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65. Shun-ichi Udagawa : (−)-Sclerotiorin, A Major Metabolite of *Penicillium hirayamae* Udagawa.

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The dextrorotatory yellow pigment sclerotiorin, $C_{21}H_{23}O_5Cl$, m.p. 206~207°, $[\alpha]_D^{20}$ +493° (ethanol), is frequently occurring in several species of the monoverticillate *Penicillia.* Originally it was isolated from laboratory cultures of *P. sclerotiorum* van Beyma by Curtin and Reilly,[1] and obtained later from *P. multicolor* Grigorieva-Manoilova et Poradielova[2,3] and *P. implicatum* Biourge.[4] In 1959, the structural formula of sclerotiorin was finally advanced by Dean *et al.*[5] as a result of extensive studies. With other three metabolites[6~9] from the genus *Monascus*, sclerotiorin constitutes a characteristic group of fungal pigments, named azaphilones,[10] which is noted as being converted readily into N-containing compound with ammonia. Meanwhile, a second analogous product rotiorin,[11] $C_{23}H_{24}O_5$, m.p. 245°, having properties similar to those of sclerotiorin, was found from the mycelium of *P. sclerotiorum.*

During a study on the phylogenetic significances of the fungal products, an optical antipode of the known (+)-sclerotiorin has been encountered in the mycelium of *P. hirayamae* Udagawa,[12] a new member in the *P. thomii* series, which is noted as it forms abundant bright yellow-orange mycelia accompanying sclerotia. It showed a good agreement in the chemical and spectral properties of (+)-sclerotiorin obtained from previously described species. As for the optical rotation, the pigment of *P. hirayamae* showed levorotation, $[\alpha]_D^{26}$ −482° (c=0.11, ethanol). It has therefore been established that the sclerotiorin is occurring in nature in both dextro- and levorotatory forms.

In addition to the major constituent, the same culture gave another hitherto unknown pigment, $C_{21}H_{21}O_4Cl$ or $C_{21}H_{23}O_4Cl$, named rubrotiorin, which crystallized from ethanol as red needles and melted at 172°. The ultraviolet spectrum in 90% ethanol shows maxima at 248 (log ε 4.03), 286 (log ε 4.29), 428 (log ε 4.46) and 580 mμ. The infrared spectrum in Nujol shows strong absorptions at 1757, 1684, 1628, and 1618 cm^{-1}. It gave

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a positive ammoniacal reaction characteristic of azaphilones. The quantity of the product was too small for comprehensive investigation. The melting point, ultraviolet and infrared spectral data and color reaction suggest that it seems a new fungal azaphilone.

Experimental

Production and Isolation of Metabolic Products——A culture of *Penicillium hirayamae* strain NHL 6046 was grown on a modified Czapek-Dox medium (glucose 50 g., $NaNO_3$ 2 g., KH_2PO_4 1 g., KCl 0.5 g., $MgSO_4 \cdot 7H_2O$ 0.5 g., $FeSO_4 \cdot 7H_2O$ 0.01 g., $ZnSO_4 \cdot 7H_2O$ 0.01 g., $CuSO_4 \cdot 5H_2O$ 0.005 g., distilled water to 1 L.) at 25°, for 3 weeks. The dried and pulverized mycelium was extracted in a Soxhlet apparatus with petr. ether (b.p. 40~60°) to remove fatty substance, and then exhaustively with Et_2O. After evaporating to dryness, the ethereal extracts (7.5 g. from 100 g. of the dried mycelium) were chromatographed on $CaHPO_4$ columns using a mixture of benzene-hexane (4:1), giving three main bands (from top to bottom yellow, orange, and yellow bands). Of these pigments the yellow substance at the top band could not be studied further on account of the insufficient amount.

(−)-Sclerotiorin——The eluate of the lower yellow band was repeatedly recrystallized from MeOH giving yellow needles of (−)-sclerotiorin, m.p. 203~204°, $[\alpha]_D^{26}$ −482° (c=0.11 in ethanol), yield 4.5 g. from 7.5 g. crude ethereal extract. The mixed m.p. with (+)-sclerotiorin obtained from *P. multicolor* showed a depression to 170~174°. *Anal.* Calcd. for $C_{21}H_{23}O_5Cl$: C, 64.53; H, 5.93; Cl, 9.07. Found: C, 64.76; H, 5.86; Cl, 9.85. UV $\lambda_{max}^{90\%\ EtOH}$ mμ (log ε): 215 (4.11), 286 (4.23), 368 (4.39). Principal IR ν_{max}^{Nujol} cm^{-1}: 1741 (s), 1726 (s), 1669 (s), 1630 (s), 1621 (s); $\nu_{max}^{CHCl_3}$ cm^{-1}: 1738 (m), 1723 (m), 1666 (broad) (m), 1629 (s), 1620 (shoulder) (s). These spectral properties were indistinguishable from those of (+)-sclerotiorin. A mixture prepared by dissolving equimolar amounts of (+)- and (−)-sclerotiorin crystallized from MeOH, melted at 170~174°, and had no measurable rotation.

(−)-Sclerotioramine——This compound was prepared with NH_3 by the method described by Eade *et al.*[5] It crystallized from aq. EtOH as red needles, m.p. 222~223°(decomp.). On admixture with authentic (+)-sclerotioramine gave mixed m.p. 210~212°. *Anal.* Calcd. for $C_{21}H_{24}O_4NCl$: C, 64.69; H, 6.20; N, 3.59. Found: C, 64.90; H, 6.21; N, 3.81. Principal IR ν_{max}^{Nujol} cm^{-1}: 1740 (m), 1706 (m), 1640 (m), 1612 (s), 1591 (s), 3190 (m), 3055 (s); $\nu_{max}^{CHCl_3}$ cm^{-1}: 1727 (m), 1705 (m), 1601 (broad) (s), 3360 (w), 3285 (w), 3072 (w). No obvious differences were observed between the IR spectrum of the product and those of (+)-sclerotioramine.

Rubrotiorin——On purification by repeated chromatography and crystallization from EtOH, the fraction eluted from the middle band rubrotiorin was obtained as red needles, m.p. 172°, yield 280 mg. from 7.5 g. crude ethereal extract. *Anal.* Calcd. for $C_{21}H_{21}O_4Cl$: C, 67.65; H, 5.68; Cl, 9,51; mol. wt. 373. Calcd. for $C_{21}H_{23}O_4Cl$: C, 67.28; H, 6.18; Cl, 9.46; mol. wt. 375. Found: C, 67.00; H, 5.82; Cl, 8.70; mol. wt. (Rast), 334. UV $\lambda_{max}^{90\%\ EtOH}$ mμ (log ε): 248 (4.03), 286 (4.29), 428 (4.46), 580. IR ν_{max}^{Nujol} cm^{-1}: 1757 (s), 1684 (s), 1628 (s), 1618 (inflex.) (s), 976 (m), 968 (m), 962 (m), 947 (m), 894 (s), 865 (m), 851 (s), 775 (m), 757 (m); ν_{max}^{KBr} cm^{-1}: 1757 (s), 1682 (s), 1626 (s), 1617 (inflex.) (s), 978 (m), 963 (m), 947 (inflex.) (w), 897 (m), 865 (inflex.) (w), 850 (s), 770 (m), 759 (m). Owing to difficulties arising from its rather high absorption at 580 mμ the specific rotation could not be observed. The pigment was readily soluble in the usual organic solvents except petr. ether, and insoluble in aqueous alkali.

In cold H_2SO_4 it formed an orange-red solution. It did not give any characteristic color with ethanolic $FeCl_3$, and ethanolic $(AcO)_2Mg$, but it developed a deep red color with NH_3.

On treatment with NH_3 rubrotiorin was converted into an ammonia compound, UV $\lambda_{max}^{90\%\ EtOH}$ mμ: 287, 368, 530. Due to the shortage of available materials, rubrotioramine has not yet been obtained in crystalline state.

Summary

From the mycelium of *Penicillium hirayamae* UDAGAWA two hitherto undescribed metabolites have been isolated. The major product is a levorotatory isomer of the known sclerotiorin. The minor component, rubrotiorin, $C_{21}H_{21\sim23}O_4Cl$, m.p. 172°, is given as red needles and appears to be a new azaphilone compound.

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