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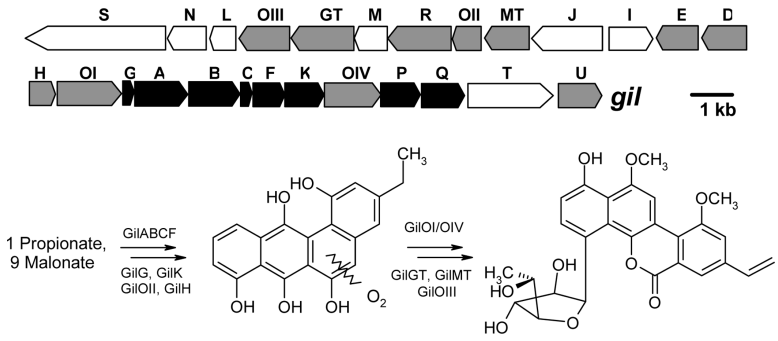

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The Complete Gene Cluster of the Antitumor Agent Gilvocarcin V and Its Implication for the Biosynthesis of the Gilvocarcins

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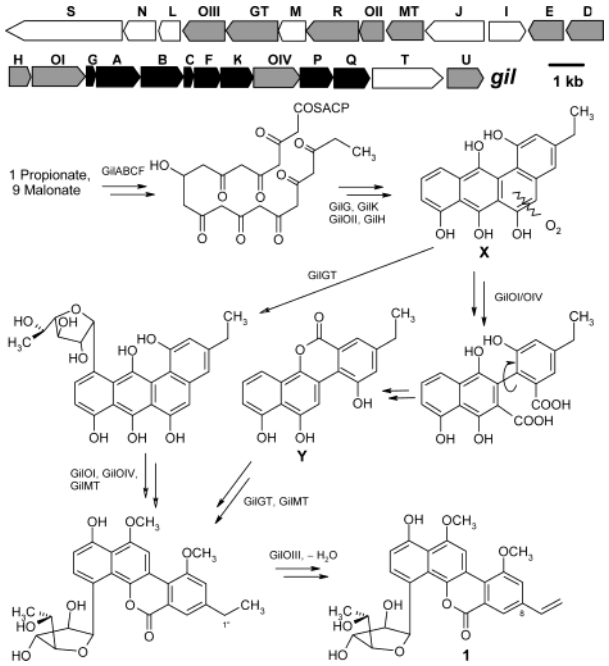
The benzo[*d*]naphtho[1,2-*b*]pyran-6-one *C*-glycoside antibiotics, often referred to as gilvocarcin-type aryl-*C*-glycosides, were discovered in Japan in the early 1980s. This distinct family of antitumor antibiotics shows excellent antitumor activity and remarkably low toxicity, and therefore has proven attractive for synthetic organic chemistry as well as for biological activity studies. The group consists of the gilvocarcins (syn. toromycins, anandimycins), ravidomycins, the ravidomycin analogues FE35A and B, the chrysomycins (syn. virenomycin, albacarcins; including recent derivatives possessing branched ketofuranose and ketopyranose sugar moieties), and BE-12406 A and B.[1−5]

Gilvocarcin V **1** (GV), the principal product of *Streptomyces griseoflavus* Gö 3592[6] and of various other *Streptomyces* strains, is the most studied member of the gilvocarcin-type aryl-*C*-glycosides, because of its potent bactericidal, virucidal, cytotoxic, and antitumor activities. GV (**1**) is one of the strongest antitumor compounds among these drugs, requiring only low concentrations and maintaining a low in vivo toxicity. The exact molecular mechanisms responsible for the in vivo mode of action of GV are still unclear. However, it was found that **1** exhibits a strong tendency to intercalate with DNA. Both equilibrium DNA binding and UV light-induced DNA adduct formation were found. GV also causes topoisomerase II inhibition.[3a] The vinyl group is essential for the antitumor activity, since the minor congeners gilvocarcins M and E, in which the vinyl group is replaced by a methyl group and an ethyl group, respectively, are significantly less effective.[7,8] Photobiological studies showed that the vinyl group undergoes a [2 + 2] cycloaddition with DNA thymine residues under photoirradiation. Moreover, it was shown recently that GV promotes protein-DNA cross-linking when photoactivated by near-UV light, and histone H3, which plays an important role in DNA replication and transcription, was identified as one of the selectively cross-linked proteins. This cross-linking with histone H3 was discussed as part of the unique molecular mechanisms of the potent antitumor activity of GV and might contribute to the better and more specific activity of GV compared to other intercalating antitumor drugs.[3b]

GV's unprecedented molecular architecture in conjunction with its unique biological activity makes **1** an excellent target for the study of its biosynthesis and the development of novel, improved anticancer drugs through combinatorial biosynthesis. The structural features and knowledge gained from biosynthetic incorporation studies[9] imply the participation of unique post-polyketide synthase (PKS) tailoring enzymes[10] promoting (i) an oxidative rearrangement of an angucyclinone intermediate, (ii) a *p*-OH-activated *C*-glycosylation, and (iii) the formation of the vinyl group side chain. None of these steps, which all provide structural elements essential for the biological activity of the drug, is fully understood, or has been studied in detail.

The identification of the *gil* gene cluster is the first step to study the intriguing mechanisms associated with GV-biosynthesis. This

Scheme 1. The Complete Gilvocarcin Gene Cluster and the Postulated Biosynthetic Sequence of Events[a]



[a] Black genes code for the PKS and associated enzymes, gray genes for post-PKS tailoring steps, and white genes for regulation, transport, resistance, and enzymes of unknown function.

was achieved by generating a *S. griseoflavus* genomic cosmid library using the *Streptomyces*−*E.coli* shuttle vector pOJ446. DNA fragments of the NDP-glucose-4,6-dehydratase[11a] (an enzyme catalyzing a key step in 6-deoxysugar biosynthesis) and also the *act*I PKS[11b] genes were used to probe the cosmid library. Cosmid DNA isolated from clones hybridizing with both probes was analyzed by restriction mapping and Southern blot experiments. One of the cosmids, cos-G9B3, was transformed into *S. lividans* TK24, where it stimulated the production of gilvocarcins V and M in the same quantities as the wild-type strain (20−30 mg/L of **1**), proving that it most likely contains the entire gene cluster of gilvocarcin biosynthesis.

Subcloning of cosG9B3-DNA fragments into pUC19 or pBluescript II SK(+) followed by sequencing revealed the entire gilvocarcin gene cluster (Scheme 1). The cluster spans a 32.9 kB region and consists of 26 open reading frames (ORFs). The database analysis (see Supporting Information) yielded expected genes encoding the PKS and associated enzymes (Scheme 1, black), several genes encoding post-PKS tailoring steps (gray), and genes of regulation and self-resistance enzymes (white, this group also includes three genes coding for proteins of unknown function, namely *gilLMN*).[12]

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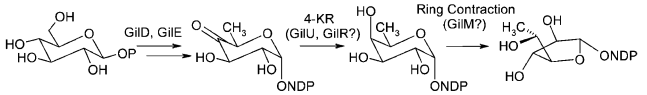
Scheme 2. Proposed Involvement of Enzymes of the Gilvocarcin Pathway in the Biosynthesis of GV's D-Fucofuranose Moiety



Not surprisingly, the genes encoding the PKS and associated enzymes are in a typical type II PKS arrangement,[13] in which the "minimal PKS" genes *gilABC* encoding KS_α, KS_β, and the acyl carrier protein ACP, respectively, are flanked by genes encoding the PKS-associated ketoreductase (KR, *gilF*) and two cyclases (*gilG* and *gilK*). More unusual are the genes *gilP* and *gilQ*, located further downstream of *gilABC*, which encode a malonyl CoA:ACP transacylase (MAT) and an acyl transferase (AT), respectively. Although essential for polyketide biosynthesis, MATs are usually not found in type-II PKS gene clusters and are often "recruited" from the fatty acid synthase.[14] The *gil* gene cluster is the third example in which such a gene was located, but the first one where the cluster is associated with a known structure.[15] GilQ resembles AT proteins found in producers of aromatic polyketides with starter units other than acetate, such as doxorubicin, enterocin, etc.[16] Therefore, GilQ might play a role in the choice of the starter unit (propionate vs acetate for the production of gilvocarcin V and gilvocarcin M, respectively).

The following genes are suspected to be responsible for the post-PKS tailoring steps including the above-mentioned intriguing biosynthetic steps toward the key structural features of GV. Four oxygenase-encoding genes were found. Genes *gilOI* and *gilOIV* encode FAD-dependent oxygenases assumed to catalyze the oxidative rearrangement of a putative angucyclinone-precursor to the unique coumarin-based aromatic core of the gilvocarcins. The corresponding enzymes GilOI and GilOIV are closely related to JadF and JadH,[16] which probably catalyze a similar rearrangement in the jadomycin pathway (see also phylogenetic tree in the Supporting Information). As one of the interesting aspects of the GV-biosynthesis, we will address in future studies whether this oxidative rearrangement occurs prior to or after the *C*-glycosylation step (Scheme 1, dotted or solid arrows). The other two oxygenases are most likely responsible for the anthrone oxidation leading to the angucyclinone intermediate **X** (GilOII) and for the generation of the 8-vinyl group. For the latter, we assume a hydroxylation in the 1"-position through GilOIII followed by dehydration, since GilOIII is a P-450 hydroxylase predestined for such a reaction. Other oxidoreductase encoding genes are *gilH* (encoding a KR presumably involved in the hydroquinone generation) and *gilR* (encoding an oxidoreductase of unclear function).

The *C*-glycosidically linked D-fucofuranose is a unique deoxysugar not found in any other polyketide,[18] and whose biosynthesis requires only a few enzymes (Scheme 2). This is confirmed by the presence of only a few typical deoxysugar biosynthesis genes in the *gil* cluster. Two of these crucial genes, *gilD* and *gilE*, encode NDP-glucose synthase and 4,6-dehydratase, respectively. A third gene possibly involved in the D-fucose biosynthesis is *gilU* located at the end of the *gil* cluster. GilU, apparently an epimerase/dehydratase, or oxidoreductase GilR, might function as 4-KR, while it is unclear how the contraction from the pyranose to the furanose is catalyzed (GilM?). Finally, *gilGT* encodes the glycosyl transferase (GT) responsible for the unusual *p*-OH activated *C*-glycosylation. Synthetic model studies suggest that the favored mechanism for *C*-glycosyltransfer is an initial *O*-glycosylation followed by a Fries-like rearrangement.[2,19] Although principally possible, it is difficult to imagine in an enzymatic environment that such a rearrangement to the *p*-position occurs. GilGT resembles most closely LanGT2 and UrdGT2,[20] both of which transfer D-olivoses to angucyclinone acceptor molecules, the latter being also a *C*-GT. However, in contrast to GilGT, UrdGT2 places its sugar moiety *ortho* to a phenolic OH group. An interesting novelty of GilGT is its unusual size, due to its N-terminal part being approximately 120 amino acids longer than any other polyketide GT found so far.

In summary, the *gil* gene cluster revealed all the genes whose corresponding enzymes are likely to be involved in the intriguing steps of **1** biosynthesis (Scheme 1) and which are crucial for establishing structural moieties important for its unique biological activities. Thus, the work presented here lays the foundation for the further study of these steps, and possibly for the generation of improved gilvocarcin-type anticancer drugs through combinatorial biosynthesis.

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Supporting Information Available: Table of deduced functions for genes of the gilvocarcin cluster (GeneBank access #AY233211), corresponding protein sizes and closest homologues. Phylogenetic relation of GilOI/GilOIV to other oxygenases. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Hirayama, N.; Takahashi, K.; Shirahata, K.; Ohashi, Y.; Sasada, Y. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 1338−1342. (b) Krohn, K.; Rohr, J. *Topics Curr. Chem.* **1997**, *188*, 127−195, and references cited therein.
(2) Hosoya, T.; Takashiro, E.; Matsumoto, T.; Suzuki, K. *J. Am. Chem. Soc.* **1994**, *116*, 1004−1015.
(3) (a) Knobler, R. M.; Radlwimmer, F. B.; Lane, M. J. *Nucleic Acid Res.* **1992**, *20*, 4553−4557. (b) Matsumoto, A.; Hanawalt, P. C. *Cancer Res.* **2000**, *60*, 3921−3926.
(4) (a) Yamashita, N.; Shin-Ya, K.; Furihata, K.; Hayakawa, Y.; Seto, H. *J. Antibiot.* **1998**, *51*, 1105−1108. (b) Nakashima, T.; Fujii, T.; Sakai, K.; Tomohiro, S.; Kumagai, H.; Yoshioka, T. U.S. Patent 6,030,951, 2000.
(5) Morimoto, M.; Okubo, S.; Tomita, F.; Marumo, H. *J. Antibiot.* **1981**, *34*, 701−707.
(6) Breiding-Mack, S.; Zeeck, A. *J. Antibiot.* **1987**, *40*, 953−960.
(7) (a) Yamashita, Y.; Nakano, H. *Nucleic Acids Res. Symp. Ser.* **1988**, *20*, 65−67. (b) Elespuru, R. K.; Gonda, S. K. *Science* **1984**, *223*, 69−71.
(8) Oyola, R.; Arce, R.; Alegria, A. E.; Garcia, C. *Photochem. Photobiol.* **1997**, *65*, 802−810, and references cited therein.
(9) Takahashi, K.; Tomita, F. *J. Antibiot.* **1983**, *36*, 1531−1535.
(10) Rix, U.; Fischer, C.; Remsing, L. L.; Rohr, J. *Nat. Prod. Rep.* **2002**, *19*, 542−580, and references cited therein.
(11) (a) Decker, H.; Gaisser, S.; Pelzer, S.; Schneider, P.; Westrich, L.; Wohlleben, W.; Bechthold, A. *FEMS Microbiol. Lett.* **1996**, *141*, 195−201. (b) Hopwood, D. A. *Chem. Rev.* **1997**, *97*, 2465−2497.
(12) Altschul, S. F.; Madden, T. L.; Schaffer, A. A.; Zhang, J. H.; Zhang, Z.; Miller, W.; Lipman, D. J. *Nucleic Acid Res.* **1997**, *25*, 3389−3402.
(13) Staunton, J.; Weissmann, K. J. *Nat. Prod. Rep.* **2001**, *18*, 380−416.
(14) Summers, R. G.; Ali, A.; Shen, B.; Wessel, W. A.; Hutchinson, C. R. *Biochemistry* **1995**, *34*, 9389−9402.
(15) Novakova, R.; Bistakova, J.; Homerova, D.; Rezuchova, B.; Kormanec, J. *Gene* **2002**, *297*, 197−208.
(16) (a) Hutchinson, C. R. *Chem. Rev.* **1997**, *97*, 2525−2536. (b) Moore, B. S.; Hertweck, C. *Nat. Prod. Rep.* **2002**, *19*, 70−99. (c) Marti, T.; Hu, Z.; Pohl, N. L.; Shah, A. N.; Khosla, C. *J. Biol. Chem.* **2000**, *275*, 33443−33448.
(17) (a) Yang, K.; Han, L.; Ayer, S. W.; Vining, L. C. *Microbiology* **1996**, *142*, 123−132. (b) Vining, L. C., personal communication.
(18) From the original X-ray resolved structure, the wrong enantiomer of GV (with an L-fucofuranose moiety) was chosen, which dominates the literature, and even remains in the newest literature on GV, although the correct structure (with D-fucofuranose) was established in 1994 by Suzuki et al. through total synthesis.[2]
(19) Palmacci, E. R.; Seeberger, P. H. *Org. Lett.* **2001**, *3*, 1547−1550.
(20) (a) Künzel, E.; Faust, B.; Oelkers, C.; Weissbach, U.; Bearden, D. W.; Weitnauer, G.; Westrich, L.; Bechthold, A.; Rohr, J. *J. Am. Chem. Soc.* **1999**, *121*, 11058−11062. (b) Westrich, L.; Domann, S.; Faust, B.; Bedford, D.; Hopwood, D. A.; Bechthold, A. *FEMS Microbiol. Lett.* **1999**, *170*, 381−387.

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