DOI: 10.1002/cbic.200500441

Biosynthesis of the Heat-Shock Protein 90 Inhibitor Geldanamycin: New Insight into the Formation of the Benzoquinone Moiety

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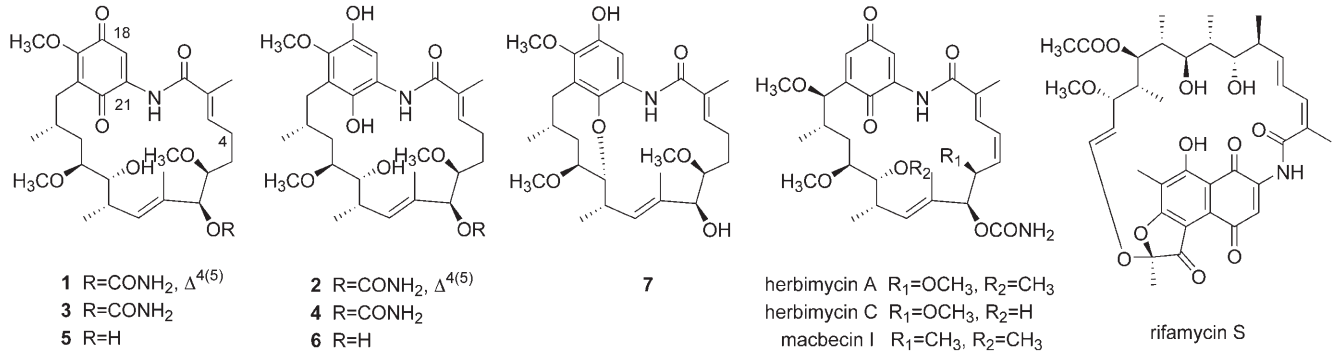
The ansamycin family, which features benzenic or naphthalenic moieties connected by an aliphatic polyketide chain, includes rifamycins and geldanamycin (**1**), which are used as antibiotics and/or anticancer agents (Scheme 1).[1,2] Geldanamycin binds to the ATP-binding site of heat-shock protein (Hsp) 90 and inhibits the chaperone activity of the protein; this leads to destabilization of the Hsp90 client proteins, which are critical in signal-transduction pathways.[3,4] Accordingly, several geldanamycin analogues, including 17-allylamino-17-demethoxygeldanamycin, are in various stages of clinical trials as novel antitumor agents.[5] Many ansamycin analogues have been produced by chemical modifications of natural products. These chemical modifications have mostly been restricted to one region of the compounds, the C-17 position of the benzoquinone moiety of geldanamycin or the C-3 position of the naphthoquinone moiety of rifamycins.[2,6,7] However, due to the occurrence of resistance and the hepatotoxicity of the current analogues, new ansamycins with improved efficacies, along with reduced toxicity and favorable pharmacological profiles, are currently in high demand.[2,8] Consequently, a clear understanding of ansamycin biosynthesis will be useful for the development of new ansamycin analogues.

Geldanamycin, produced by *Streptomyces hygroscopicus*, is a 19-membered macrocyclic lactam that is related to benzoquinone ansamycins, such as herbimycin and macbecin (Scheme 1).[9–11] The biosynthesis of this class of compounds involves the assembly of 3-amino-5-hydroxybenzoic acid (AHBA) as a starter unit, followed by the sequential addition of extender units, such as acetate, propionate, and glycolate. This process of assembly forms a polyketide backbone, which then undergoes further tailoring processing. The genes required for geldanamycin biosynthesis, including a set of polyketide synthase (PKS) genes, have been cloned, sequenced, and analyzed from several strains of *Streptomyces*. The initial PKS product, progeldanamycin, is then converted to geldanamycin by the post-PKS modification steps, which include C-17 hydroxylation, C-17 *O*-methylation, C-21 oxidation, C-7 carbamoylation, and C-4,5 oxidation.[12–16]

Floss and colleagues reported that the naphthoquinone moiety of rifamycins was formed by oxidative cyclization during, not after, PKS assembly, presumably between the third and fourth polyketide-chain-extension steps. This transformation requires enzymatic oxidation to the benzoquinone, followed by a Michael addition driven by reoxidation of the naphthohydroquinone to the quinone.[1,17] However, until recently, very little was known about the biosynthetic process of the benzoquinone moiety of benzenic ansamycins. We recently reported that 4,5-dihydro-7-*O*-descarbamoyl-7-hydroxygeldanamycin (**5**) is the main product of a culture of a *gel8* gene-inactivated strain of *Streptomyces hygroscopicus* subsp. *duamyceticus* JCM4427, and refined the post-PKS modification steps of geldanamycin biosynthesis.[12] Herein, we report the finding and structure elucidation of a tricyclic geldanamycin derivative (ACDL3172, **7**),[18] which provides a clue to understanding the formation of the benzoquinone in the process of geldanamy-



Scheme 1. Geldanamycin and related ansamycins.

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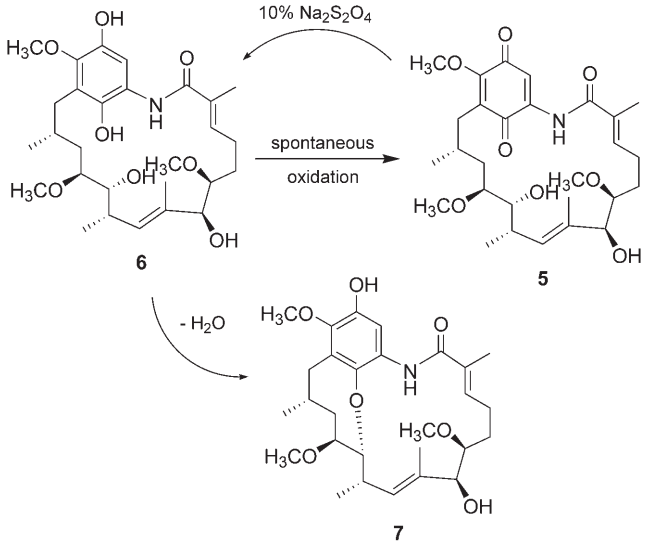
cin biosynthesis. This new metabolite is produced in trace amounts (~1 mg L^{-1}) and was initially overlooked in the study of the *gel8* gene-inactivated strain.

ACDL3172 (**7**) was obtained as an amorphous yellowish powder, and its ^{1}H NMR data are closely comparable to those of compound **5**; this indicates the absence of the carbamoyl group and the C-4,5 *cis* double bond of geldanamycin.[12] In **7**'s ^{13}C NMR spectrum, however, the absence of signals corresponding to the benzoquinone at C-18 and C-21, and the presence of two additional oxygenated aromatic signals at $\delta_C =$ 146.68 and 143.13, led to the inference that **7** is a hydroquinone of **5**, a prospective intermediate of geldanamycin biosynthesis. This inference was supported by the HMBC cross peaks for H-15/C-21 and H-19/C-18, C-21. A complete analysis of 1D and 2D NMR data confirmed that **7** is a hydroquinone compound of **5**, except for the difference of 18 mass units. The downfield shift of the H-11 signal at $\delta_H = 3.77$, relative to those of **5** and related compounds, and the mass data, including the ESI-MS^n fragmentation pattern, indicated that **7** forms an additional ether ring that connects C-11 and C-21. This was consistent with the molecular formula $C_{28}H_{41}NO_7$ obtained by HRESI-TOFMS. Accordingly, the structure of this new metabolite was determined to be that given in Scheme 1.[18]

Of interesting note, the structure of **7** indicates that it is not an intermediate of geldanamycin biosynthesis, but is, in fact, a shunt product that is likely to be derived from 4,5-dihydro-7-*O*-descarbamoyl-7-hydroxygeldanamycin hydroquinone (**6**, $M_W =$ 521) by dehydration. To verify this assumption, the metabolites of the culture of the *gel8* gene-inactivated strain were analyzed, and the presence of a compound with molecular ion of *m/z* 520 $[M-H]^-$ was detected by LC/MS^n.[19] However, it was observed that this metabolite was unstable and was spontaneously converted to **5** during the process of purification from the culture extract. To determine the identity of the metabolite at *m/z* 520, **5**, a major metabolite of the *gel8* gene-inactivated strain, was chemically reduced to the corresponding hydroquinone (**6**) by treatment with aqueous $Na_2S_2O_4$.[6] The identity of the metabolite at *m/z* 520 was confirmed by LC/MS^n analysis to be the same as that of the reduction product (**6**). Interestingly, it was observed that **7** was readily produced from the chemically formed **6** under neutral conditions; this confirms that **7** is derived from **6** by dehydration (Scheme 2).

These results suggest that the benzoquinone moiety of geldanamycin (**1**) is spontaneously produced from a corresponding hydroquinone after hydroxylation of the AHBA moiety at C-21. To confirm this suggestion, chemical conversion of **1** and 4,5-dihydro-geldanamycin (**3**), and analysis of the metabolites of the *S. hygroscopicus* JCM4427 wild-type strain were performed.[19] Compounds **1** and **3** were converted by $Na_2S_2O_4$ to the corresponding hydroquinones, **2** and **4**, respectively, and these hydroquinones were also detected in the culture of the wild-type strain in the LC/MS^n analysis.[19] As in the case of **6**, 4,5-dihydro-geldanamycin hydroquinone (**4**) was auto-oxidized back to **3**. However, **2**, which was also produced in trace amounts in the culture, was quite stable and was purified by HPLC without difficulty.



Scheme 2. Production of **5** and **7** from **6**, which was readily regenerated by chemical reduction of **5**, in the culture of a *gel8* gene-inactivated strain of *Streptomyces hygroscopicus* subsp. *duamyceticus* JCM4427.

Furthermore, the production of **1** and **4** was monitored during the culture of the wild-type strain.[20] This strain produced a relatively large amount of **4** during the early stage of the culture. However, at a late stage of the culture, **4** was consumed, and the production of **1** increased. This result supported the spontaneous transformation of the benzoquinone of **1** from a corresponding hydroquinone.

Based on the results obtained, we conclude that the benzoquinone of **1** is formed by spontaneous oxidation from a corresponding hydroquinone during post-PKS modification, not PKS-bound polyketide assembly. We also concluded that the conversion of 4,5-dihydrogeldanamycin hydroquinone (**4**) to 4,5-dihydrogeldanamycin (**3**) occurs after a C-21 hydroxylase has operated (Scheme 3). Therefore, the oxidation of a hydroquinone to the corresponding quinone in naphthalenic or benzenic ansamycin biosynthesis depends on the hydroxylation of the AHBA moiety by hydroxylases during the process of polyketide assembly or after the formation of the macrocyclic ring, respectively.

Geldanamycin (**1**) is a competitive Hsp 90 inhibitor, which has been the subject of much study during the past few decades. ACDL3172 (**7**), a tricyclic geldanamycin derivative produced by the *gel8* gene-inactivated strain of *S. hygroscopicus* subsp. *duamyceticus* JCM4427, provided a clue towards determining the biosynthetic origin of ansamycin benzoquinone formation from a corresponding hydroquinone by spontaneous oxidation. This might contribute to a better understanding of geldanamycin biosynthesis, which would provide valuable information for the design of new geldanamycin analogues with enhanced biological activity.

Acknowledgements

The work was supported by the 21C Frontier Microbial Genomics and Application Center (Grant MG05-0303-2-0 to Y.-S.H.), the

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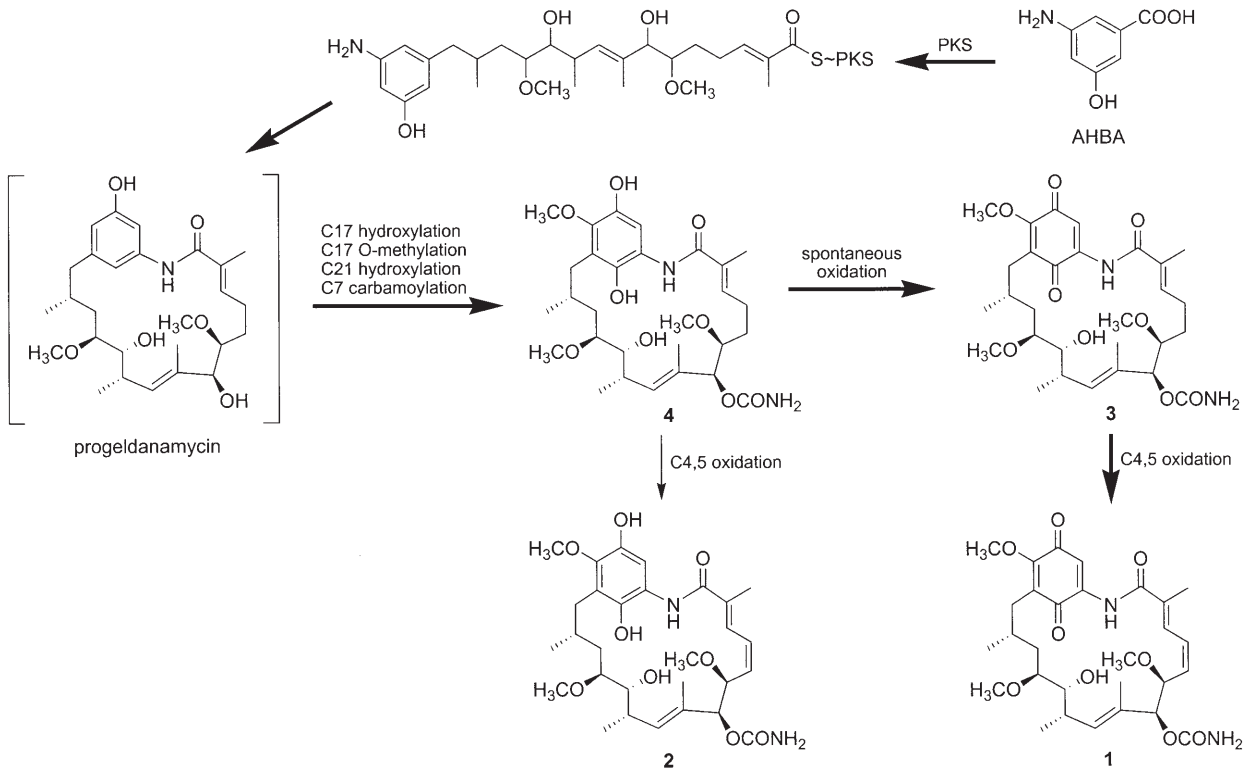

Scheme 3. Biosynthesis of geldanamycin and benzoquinone formation. Thick and thin arrows indicate the major and minor pathways, respectively.

Ministry of Science and Technology, Republic of Korea and by a grant from the KRIBB Research Initiative Program. We thank the Korea Basic Science Institute for providing certain instruments used in this study.

Keywords: antitumor agents · autooxidation · biosynthesis · polyketides

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[18] ACDL3172 (**7**). Amorphous yellowish powder; m.p. 125–128 °C; $[\alpha]_D = +2.3^\circ$ ($c = 0.01$, MeOH); UV (MeOH): λ_{max} (log ε) 317 (3.46), 274 (3.42), 207 nm (3.72); NMR and MS data, see Supporting Information.
[19] LC/MS^n analysis data of A: the culture of *gel8* gene-inactivated strain of *S. hygroscopicus* subsp. *duamyceticus* JCM4427, B: the chemically reduced product from treatment of **5** with 10% $Na_2S_2O_4$, C: the culture of wild-type strain *S. hygroscopicus* subsp. *duamyceticus* JCM4427, D: the chemically reduced product from treatment of **1** with 10% $Na_2S_2O_4$, E: the chemically reduced product from treatment of **3** with 10% $Na_2S_2O_4$, see Supporting Information.
[20] Accumulation of **1** and **4** in the culture of wild-type strain *S. hygroscopicus* subsp. *duamyceticus* JCM4427 was monitored by HPLC, see Supporting Information.

Received: October 26, 2005
Published online on December 28, 2005

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