GILVOCARCINS, NEW ANTITUMOR ANTIBIOTICS
2. STRUCTURAL ELUCIDATION

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(Received for publication December 27, 1980)

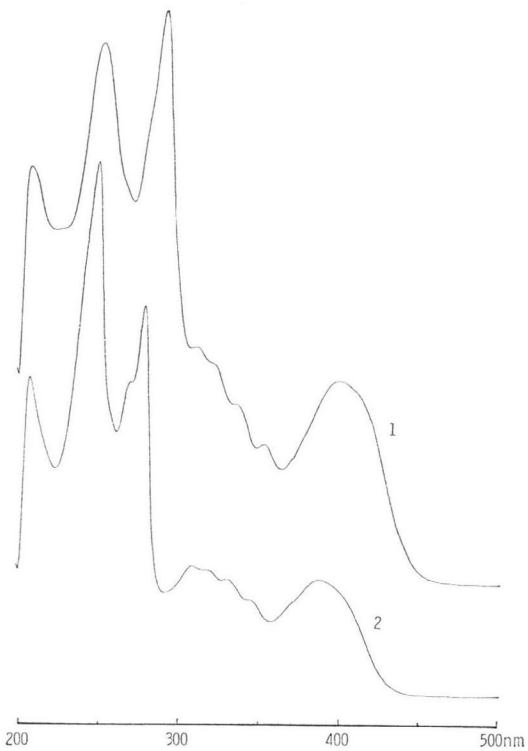
Gilvocarcin V(**1**), $C_{27}H_{26}O_9$, m.p. 264~267°C (dec.), and gilvocarcin M(**2**), $C_{26}H_{26}O_9$, m.p. 245~248°C (dec.), are new antitumor antibiotics produced by *Streptomyces gilvotanareus*. The structure of gilvocarcins has been determined by chemical degradation, nmr and mass spectra. They have a benzonaphtopyran-one system, to which the furanose moiety is linked through a C-C glycosyl bond.

The fermentation and isolation of gilvocarcins have been reported in a preceding paper[1]. In this paper we report on studies dealing with the structural elucidation of gilvocarcins V and M which have resulted in the assignments of structures **1** and **2** to these new antibiotics.

Gilvocarcin V (**1**), mp 264~267°C (dec.), $[\alpha]_D^{20}$ −216° (*c* 0.16, DMSO), MS: *m/z* 494.1596 (M^+) (Calcd. for $C_{27}H_{26}O_9$: 494.1576), showed UV λ_{max}^{MeOH} (log ε) 248(4.63), 287(4.65), and 398 nm (4.23) (Fig. 1), and IR ν_{max}^{KBr} 3380 (OH) and 1690 cm^{-1} (C=O) (Fig. 2). ^{1}H-NMR (DMSO-d_6, Table 1) of **1** indicated the presence of five aromatic protons, a vinyl group, five methine protons attached to oxygen functions, two methoxy groups, doublet methyl group, and four exchangeable protons by CD_3OD.*

Gilvocarcin M (**2**), mp 245~248°C (dec.), $[\alpha]_D^{20}$ −209° (*c* 0.2, DMSO), MS: *m/z* 482.1608 (M^+) (Calcd. for $C_{26}H_{26}O_9$: 482.1576), had UV λ_{max}^{MeOH} (log ε) 245(4.61), 267sh(4.40), 275(4.48), and 387 nm(4.00) (Fig. 1), IR ν_{max}^{KBr} 3380 (OH) and 1690 (C=O) (Fig. 2). ^{1}H-NMR (DMSO-d_6, Table 1) of **2** was similar to these of **1** except that the vinyl group was replaced by a methyl group. The above two compounds are soluble in DMSO and THF, slightly soluble in methanol

Fig. 1. UV absorption spectra of gilvocarcins V(**1**) and M(**2**) in MeOH.



* Gilvocarcin V is very similar to toromycin[2] in physico-chemical properties but differs in UV spectrum (247, 277, 288, 398 nm for toromycin). UV spectrum of chrysomycin[3] is closely related to that of gilvocarcin V but these two compounds differ in optical rotation and molecular weight.

Fig. 2. IR spectra of gilvocarcins V(**1**) and M(**2**). (KBr pellets).

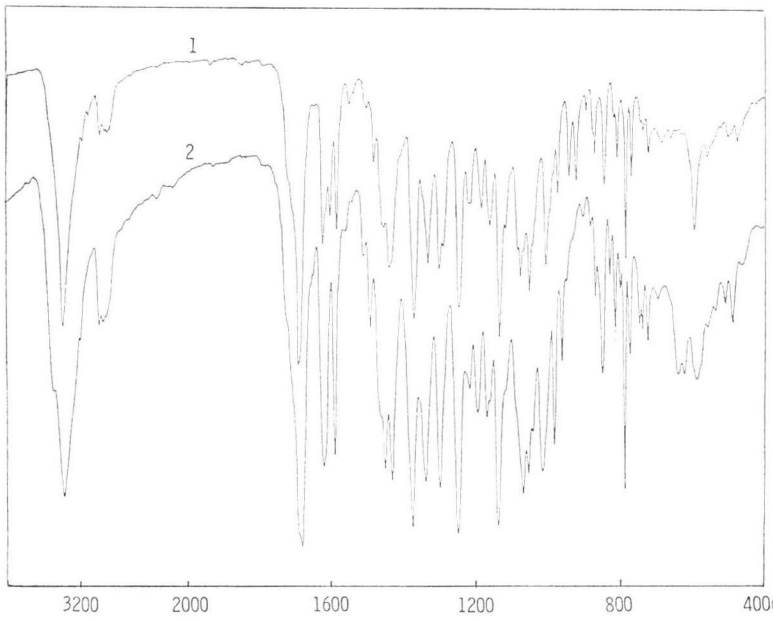
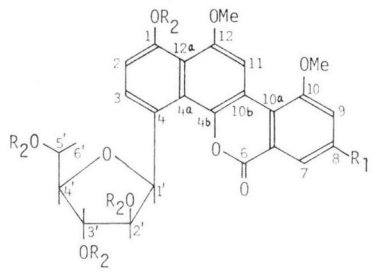


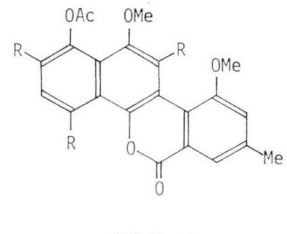
Table 1. ^{1}H-NMR data[a] for **1**, **2**, **3** and **7**.

Proton	**1**	**2**	**3**	**7**
1-R_2	9.66 (1H, s)	9.66 (1H, s)	2.14 (3H, s)	2.15 (3H, s)
2-H	6.92 (1H, d, 8.3Hz)	6.91 (1H, d, 8.3Hz)	7.15 (1H, d, 8.3Hz)	7.21 (1H, d, 8.3Hz)
3-H	8.05 (1H, d, 8.3Hz)	8.05 (1H, d, 8.3Hz)	8.04 (1H, dd, 0.9 & 8.2Hz)	8.07 (1H, dd, 0.7 & 8.3Hz)
7-H	7.93 (1H, d, 1.5Hz)	7.71 (1H, b.s)	7.89 (1H, b.s)	8.50 (1H, d, 1.7Hz)
9-H	7.69 (1H, b.s)	7.40 (1H, b.s)	7.14 (1H, d, 1.5Hz)	7.76 (1H, d, 1.5Hz)
11-H	8.39 (1H, s)	8.37 (1H, s)	8.49 (1H, s)	8.46 (1H, s)
10-OMe	4.14 (3H, s)	4.08 (3H, s)[b]	4.01 (3H, s)	4.08 (3H, s)
12-OMe	4.08 (3H, s)	4.06 (3H, s)[b]	3.97 (3H, s)	3.97 (3H, s)
1′-H	6.18 (1H, d, 4.9Hz)	6.17 (1H, d, 4.9Hz)	6.56 (1H, d, 3.2Hz)	6.44 (1H, d, 3.4Hz)
2′-H	4.67 (1H, m)	4.64 (1H, m)	6.16 (1H, dd, 1.1 & 3.3Hz)	6.12 (1H, dd, 0.7 & 3.2Hz)
3′-H	3.88 (1H, m)	3.88 (1H, m)	5.18 (1H, dd, 1.0 & 3.9Hz)	5.17 (1H, dd, 0.9 & 4.0Hz)
4′-H	3.53 (1H, dd, 3.9 & 5.6Hz)	3.53 (1H, dd, 4.2 & 5.6Hz)	4.19 (1H, dd, 3.9 & 6.4Hz)	4.20 (1H, dd, 3.8 & 6.5Hz)
5′-H	3.88 (1H, m)	3.88 (1H, m)	5.38 (1H, dq, 6.4 & 6.4Hz)	5.39 (1H, dq, 6.5 & 6.5Hz)
6′-Me	1.26 (3H, d, 6.3Hz)	1.26 (3H, d, 6.3Hz)	1.43 (3H, d, 6.4Hz)	1.43 (3H, d, 6.5Hz)
5′-R_2	5.07 (1H, d, 4.9Hz)[b]	5.05 (1H, d, 4.9Hz)[c]	2.38 (3H, s)[b]	2.38 (3H, s)[b]
3′-R_2	4.83 (1H, d, 4.9Hz)[b]	4.82 (1H, d, 4.9Hz)[c]	2.31 (3H, s)[b]	2.32 (3H, s)[b]
2′-R_2	4.48 (1H, d, 6.8Hz)	4.45 (1H, d, 6.8Hz)	1.52 (3H, s)	1.52 (3H, s)
	5.48 (1H, d, 9.2Hz)			
R_1	6.11 (1H, d, 18.6Hz)	2.50 (3H, s)	2.49 (3H, s)	10.08 (1H, s)
	6.93 (1H, dd, 9.2 & 18.6Hz)			

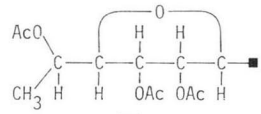
[a] δ in ppm 100 MHz with TMS as an internal standard.
[b],[c] Assignments within any vertical column may be reversed.



(1) R_1= $CH=CH_2$ R_2= H
(2) R_1= CH_3 R_2= H
(3) R_1= CH_3 R_2= $COCH_3$
(7) R_1= CHO R_2= $COCH_3$
(8) R_1= $CH=CH_2$ R_2= $COCH_3$



(5) R= H
(6) R= D



(4)

and acetone, and insoluble in water, ether, and *n*-hexane.

Treatment of **2** with acetic anhydride and pyridine gave a tetraacetyl derivative (**3**), mp 217~218°C, $[\alpha]_D^{17}$ −170.5° (*c* 1.0, $CHCl_3$), MS: *m/z* 650.2012 (M^+) (Calcd. for $C_{34}H_{34}O_{13}$: 650.1999), which showed IR ν_{max}^{KBr} 1748 and 1728 cm^{-1}. In ^1H-NMR of **3** ($CDCl_3$, Table 1), four acetyl signals were observed and one of which appeared at abnormally high field (1.52 ppm). Detailed spin decoupling experiments on **3** clarified the presence of pairs of ortho coupling protons, meta coupling protons, and a low field proton at δ 8.49 in aromatic region and the presence of the structural unit **4**, namely a furanose moiety.

Hydrolysis of **3** with HCl gas in methanol under refluxing, followed by reacetylation with acetic anhydride and pyridine, afforded its chromophore part (**5**) in a moderate yield. ^1H-NMR of **5**($CDCl_3$) showed signals at δ 2.40 (3H, s, OCOMe), 2.48 (3H, s, Ar-Me), 3.98, 4.02 (each 3H, s, OMe), 7.05 (1H, b.s, 9-H), 7.11 (1H, dd, 1.2 & 7.6, 2-H), 7.50 (1H, dd, 7.6 & 8.6, 3-H), 7.85 (1H, b.s, 7-H), 8.31 (1H, s, 11-H), and a newly appeared proton at 8.43 (1H, dd, 1.2 & 8.5, 4-H), and **5** showed a molecular ion at *m/z* 378.1094 (Calcd. for $C_{22}H_{18}O_6$: 378.1103) in the mass spectrum.

These facts suggested that gilvocarcins have a benzonaphtopyran-one system, to which the furanose moiety must be linked through a C-C glycosyl bond. The acid hydrolysis of **3** in 20% DCl-CD_3OD, followed by reacetylation, gave a deuterated chromophore (**6**): MS: *m/z* 381.1277 (M^+) (Calcd. for $C_{22}H_{15}D_3O_6$: 381.1291), ^1H-NMR ($CDCl_3$): δ 2.40 (3H, s, OCOMe), 2.50 (3H, s, Ar-Me), 3.99, 4.05 (each 3H, s, OMe), 7.16 (1H, b.s, 9-H), 7.57 (1H, s, 3-H), and 7.95 (1H, b.s, 7-H). This experiment suggested that the 2, 4 and 11-hydrogens were exchanged with D, and a phenolic OH was attached at 1-position. ^{13}C-NMR ($CDCl_3$, Table 2) of **3** indicates 34 signals, which were assigned by analysis of off-resonance proton-decoupled ^{13}C-NMR, fully ^1H-coupled ^{13}C-NMR and selective proton decoupled spectra. The precise arrangement of substituents on aromatic ring was defined by long-range selective proton decoupling. For example the C-1 resonance, 145.9 ppm, appeared as a doublet of doublets (11.0 & 3.7 Hz) due to coupling to 3-H and 2-H, collapsing to a doublet on selective low-power irradiation of 2-H or 3-H. Irradiation of 7-H (δ 7.89) in the similar way changed the doublet (3.7 Hz) at 159.8 ppm (C-6) to a singlet. Irradiation of the high field acetyl signal at δ 1.52 changed the multiplet at 168.3 ppm (2′-CO) to a doublet (3.7 Hz) coupled with 2′-H. This multiplet was also collapsed to a quartet (7.3 Hz) by irradiation of 2′-H at δ 6.16. The aldehyde derivative (**7**), which was

Table 2. ^{13}C-NMR data[a] for **1**, **2** and **3**.

Carbon	1[b]	2[b]	3[c]	Carbon	1[b]	2[b]	3[c]
1	152.5	152.5	145.9	8-R_1	116.5, 135.0	21.0	21.3[e]
2	111.7	111.4	119.9	10-OMe	56.2[d]	56.0[d]	55.8[d]
3	128.7	128.8	127.3	12-OMe	55.7[d]	55.7[d]	55.7[d]
4	125.7	125.5	129.6	1′	80.8	80.9	81.8
4a	123.3	123.4	124.0	2′	78.8	79.0[e]	77.8
4b	141.9	141.3	141.0	3′	78.8	78.7[e]	78.9
6	159.3	159.4	159.8	4′	85.8	86.1	83.4
6a	121.6	121.1	121.7	5′	66.5	66.6	69.7
7	118.7	120.7	121.8	6′-Me	20.1	20.1	16.4
8	138.1	139.7	140.0	1-CO			169.7
9	113.8	118.0	117.7	2′-CO			168.3
10	156.8	156.4	156.8	3′-CO			170.4
10a	122.5	120.5	121.0	5′-CO			170.4
10b	112.5	112.7	114.4	1-COMe			21.3
11	100.9	101.1	104.6	2′-COMe			20.1
12	151.3	151.3	150.5	3′-COMe			21.1[e]
12a	114.5	114.5	119.3	5′-COMe			20.9[e]

[a] δ in ppm 25.2 MHz with TMS as an internal standard. [b] Solvent, DMSO-d_6.
[c] Solvent, $CDCl_3$. [d],[e] Assignments within any vertical column may be reversed.

obtained by oxidative cleavage of tetraacetylgilvocarcin V (**8**) with osmium tetroxide and sodium periodate in dioxane and water, showed 48% and 13% enhancement of the integrated area of 11-H and 9-H, respectively, when its OMe at δ 3.97 was irradiated. The similar Nuclear Overhauser effects of 25% and 36% were also observed at 11-H and 9-H, respectively, when the another OMe at δ 4.08 was irradiated.

These observations can only be accommodated by the structure **3** for tetraacetylgilvocarcin M. The configuration of furanose was not confirmed by the coupling constants of ^{1}H-NMR of **3**. X-Ray crystallography confirmed the gross structure **2** and yielded the relative stereochemistry.[4] Gilvocarcin V (**1**) have a unique structure, 4-fucofuranosyl-1-hydroxy-10,12-dimethoxy-8-vinyl-6H-benzo[d]-naphtho[1,2b]pyran-6-one, and gilvocarcin M (**2**) is its 8-methyl isomer.

Experimental

General

The spectrometric data were obtained by the following instruments. Infrared spectra; Shimadzu IR-27G. Mass spectra; JEOL JMS-O1SG-2. ^{13}C- and ^{1}H-NMR spectra; JEOL FX-100. Optical rotations; Perkin-Elmer 141 polarimeter. Ultra violetspectra; Hitachi Model 200-20 spectrophotometer.

Tetraacetylgilvocarcin M(3)

To a suspension of gilvocarcin M (**2**, 550 mg) in pyridine, acetic anhydride (3 ml) was added and the suspension was stirred for 19 hours at room temperature. The reaction mixture was poured into ice-water, the precipitates were collected by filtration, washed with water, and then dissolved in chloroform. The chloroform layer was washed with water, dried over sodium sulfate, and evaporated to leave a yellow solid, which was recrystallized from benzene to give **3** as pale yellow needles (660 mg).

Anal. Calcd. for $C_{34}H_{34}O_{13}$: C, 62.76; H, 5.28.
Found: C, 62.96; H, 5.30.

Hydrolysis of Tetraacetylgilvocarcin M

a) A solution of **3** (200 mg) in 10% dry HCl-methanol (30 ml) was refluxed for 16 hours. After evaporation of solvent, the residue was dissolved in pyridine (5 ml) and acetic anhydride (1 ml), and stirred for 3 hours at room temperature. The reaction mixture was poured into ice-water and extracted with chloroform. The extracts were washed with 5% HCl and water, dried over sodium sulfate, and evaporated to leave a brown viscous syrup, which was purified on silica gel chromatography using chloroform as eluant to give a plae yellow viscous syrup. Further purification of its was done by a preparative TLC ($CHCl_3$ - MeOH, 50: 1) to afford **5** as a pale yellow powder (15 mg).

b) A solution of **3** (100 mg) in 20% DCl (0.5 ml) and CD_3OD (3 ml) was heated at 100°C for 24 hours in a sealed tube. After evaporation of solvent, the residue was dissolved in pyridine (2 ml) and acetic anhydride (0.5 ml), and kept standing for 4 hours. The reaction mixture was poured into ice-water and extracted with chloroform. The extract was washed with 5% HCl and water, dried over sodium sulfate, and evaporated to leave a brown syrup, which was purified by a preparative TLC ($CHCl_3$ - MeOH, 50: 1) to afford **6** as a pale yellow powder.

Tetraacetylgilvocarcin V (**8**)

To a suspension of gilvocarcin V (**1**, 100 mg) in pyridine (2 ml), acetic anhydride (0.5 ml) was added and the suspension was stirred for 15 hours at room temperature. The reaction product was poured into ice-water, and extracted with chloroform. The extract was washed with 5% HCl and water, dried over sodium sulfate, and evaporated to give **8** as a yellow solid, which was recrystallized from benzene - pet. ether to afford yellow needles (120 mg). mp. 195~196°C, IR (KBr) 1740 cm^{-1}, MS *m/z*: 662 (M^+), 620, 483, 441, 377.

Anal. Calcd. for $C_{35}H_{34}O_{13}$: C, 63.44; H, 5.17.
Found: C, 63.58; H, 5.12.

Oxidative Cleavage of **8**

To a stirred solution of **8** (200 mg) in dioxane (15 ml) and water (3 ml) was added a solution of osmium tetroxide (100 mg) in dioxane (5 ml), and the solution was stirred for 10 minutes. To the above reaction mixture was added sodium periodate (800 mg) in small portions during 30 minutes and the mixture was stirred for 2 hours. It was diluted with water and extracted with chloroform. The extract was washed with water, dried over sodium sulfate, and evaporated to leave a brown syrup, which was purified on silica gel chromatography using ethyl acetate - chloroform as eluant. Fraction of ethyl acetate - chloroform (1: 9) gave **7** (110 mg) as yellow solid, which was recrystallized from benzene to give yellow needles, mp. 243~244°C, $[\alpha]_D^{17}$ −184° (*c* 1.35, $CHCl_3$), IR (KBr) 1735, 1693, 1605, 1370, 1240, 1215 cm^{-1}, UV λ_{max}^{MeOH} 245, 269, 278, 400 nm, MS *m/z*: 664 (M^+), 622, 485, 443.

Anal. Calcd. for $C_{34}H_{32}O_{14}$: C, 61.44; H, 4.85.
Found: C, 61.69; H, 4.86.

Added in Proof

After submitting this paper, the chromophore of toromycin was found to be same as that of gilvocarcin V. (HORII, S., *et al.*: Chemistry of toromycin. Chem. Pharm. Bull. 28: 3601~3611, 1980).

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