform (IX)—A solution of VIII (10 g.) in dry ether (30 ml.) was added dropwise to an ethereal CH$_2$N$_2$ (prepared from 18 g. of of N-nitosomethylurea) under ice cooling and vigorous stirring. After standing overnight, the solvent was removed under reduced pressure at room temperature, and finally at 30°. The crystalline yellow residue was recrystallized from benzene-light petroleum to pale yellow plate, m.p. 71–72°. Yield: 10 g. (91%), Anal. Calcd. for C$_{18}$H$_{20}$N$_2$: C, 58.25; H, 4.85; N, 13.59. Found: C, 58.48; H, 4.92; N, 13.75. IR $\nu_{\text{max}}$ cm$^{-1}$: 2130, 2100 (COCHN$_2$).

3.5-Dimethoxyphenylacetic acid (X)—To a mixture of freshly prepared AgNO$_3$ (4 g.), Na$_2$CO$_3$ (4 g.) and sodium thiosulfate (10 g.) in water (140 ml.), the solution of K (10 g.) in dioxane (40 ml.) was added slowly dropwise under vigorous stirring at 65–70°. After the addition was completed, stirring was continued for 30 min. at 70–80° and subsequent 30 min. at 80–90°. The solution was filtered to remove Ag$_2$O, diluted with water and acidified with conc. HNO$_3$. The separated orange needles were collected and dissolved in ether from which acidic product was separated by shaking with 5% NaHCO$_3$ solution. The pale yellow needles which were separated on acidification were recrystallized from water using charcoal to colorless needles, m.p. 100–101°. Yield: 6 g. (63%). This product showed the almost same melting point with 3,5-dimethoxyphenylacetic acid (m.p. 99–100°) prepared by another synthetic method of F. Mauthner, et al.$^{15}$IR $\nu_{\text{max}}$ cm$^{-1}$: 1710 (C=O).

3.5-Dimethoxyphenylacetil chloride (XI)—A solution of X (10 g.) and PCl$_3$ (3 ml.) in dried benzene (40 ml.) was refluxed for 1 hr. After filtration and evaporation in vacuo, a yellow syrupy oil (10 g., 92%), was obtained, which was employed for next step of reaction. It was characterized as an amide, colorless needles, m.p. 126–127° (from benzene). IR $\nu_{\text{max}}$ cm$^{-1}$: 3410, 3220 (NH$_2$), 1640 (C=O).

Ethyl 2-(3,5-Dimethoxyphenylacetyl) acetate (XII)—1) By the Spassow reaction, XI was prepared from X (10 g.), ethyl acetoacetate (7 g.), and Mg (1.3 g.) by the modified method of M. Viscontini, et al.$^{9}$ XI was obtained as an yellow oil, Yield: 1.3 g. (90.7%), which was confirmed as the copper salt, bluish white needles, m.p. 178–179° (from benzene). It gives a red FeCl$_3$ reaction. Anal. Calcd. for C$_{18}$H$_{19}$O$_4$·½Cu: C, 56.67; H, 5.61. Found: C, 56.86; H, 5.63. IR $\nu_{\text{max}}$ cm$^{-1}$: 1700 (C=O).

2) By the Caisen reaction, a solution of ethyl acetoacetate (7.3 g.) in dry ether (30 ml.) was added dropwise under stirring and ice cooling into a suspension of NaH (1.4 g.) in dry ether (10 ml.). The reaction immediately took place under the evolution of hydrogen. After standing for 1 hr., a solution of XI (10 g.) in dry ether was added dropwise slowly into the above mixture at 0° under ice-cooling. Standing overnight, the reaction mixture was refluxed for 3 hr. and treated with 5% H$_2$SO$_4$ and ice by usual method. The ethereal layer was separated, shaken with 5% NaHCO$_3$ and washed with water to remove the unreacted free acid (X). The solvent and ethyl acetacetate recovered were evaporated in vacuo to obtain orange yellow oil, Yield: 12 g. (76%).

2-Acetyl-6,8-Dimethoxy-1,5-naphthalenediol (XIII)—The above oil (XI) (12 g.) was distilled in a high vacuum (0.001–0.005 mm./Hg) to remove the volatile portion (b.p. 152–165°). The brown residue sublimed

at bath temp. 200~220°C, for 2~3 hr. to form yellow crystals. The residue was extracted with CHCl₃ to obtain yellow solid. The yellow sublimate and solid obtained as above were chromatographed on silica gel column using CHCl₃ as the solvent. The bottom yellow band was eluted and recrystallized from benzene: MeOH (3:2) mixture to form pale yellow prisms, m.p. 193.5~194°C. Yield: 920 mg. (18%). It dissolves in 5% NaOH and gives a dark green color with 1% FeCl₃ in EtOH and dark red color with Gibbs' reagent. *Anal. Calcd. for C₄₇H₃₂O₇: C, 64.12; H, 5.34. Found: C, 64.19; H, 5.37.*

1.3-Dicarboxy-2-acetyl-6,8-dimethoxynaphthalene — The compound (XII) (0.1 g.) was acetylated with Ac₂O (3 ml.) and pyridine (0.5 ml.) on standing overnight at room temperature. On recrystallization from EtOH, colorless needles, m.p. 126~127°C, were obtained. Yield: 100 mg. (76%). *Anal. Calcd. for C₁₅H₁₃O₅: C, 62.23; H, 5.20. Found: C, 62.45; H, 5.17. IR ν(CH₃) cm⁻¹: 1770 (phenolic acetate C=O), 1700 (C=O).*

2. Acetyl-1,3,6,8-tetramethoxynaphthalene (XV) — A mixture of XII (1 g.), anhydhr. K₂CO₃ (5 g.), Me₃SO₄ (2.5 ml.) and acetone (80 ml.) was refluxed for 5 hr. under vigorous stirring. The product on recrystallization from 60% MeOH gave colorless needles, m.p. 99~100.5°C. Yield: 1 g. (90%). *Anal. Calcd. for C₃₅H₃₃O₇: C, 66.21; H, 6.21. Found: C, 66.19; H, 6.16. UV λmax mp (log e): 233 (4.59), 240 (4.60), 300 (3.68). IR ν(CH₃) cm⁻¹: 1710 (C=O).*

Methylation with diazomethane of XV gave no good result.

Ethyl 3-Hydroxy-6,8-dimethoxy-1-methyl-2-naphthoate (XIV) — A mixture of XII (4 g.) and PPA (prepared from phosphoric acid (5 ml.) and anhydhr. P₂O₅ (5 g.)) was kept at 100°C for 5 min. under stirring. The reaction mixture was poured into ice-water and extracted with CHCl₃. The extract was washed with 5% NaHCO₃ and water, dried, and chromatographed on silica gel column, using CHCl₃ as the solvent.

A yellow fluorescent band which was made visible under UV-illumination was eluted and recrystallized from petr. benzene (b.p. 70~80°C) to form colorless needles, m.p. 130~131°C. Yield: 2.2 g. (58.2%). *Anal. Calcd. for C₃₅H₃₃O₇: C, 66.21; H, 6.21. Found: C, 66.49; H, 6.35. It gives no color reaction with FeCl₃ and a blue color with Gibbs' reagent. Moreover, a small amount of the free acid corresponding to XIV was obtained from the bicanonate washings.

The Cu-salt of XII was treated with PPA as above described. Yield: 0.6 g. (56.5%).

2. Hydroxy-6,8-dimethoxy-1-methyl-2-naphthoic Acid — A solution of 0.5 g. of XIV in 15% NaOH (20 ml.) and EtOH (5 ml.) was refluxed for 1 hr. at 130~140°C in a sealed tube and treated by usual method. Recrystallization of the product from benzene: Me₂CO (7:3) gave pale yellow needles, m.p. 202°C (decomp.). Yield: 0.34 g. (84%). *Anal. Calcd. for C₃₅H₃₃O₇: C, 64.12; H, 5.34. Found: C, 63.80; H, 5.39. IR ν(CH₃) cm⁻¹: 1658 (C=O).*

3. Acetoxy-2-acetyl-1-hydroxy-6,8-dimethoxynaphthalene (XVI) — XII (3 g.) was warmed for 1 hr. at 60°C with Ac₂O (90 ml.) and AcONa (0.6 g.) and treated by usual method. The yellow product was chromatographed on silica gel column using CHCl₃ as the solvent. The starting material (XII) was recovered from a yellow band at the bottom. Yield: 0.21 g. (7%). Then a pale yellow fluorescent band was eluted and recrystallized from MeOH to form pale yellow needles, m.p. 126~127°C. Yield: 1.86 g. (53.6%). *Anal. Calcd. for C₃₅H₃₃O₇: C, 63.16; H, 5.26. Found: C, 63.43; H, 5.32. IR ν(CH₃) cm⁻¹: 3400 (OH), 1770, 1685, 1630 (C=O). Moreover, the diacetate of XII was obtained from the blue fluorescent band immediately above the bottom one. Yield: 0.66 g.

2. Acetyl-3-hydroxy-1,6,8-trimethoxynaphthalene (XVIII) — A mixture of XVI (0.67 g.), K₂CO₃ (2.5 g.), Me₃SO₄ (1 ml.) and acetone (50 ml.) was refluxed for 1.5 hr. under stirring. After removal of K₂CO₃, acetone was evaporated in vacuo. Th residue (XVII) dissolved in MeOH (5 ml.) and 5% NaOH (10 ml.) was warmed for 15 min. on a water bath. After cooling, MeOH was evaporated and acidified with conc. HCl. The yellow solid separated was chromatographed on silica gel using CHCl₃ as the solvent. The yellow bottom band was eluted and recrystallized from MeOH to give orange yellow needles, m.p. 106~107°C. Yield: 0.52 g. (85.5%). *Anal. Calcd. for C₃₅H₃₃O₇: C, 65.22; H, 5.80. Found: C, 65.42; H, 5.64.*

5. Hydroxy-6,8-dimethoxy-2-methyl-1H-naphtho[2,3-b]pyran-4-one (XIX) (Rubrofusarin Monomethyl Ether B) — A solution of XIV (1 g.) in dried AcOEt (2 ml.) was added dropwise to a suspension of NaH (0.8 g.) in dried AcOEt (1 ml.) at 0°C under stirring. After 30 min. the ice bath was removed, and the mixture was kept under stirring at room temperature for 2 hr., and then refluxed for 30 min. Pouring the mixture into ice-water and acidifying the aqueous layer with AcOH, orange precipitates were separated, which were failed to crystallize. Methanolic solution (10 ml.) of this product was added with one drop of conc. HCl, and refluxed for 5 min. After cooling, a brownish orange substance was separated, which was chromatographed on CaHPO₄ column using benzene as a solvent. The second yellow band from the bottom was eluted and recrystallized from EtOH to give orange yellow needles, m.p. 213°C. Yield: 0.42 g. (35.7%). It is insoluble in 5% NaOH and gives a green color with FeCl₃ and blue color with Gibbs' reagent. *Anal. Calcd. for C₅₃H₄₃O₇: C, 67.13; H, 4.88. Found: C, 67.08; H, 4.81. UV λmax mp (log e): 225 (4.44), 275 (4.69), 322 (3.31), 395 (3.81). IR ν(CH₃) cm⁻¹: 1655 (C=O).*

On methylation of this product with an ethereal diazomethane in a mixture of benzene and MeOH, rubrofusarin dimethyl ether (XXI) was obtained. Yield: 47.6%. It was identified with the dimethyl ether of natural rubrofusarin, m.p. 186~187°C, by a mixed fusion (mixed m.p. 186~187°C) and comparison of IR spectra (KBr) and thin-layer chromatograms.
Synthesis of Rubrofusarin Monomethyl Ether B by Partial Methylation of Natural Rubrofusarin

A mixture of I (0.1 g), K₂CO₃ (0.5 g), Me₃SO (0.2 ml) and acetone (10 ml) was refluxed for 7 h under stirring and treated by usual method. The product obtained as above was chromatographed on silica gel using a mixture of benzene-acetone (4:1) as the solvent. The yellow bottom band was eluted and recrystallized from EtOH to give orange yellow needles, named rubrofusarin monomethyl ether B, m.p. 213°. Yield: 0.05 g. (47.5%). From the next yellow fluorescent band, Yield 0.05 g. (45.5%), rubrofusarin dimethyl ether, pale yellow needles, m.p. 186-187°, was obtained. Anal. Calcd. for C₁₃H₁₂O₃: C, 67.13; H, 4.89. Found: C, 66.89; H, 4.87. Rubrofusarin monomethyl ether B was proved to be identical with XIX by a mixed fusion (m.p. 213°), IR spectra (KBr) and thin-layer chromatogram.

5.6.8-Trimethoxy-2-methyl-4H-naphtho[2,3-b]pyran-4-one (XXI) (Rubrofusarin Dimethyl Ether)—
A solution of 0.5 g. of XVII in dried AcOEt (2 ml.) was added dropwise at 0° under stirring to a suspension of NaOH (0.6 g.) in dried AcOEt (3 ml.) and the reaction mixture was treated in the same way, as described above (see XIX) to yield orange yellow solid. Yield: 0.43 g. (75%).

The product which was not able to crystallize was refluxed in a mixture of MeOH and conc. HCl for cyclization. A gray solids obtained were chromatographed on alumina column using a mixture of benzene: MeOH (3:2) as the solvent. The purified pale yellow solids recrystallized from 60% MeOH to give colorless needles, m.p. 186-187°. Yield: 0.175 g. (69%). Anal. Calcd. for C₁₃H₁₂O₃: C, 68.00; H, 5.37. Found: C, 68.28; H, 5.33. UV λ_max (μm) (log ε): 226 (4.47), 271 (4.67), 326 (3.53), 342 (3.62), 373 (3.79). IR ν_max cm⁻¹: 1650 (C=O).

This product was identified with the dimethyl ether of natural rubrofusarin, m.p. 186-187°, by a mixed fusion (mixed m.p. 186-187°) and comparison of IR spectra (KBr) and thin-layer chromatograms.

5.6.8-Trihydroxy-2-methyl-4H-naphtho[2,3-b]pyran-4-one (XX) (Nor-rubrofusarin)—A solution of XV (0.5 g.) in dried AcOEt (2 ml.) was added dropwise at 0° under stirring to a suspension of NaOH (0.25 g.) in dried AcOEt (5 ml.). The reaction mixture was treated in the same way as described for XIX to yield the pale brown amorphous solids. Yield: 0.4 g. (70%). This was cyclized by refluxing for 8 hr. in a mixture of AcCl (12 ml.) and HI (sp. gr. 1.7; 20 ml.). The reaction mixture was poured into a cold solution of 3% NaHSO₃. The separated solids were collected, washed with water and then chromatographed on silicic acid (Mallinckrodt) column using benzene-acetone (4:1) mixture as the solvent. An orange red band was eluted and recrystallized from 75% dioxane to give orange red needles, m.p. 298-299°(decomp). Yield: 0.2 g. (44%). This product was identified with nor-rubrofusarin, which was obtained by the demethylation of natural rubrofusarin, by the comparison of IR spectra (KBr) and thin-layer chromatograms. Anal. Calcd. for C₁₃H₁₂O₃: C, 65.12; H, 3.87. Found: C, 64.95; H, 3.86. UV λ_max (μm) (log ε): 225 (4.43), 278 (4.65), 329 (3.43), 415 (3.73). IR ν_max cm⁻¹: 1645 (C=O).

6-Hydroxy-5,8-dimethoxy-2-methyl-4H-naphtho[2,3-b]pyran-4-one (XXII) (Rubrofusarin Monomethyl Ether A)—XX (0.15 g.) dissolved in a mixture of benzene and tetrahydrofuran (15 ml.: 5 ml.) was treated with an excess of ethereal diazomethane and kept at room temperature for 4 hr. The reaction mixture was filtered to remove the purple precipitates and the filtrate was evaporated in vacuo. The residue was chromatographed on CaHPO₄ column using benzene as the solvent. The blue fluorescent bottom band was eluted and recrystallized from MeOH to give pale yellow needles, m.p. 203-204°. Yield: 0.05 g. (31%). This product was identified with rubrofusarin monomethyl ether A (I) by a mixed fusion (the mixed m.p. 205-204°), IR spectra and thin-layer chromatograms as the comparison. Anal. Calcd. for C₁₃H₁₂O₃: C, 67.13; H, 4.89. Found: C, 67.37; H, 4.94. IR ν_max cm⁻¹: 3370 (OH), 1642 (C=O).

6-Hydroxy-5,8-dimethoxy-2-methyl-4H-naphtho[2,3-b]pyran-4-one (XXIII) (Rubrofusarin Diacetate)—To a solution of XXII (0.1 g.) in dioxane (4 ml.) 5N HCl (3 ml.) was added and the mixture was refluxed for 1 hr., when the color turned into dark red. On addition of water orange red solids were separated. Red crystals obtained by sublimation of the product in vacuo at 200° were recrystallized from benzene to form orange red needles, m.p. 210°. Yield: 0.05 g. (52.6%). This product was identified with natural rubrofusarin by a mixed fusion (the m.p. 210°) and comparison of IR spectra and thin-layer chromatograms. Anal. Calcd. for C₁₃H₁₂O₃: C, 66.18; H, 4.41. Found: C, 66.15; H, 4.40. UV λ_max μm (log ε): 224 (4.41), 277 (4.66), 325 (3.51), 340 (3.34), 410 (3.74). IR ν_max cm⁻¹: 1660 (C=O).

6-Hydroxy-5-hydroxy-2-methyl-4H-naphtho[2,3-b]pyran-4-one (XXIV) (Nor-rubrofusarin Diacetate)—On acetylation with pyridine (0.5 ml.) and Ac₂O (5 ml.), standing overnight at room temperature, nor-rubrofusarin (0.2 g.) afforded diacetate (XXIV) which had been obtained by H. Raisank, et al. (1957) while its structure was remained unestablished. Pale yellow needles (from AcOH, m.p. 205-206°). Yield: 0.2 g. (76%). Anal. Calcd. for C₁₃H₁₂O₄: C, 65.16; H, 4.03. Found: C, 65.04; H, 4.22. UV λ_max μm (log ε): 253 (4.65), 267 (4.69), 299 (3.45), 313 (3.52), 326 (3.27), 385 (3.68).

6-Hydroxy-5-hydroxy-2-methyl-4H-naphtho[2,3-b]pyran-4-one (XXVI) The mixture of XXIV (0.1 g.), Me₃SO (4 ml.), K₂CO₃ (0.8 g.), and acetone (15 ml.) was refluxed for 4 hr. After treatment by usual method, product was purified by chromatography on silica gel column using a mixture of benzene and acetone (4:1). From the bottom blue fluorescent band colorless needles, m.p. 156-157°(from MeOH) were isolated. Yield: 0.09 g. (86.9%). Anal. Calcd. for C₁₃H₁₄O₃: C, 64.02; H, 4.50. Found: C, 64.07; H, 4.57. IR ν_max cm⁻¹: 1770, 1660 (C=O).
6.8-Dihydroxy-5-methoxy-2-methyl-4H-naptho[2,3-b]pyran-4-one (XXVII) (Nor-rubrofusarin 5-Monomethyl Ether) —— XXVI (0.1 g.) dissolved in MeOH (10 ml.) and 10% H$_2$SO$_4$ (10 ml.) was refluxed for 3 hr. on a water bath. After cooling, the resulting solid was collected, washed with H$_2$O and dried. It was chromatographed on silica gel column using a mixture of benzene-acetone (4:1). The bottom orange yellow fluorescent band was eluted and recrystallized from EtOH to give orange yellow needles, m.p. 235~237° (decomp.). Yield: 0.05 g. (65.3%). Anal. Calcd. for C$_{13}$H$_{12}$O$_5$: C, 66.18; H, 4.41. Found: C, 66.18; H, 4.43. UV $\lambda_{max}^{\text{EIOH}}$ m$\mu$ (log $\varepsilon$): 226 (4.43), 277 (4.68), 333 (3.43), 350 (3.43), 396 (3.69). IR $\nu_{max}^{\text{IR}}$ cm$^{-1}$: 1630~1640 (C=O).

The authors are grateful to Prof. H. Raisitck and Mr. G. Smith, for supplying the strain of Fusarium culmorum from which rubrofusatin was isolated and to Prof. S. Fukushima, Shizuoka College of Pharmacy, for discussion.

The starting material (α-Resorcylic acid) was supplied by Dr. S. Matsuura, Gifu College of Pharmacy, and NMR spectral measurements were carried out by Dr. F. Nagasawa and Dr. S. Morita of the Research Laboratory of Mitsubishi Kasei Co., Ltd., to whom the authors are much indebted. Microanalysis and UV and IR spectral measurements were carried out by the members of microanalytical Laboratory of this Faculty, to whom the authors' thanks are due.