Notes

A NEW PIGMENT FROM *STREPTOMYCES LAVENDULAE*

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A saframycin-non-producing mutant of *Streptomyces lavendulae* IFM 1291 was found to produce a new tyrosine-derived indicator SL-1 pigment with anti-dermatophyte activity. The present paper describes the isolation, structural elucidation and biological properties, especially the antimicrobial activity against dermatophytes.

Ten saframycin-non-producers were isolated by treatment with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine from *Streptomyces lavendulae* No. 314[1~4]. All these mutant strains were found to produce a large amount of reddish brown pigment around colonies in organic solid media such as nutrient and glucose-starch agars (GSB agar medium). SL-1 pigment was also produced in liquid media exhibiting wine red color. It is yellow or orange in acid to neutral solutions and wine red to reddish brown in alkaline solutions. The pigment was isolated from the culture broth of *S. lavendulae* No. 314-39 strain by the following fermentation and procedures.

Fermentation was carried out at 27°C with agitation of 400 rpm and aeration of 20 liters/minute for 18 hours in a 25-liter jar fermentor containing 20 liters of GSB medium (soluble starch 0.72%, glucose 0.5%, meat extract 0.5% and peptone 1.0%: pH 7.2 before sterilization). SL-1 pigment was isolated from the culture filtrate as follows. The culture broth (*ca.* 17 liters) was harvested, adjusted to pH 4.0 with 5 N HCl and filtered. The filtrate was extracted with one-fifth volume of ethyl acetate and the extract washed with water and dried over anhydrous Na_2SO_4. The pigment was transferred from the solvent to the aqueous layer at alkaline pH, suggesting that it is an acidic substance. The solvent extract was evaporated to dryness and then subjected to silica gel column chromatography (Merck, developer: ethyl acetate). The crude material thus obtained was further chromatographed on a Sehadex LH-20 column with MeOH and the yellow-pigmented fractions were combined and evaporated *in vacuo* to dryness. After rechromatography of the pigment on Sephadex LH-20, the SL-1 pigment fraction was dissolved in ethyl ether and crystallized. One hundred mg of purified SL-1 brown pigment were obtained from 17 liters of culture broth. SL-1 (**1**) was obtained as prisms, mp 157~159°C (dec, EtOAc). It is freely soluble in methanol and ethanol, soluble in acetone and ethyl acetate, slightly soluble in chloroform, and practically insoluble in *n*-hexane and H_2O. Electron ion (EI) mass spectrum of **1** gave a molecular ion at *m/z* 181 as a base peak, together with fragment ions at *m/z* 134(75) and 89(57). The molecular formula of **1** was assigned $C_8H_7NO_4$ from the molecular ion peak and elemental analysis. The UV and IR spectra of SL-1 pigment (**1**) are as follows: UV λ_{max}^{MeOH} nm (log ε) 242(sh, 3.54), 262 (3.69), 322(sh, 3.68), 3.76(4.15), 490(sh, 2.80); UV λ_{Min}^{MeOH} nm (log ε) 225(3.45), 286(3.25). IR ν_{max}^{KBr} cm^{-1} 3375, 1630, 1605, 1595, 1530, 1495, 1445, 1380, 1345, 1300, 1270, 1240, 1195, 1180.

The ^1H NMR spectrum in $CDCl_3+CD_3OD$ at 400 MHz exhibited signals at δ 6.869 (1H, d, J= 8.1 Hz), 7.006 (1H, dd, J=8.1 and 2.2 Hz), 7.071 (1H, d, J=2.2 Hz), which were assigned to 1,2,4-trisubstituted benzene ring protons, and δ 7.539 (1H, d, J=13.4 Hz), 7.913 (1H, d, J=13.4 Hz), which were ascribed to *trans* olefinic protons. Exchange of two phenolic protons was observed. The ^{13}C NMR spectrum in $CDCl_3+CD_3OD$ at 100 MHz showed resonances at δ 115.27(d), 116.14(d), 122.43(s), 124.47(d), 134.52(d), 140.69 (d), 145.67(s) and 150.02(s). Treatment of **1** with acetic anhydride in pyridine provided the

Fig. 1. Possible partial structure of SL-1 pigment.

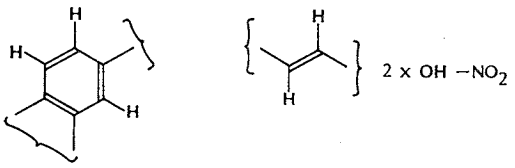
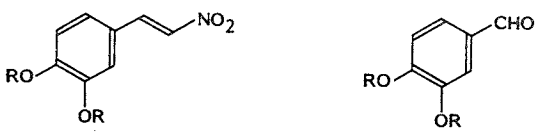


Fig. 2. Structure of SL-1 and related synthetic pigments.



1 R = H
2 R = $COCH_3$
5 R = MOM

3 R = H
4 R = MOM

diacetate (**2**), mp 113.5~115°C, MS *m/z* 265 (M^+) $C_{12}H_{11}NO_6$. Its ^1H NMR spectrum in $CDCl_3$ at 400 MHz showed signals at 2.315(3H, s), 2.320(3H, s), 7.282(1H, d, J=8.3 Hz), 7.391 (1H, br s), 7.423(1H, d, J=8.3 Hz), 7.517(1H, d, J=13.7 Hz), 7.927(1H, d, J=13.7 Hz).

The foregoing evidence was suggestive of the following structural fragments as shown in Fig. 1. The structure of the SL-1 pigment (*E*)-1,2-dihydroxy-4-[2-nitroethenyl]benzene was determined unambiguously by its synthesis as shown in Fig. 2. 3,4-Dihydroxybenzaldehyde (**3**) was reacted with chloromethylmethyl ether in the presence of sodium hydride in dimethylformamide to give the corresponding methoxymethyl (MOM) ether derivative (**4**), mp 56~57°C, in 90.4% yield, which was condensed with nitromethane by refluxing to afford the β-nitrostyrene derivative (**5**), mp 86~88°C, in 78.2% yield. Removal of the MOM groups of **5** was accomplished by hot conc HCl in MeOH with a yield of 76.2%. The product was identical in all respects (mmp, IR, UV, MS, ^1H NMR, TLC) with the natural pigment (**1**). This paper is the first report on the isolation of SL-1 pigment from natural sources.

SL-1 pigment showed moderate inhibitory activity against certain Gram-positive bacteria such as *Streptococcus pyogenes* and *Brucella abortus*. In addition, SL-1 pigment was active against some dermatophytes and MIC values are shown in Table 1. It is also of interest that the producer itself is sensitive to the pigment. The pigment also exhibited moderate cytocidal activities against cultured L1210 leukemic cells *in vitro* and ED_{50} value against the tumor cells was 1.0 μg/ml.

Table 1. Antimicrobial spectrum of SL-1 pigment.

Test organisms	IFM No.	MIC (μg/ml)
Bacillus subtilis PC1219	2026	>100.0
Streptococcus pyogenes	2006	25.0
Brucella abortus	3032	25.0
Streptomyces lavendulae (SL-1 pigment producer)	1291	12.5
Trichophyton mentagrophytes	40736	12.5
	40737	50.0
T. rubrum	40733	12.5
T. violaceum	40738	25.0
T. interdigitale	40742	25.0
T. concentricum	40734	12.5
Microsporum gypseum	40766	25.0
M. canis	40767	50.0
Epidermophyton floccosum	40770	50.0

Agar dilution streak method was used to determine the MIC values. Glucose (0.5%) nutrient agar for bacteria and 2% glucose Sabouraud agar for fungi were used.

Erbstatin, [(*E*)-2-(2-formamidovinyl)-1,4-hydroquinone][5], and WF-5239[6] which show structural similarity with the present SL-1 pigment, were recently reported to have such interesting biological activities as inhibition of epidermal growth factor-receptor kinase or of platelet aggregation. Detailed studies on these activities of SL-1 and related newly synthesized compounds will be reported elsewhere.

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