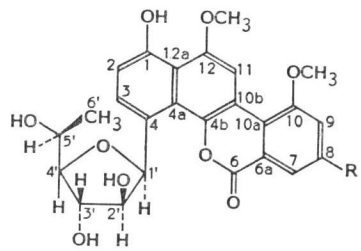
Communications to the Editor

DEFUCOGILVOCARCIN V, A NEW ANTIBIOTIC FROM *STREPTOMYCES ARENAE* 2064: ISOLATION, CHARACTERIZATION, PARTIAL SYNTHESIS AND BIOLOGICAL ACTIVITY*

Sir:

During the course of our studies with the large-scale production of the antitumor antibiotic gilvocarcin V[1~9] (**1**) from *Streptomyces arenae* 2064, a new antibiotic designated as defucogilvocarcin V was isolated from the acetone extract of the mycelia (cell-paste). The new antibiotic was detected in high performance liquid chromatography (HPLC) by WEI *et al.* in 1982[8] in a biochemical prophage induction assay (BIA), a test which detects DNA-interacting compounds.[10] In this paper, we describe the isolation, characterization, partial synthesis, and biological activity of defucogilvocarcin V.

Fermentations were carried out under the conditions described earlier.[8] The whole broth was centrifuged and the mycelia were extracted three times with acetone. The spent mycelia were discarded, and the combined acetone extract was concentrated. The precipitated complex was filtered and washed with hexane. The crude complex was then chromatographed on a silica gel column and eluted with $CHCl_3$ and $CHCl_3$ - MeOH (90: 10 and 75: 25). The new antibiotic eluted first followed by the mixture of gilvocarcin M[2,6,11] (**2**) and gilvocarcin V (**1**). Various fractions were combined based on HPLC and thin layer chromatography (TLC) analysis. Solvents were removed until the products started to crystallize. These were filtered and dried. Defucogilvocarcin V was isolated as a yellow crystalline powder from $CHCl_3$ - MeOH (mp



Gilvocarcin V (**1**) R=CH=CH$_2$
Gilvocarcin M (**2**) R=CH$_3$
Gilvocarcin E (**3**) R=CH$_2$CH$_3$

Table 1. Physico-chemical properties of defucogilvocarcin V.

Producing organism:	*Streptomyces arenae* 2064
Type of compound:	Gilvocarcin type
Nature:	Yellow crystalline powder
Melting point:	253~257°C
Solubility:	Partly soluble in DMSO, DMF, and pyridine Very sparingly soluble in $CHCl_3$, EtOAc, acetone, MeOH, methyl isobutyl ketone, and acetic acid Insoluble in H_2O, petroleum ether, and ether
TLC[a] (Rf)	S1: 0.91, S2: 0.95, S3: 0.92, S4: 0.90
HPLC[b] (Retention time):	21.6 minutes
UV, λ_{max}^{MeOH} nm:	388, 349, 283, 251, and 205
IR (KBr) cm^{-1}	3320, 1712, 1618, 1597, 1575, 1408, 1374, 1321, 1229, 1156, 1119, 1051, 898, 798, 770, 746, and 618
MW and molecular formula:	348.1013 (Δm−1.8 mmu), $C_{21}H_{16}O_5$ (HREIMS)

[a] Using analytical silica gel plates, solvent system S1: EtOAc (100%); S2: methyl isobutyl ketone (100%); S3: $CHCl_3$ - MeOH (90: 10); S4: EtOAc - CH_3COOH (90: 10).

[b] On a C_{18} μBondapak column. Solvent system, MeOH - H_2O (70: 30); flow, 1.5 ml/minute; UV, 254 nm; AUFS, 0.2; chart speed, 0.5 cm/minute.

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Table 2. ^{1}H NMR comparison of gilvocarcins V and M, and defucogilvocarcin V.

Proton	Gilvocarcin V[6]	Gilvocarcin M[2]	Defucogilvocarcin V
1OH	9.66	9.66	
2H	6.92 (d, J=8.3 Hz)	6.91 (d)	7.01 (dd, J=0.8 and 7.7 Hz)
3H	8.05 (d, J=8.3 Hz)	8.05 (d)	7.54 (t, J=8.0 Hz)
4H	—	—	7.87 (dd, J=0.8 and 7.7 Hz)
7H	7.93 (d, J=1.5 Hz)	7.71	8.03 (d, J=1.5 Hz)
9H	7.69 (br s)	7.40	7.84 (d, J=1.3 Hz)
11H	8.39	8.37	8.41
10OCH_3	4.14 (3H)	4.08 (3H)	4.21 (3H)
12OCH_3	4.08 (3H)	4.06 (3H)	4.20 (3H)
13H	6.93 (dd, J=9.29 and 18.6 Hz)	—	6.94 (m)
14H	5.48 (d, J=9.2 Hz)	—	5.50 (d, J=11.1 Hz)
14H	6.11 (d, J=18.6 Hz)	—	6.16 (d, J=17.7 Hz)

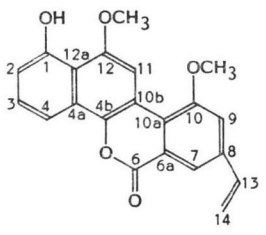
Spectra were determined on a 300 MHz instrument in DMSO-d_6 using $(CH_3)_4Si$ as the internal standard.

Table 3. Comparison of antimicrobial spectra of gilvocarcin V and defucogilvocarcin V.

Test organism	ATCC	MIC (μg/ml)	
		Gilvocarcin V	Defucogilvocarcin V
Staphylococcus aureus	6538P	0.125	0.125
Micrococcus luteus	9341	0.008	0.032
Bacillus subtilis	6633	0.008	0.008
Escherichia coli	10536	4.0	>250
Candida albicans	10231	4.0	>250
Biochemical induction assay[a] (BIA)		0.01	0.01

Determined by serial agar dilution method. The plates were allowed to remain under room light for 1 hour to activate the compound after spotting.

[a] The BIA is a β-galactosidase prophage induction assay for possible DNA interaction, according to the method of ELESPURU and WHITE.[10] Under similar conditions, the minimal inducing concentration for bleomycin and chrysomycin A was 1.0 and 0.01 μg/ml, respectively.



Defucogilvocarcin V (**4**)

253~257°C). Its physico-chemical properties are summarized in Table 1.

The ^{1}H NMR spectrum in DMSO-d_6 indicated the presence of two methoxy groups at δ 4.20 and 4.21; three vinylic protons at 5.50 (d, J=11.1 Hz), 6.16 (d, J=17.7 Hz), and 6.94 (m); six aromatic protons at 7.01 (dd, J=0.8 and 7.7 Hz), 7.54 (t, J=8.0 Hz), 7.84 (d, J=1.3 Hz), 7.87 (dd, J=0.8 and 7.7 Hz), 8.03 (d, J=1.5 Hz), and 8.41. A comparison of these values with the ^{1}H NMR spectral values of gilvocarcin V and M, as shown in Table 2, clearly indicate that the new antibiotic is defucogilvocarcin V (**4**), (1-hydroxy-10,12-dimethoxy-8-vinyl-6*H*-benzo-[d]naphtho[1,2b]pyran-6-one). The identity was further confirmed by the electron impact mass spectrum (EIMS) which showed a molecular ion at m/z 348.1013 ($C_{21}H_{16}O_5$, Δ m−1.8 mmu) and fragment ions at m/z 333, 305, 290, 262, 234, 205, 174, 147, 124, 95 and 75 amu. Its UV spectrum was superimposable on the UV spectrum of gilvocarcin V and the IR spectrum showed bands at 3320, 1712, 1618, 1597, 1575, 1408, 1374, 1321, 1229, 1156, 1119, 1051, 898, 770, 746, 618 cm^{-1}.

The structure was finally confirmed by its partial synthesis from gilvocarcin V. Gilvocarcin V (200 mg) and methanol containing 15% dry HCl (30 ml) were heated to reflux for 18 hours

(the course of the reaction was followed by TLC and HPLC). After this time, the solvent was removed and the residue was chromatographed using a column of TLC grade silica gel and eluting with $CHCl_3$ and $CHCl_3$ - MeOH (90: 10). Pure defucogilvocarcin V, which eluted first, after solvent removal gave a yellow crystalline powder, identical in all respects (TLC, HPLC, mp, mixture mp, UV, IR, ^1H NMR, and EIMS) with the natural product.

The prophage-inducing and DNA-strand breaking[7] activities of gilvocarcin V were shown to be dependent on the irradiation of the complex with visible or near-ultraviolet light. Experiments with defucogilvocarcin V have shown a light dependent prophage-inducing activity that is identical to that observed with gilvocarcin V, and is therefore, independent of the sugar moiety.

Defucogilvocarcin V shows antimicrobial activity against *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis*, and membrane-permeable *Escherichia coli* (BIA) but is inactive against wild type *E. coli* and *Candida albicans*. A comparison of the minimum inhibitory concentration (MIC) of light-activated gilvocarcin V against these organisms is shown in Table 3.

These results suggest that Gram-negative organisms and yeast discriminate between gilvocarcins with and without sugar moiety, while Gram-positive organisms do not.

The isolation of defucogilvocarcin V from fermentation broth is important from a biogenetic point. It suggests that the sugar moiety is probably attached to the chromophore in a subsequent step. Further, the biological data suggested that the sugar may affect the uptake of gilvocarcins but not the photochemical reactions responsible for biological activity.[12] Besides gilvocarcins V and M, gilvocarcin E,[2] chrysomycins A[13] and B,[13] and ravidomycin,[14~17] all possess the same chromophoric system.

Acknowledgments

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