Communications to the Editor

LAVANDUCYANIN, A NEW ANTITUMOR SUBSTANCE PRODUCED BY *STREPTOMYCES* SP.

Sir:

In the course of our screening program for new cytotoxic compounds, *Streptomyces* sp. CL190, which was isolated from Ishigaki Island, Okinawa Prefecture, Japan, was found to produce a new phenazine type antibiotic active against P388. In this communication, the isolation, characterization and structural elucidation of the new compound named lavanducyanin are reported.

Streptomyces sp. CL190 was cultivated at 27°C in a 60-liter jar fermenter with an agitation rate of 400 rpm and an air flow of 30 liters/minute. The medium consisted of glucose 2.5%, soybean meal 1.5%, dry yeast 0.2% and calcium carbonate 0.4% and pH was adjusted to 6.2. After fermentation for 28 hours, the mycelium was collected by centrifugation from 30 liters of the fermentation broth and extracted with acetone (4 liters) added with a few drops of 10 N NaOH solution. After neutralization with 2 N HCl, the extract was concentrated to a small volume, and the aqueous residue was adjusted to pH 10 and extracted twice with EtOAc. The solvent layer was extracted with acidic water (pH 3) and after being adjusted to pH 10, the active material was extracted with EtOAc. The solvent layer was concentrated to dryness and the residue was subjected to silica gel column chromatography ($CHCl_3$ - MeOH, 50:1). The combined active fraction was purified by Sephadex LH-20 column chromatography ($CHCl_3$ - MeOH, 1:1). Concentration of the pooled active fraction gave a blue powder of lavanducyanin (19.3 mg).

The physico-chemical properties of lavanducyanin were as follows: MP 135~136°C; IR $\nu_{max}^{CHCl_3}$ (cm^{-1}) 2980, 1635, 1602, 1565, 1495, 1467, 1350, 1297, 1282, 1242, 1157, 835; the high resolution-electron impact mass spectrum (HREI-MS) of lavanducyanin showed the molecular ion at *m/z* 332.1914 indicating its molecular formula to be $C_{22}H_{24}N_2O$ (calcd 332.1939).

The UV and visible spectra of lavanducyanin

Table 1. ^{13}C and ^{1}H NMR spectral data of lavanducyanin.

Carbon No.	δ_C	Functional group	δ_H (Hz)
C-1	179.75	C=O	
C-2	117.13	CH=	6.543 d (J=9.0)
C-3	143.15	CH=	7.570 dd (J=8.0, 9.0)
C-4	91.35	CH=	5.868 d (J=8.0)
C-4a	135.06	C=	
C-5a	135.71	C=	
C-6	124.31	CH=	7.453 d (J=8.0)
C-7	134.56	CH=	7.732 dd (J=8.0, 7.0)
C-8	113.30	CH=	7.462 dd (J=8.5, 7.0)
C-9	133.83	CH=	8.347 d (J=8.5)
C-9a	133.63	C=	
C-10	147.44	C=	
C-11	49.57	CH_2	5.012 s
C-12	121.30	C=	
C-13	130.94	C=	
C-14	46.25	CH_2	1.870 s
C-15	28.85	C	
C-16	34.87	CH_2	1.202 t (J=6.5)
C-17	22.75	CH_2	1.549 br
C-18	19.38	CH_3	1.870 s
C-19	27.95	CH_3	0.826 s
C-20	27.95	CH_3	0.826 s

Measured at 500 MHz (^{1}H) and 125 MHz (^{13}C) in $CDCl_3$.

Fig. 1. Partial structures of lavanducyanin.

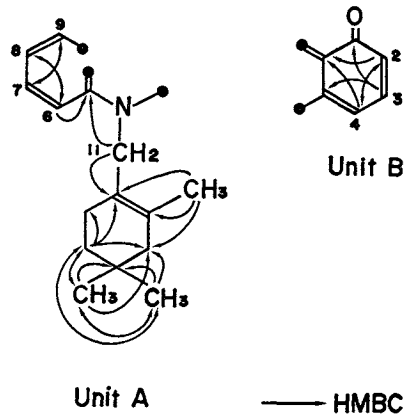
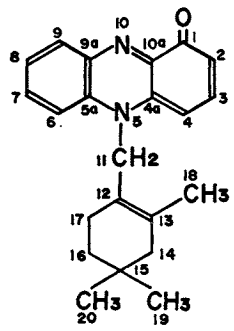


Fig. 2. Structure of lavanducyanin.



in neutral and alkaline MeOH solution showed absorption maxima at 237 nm (ε 12,400), 319 (9,700) and 705 (br, 1,700) with shifted to 274 nm (ε 9,700), 386 (5,000) and 508 (br, 1,400) in 0.1 N HCl. These spectral data were very similar to those of pyocyanin[1] suggesting that lavanducyanin belonged to the group of phenazine antibiotics.

The ^1H NMR spectrum of lavanducyanin (Table 1) showed the presence of 24 unexchangeable protons. The carbons of lavanducyanin as revealed by ^{13}C NMR spectra were classified as follows; $CH_3 \times 3$, $CH_2 \times 4$, CH= $\times 7$, C $\times 1$, C= $\times 6$ and C=O $\times 1$. Detailed NMR spectral experiments such as ^1H-^1H selective decoupling, ^1H-^1H long range correlation spectroscopy (COSY), ^1H-^{13}C COSY and heteronuclear multiple-bond correlation (HMBC)[2,3] revealed the partial structural Units A and B as shown in Fig. 1. The linkage of C-11 methylene to a nitrogen atom was suggested by its ^{13}C and ^1H NMR chemical shifts (49.57 and 5.012 ppm, respectively). The remaining units to be assigned were one quaternary sp^2 carbon and a nitrogen atom. By taking into consideration of the degree of unsaturation and the very similar UV and visible spectral data to those of pyocyanin (*vide supra*), the total structure of lavanducyanin was established as shown in Fig. 2. This structure was corroborated by observing nuclear Overhauser effects with 4-H and 6-H upon irradiation of 11-H.

Lavanducyanin possesses a presumably monoterpene derived unit in place of the methyl residue of pyocyanin. This hydrocarbon unit is present in cyclolavandulol[4].

Lavanducyanin was active against P388 and L1210 at the concentration (IC_{50}) of 0.09 and 0.10 μg/ml, respectively. Biological activities of lavanducyanin will be reported elsewhere in detail.

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