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Bioconversion of the Anthracycline Analogue Desacetyladriamycin by Recombinant DoxA, a P450-Monooxygenase from *Streptomyces* sp. Strain C5

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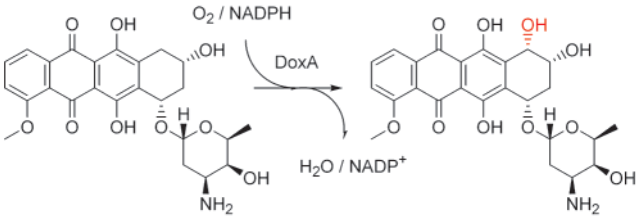
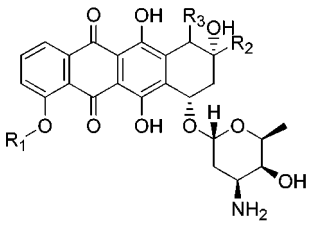
ABSTRACT



A recombinant P450-monooxygenase, DoxA, obtained from *Streptomyces* sp. strain C5, the producer of the anticancer compound daunorubicin, was expressed in *S. lividans* TK24 and therein used to catalyze the conversion of the anthracycline analogue desacetyladriamycin into the new anthracycline, 10-hydroxydesacetyladriamycin. This work establishes a new function for DoxA and demonstrates the use of a recombinant enzyme to prepare a new anthracycline analogue.

Daunorubicin (**1**, daunomycin[1,2]) and doxorubicin (**2**, 14-hydroxydaunorubicin; adriamycin[3]) are clinically useful anthracycline chemotherapeutic agents obtained from *Streptomyces* sp. strain C5[4] and *Streptomyces peucetius* (Figure 1).[3] The anthracycline aglycone is made by the action of a type II polyketide synthase (PKS) which catalyzes the condensation of nine malonyl-CoA "extender" units onto a propionate "starter" unit, with subsequent processing to give



1	R_1=Me	R_2=COMe	R_3=H	daunorubicin (adriamycin)
2	R_1=Me	R_2=$COCH_2OH$	R_3=H	doxorubicin
3	R_1=Me	R_2=CH_2Me	R_3=CO_2Me	rhodomycin D
4	R_1=H	R_2=CH_2Me	R_3=H	deoxycarminomycin
5	R_1=Me	R_2=CH_2Me	R_3=H	deoxydaunomycin
6	R_1=Me	R_2=Me	R_3=OH	feudomycin D
7	R_1=Me	R_2=Me	R_3=H	feudomycin C
8	R_1=Me	R_2=H	R_3=H	desacetyladriamycin

Figure 1. Structures of anthracycline compounds.

the first free intermediate, aklanonic acid.[5] The first glycone intermediate, rhodomycin D (**3**), is converted into deoxy-

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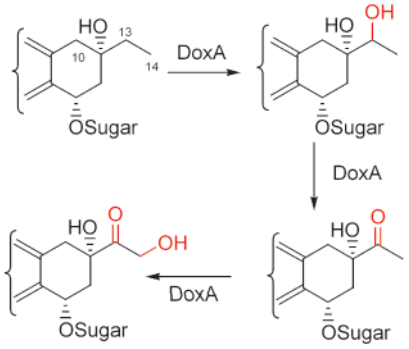
carminomycin (**4**) and deoxydaunomycin (**5**) in part by the actions of an esterase (DauP) and a methyl transferase (DauK).[4] We have recently shown that *one* enzyme, DoxA, a P450-like monooxygenase, catalyzes the last *three* steps of the biosynthesis, these being the oxidative transformations at C-13 and C-14 (Scheme 1).[6,7]

Scheme 1. Oxidative Reactions Catalyzed by P450-Monooxygenase, DoxA, in the Late Stages of Doxorubicin Biosynthesis



In other work we have shown that disruption of two of the PKS encoding genes, genes *dpsCD*, leads to a relaxation of starter unit fidelity such that an acetate, rather than a propionate, can be used.[8] In this case feudomycin D (**6**), a C-9 methyl-substituted anthracycline, is the terminal biosynthesis product. We had hypothesized that feudomycin C (**7**) is the precursor of feudomycin D, the latter being formed by an oxidation at C-10 catalyzed by DoxA. Since DoxA appears to be somewhat promiscuous in its oxidative capabilities, we sought to use DoxA to carry out the oxidation of a number of anthracycline analogues to afford potentially new anthracyclines.

In this study we first determined the potential for oxidation by measuring the catalytic activity[7] of DoxA with a panel of anthracycline analogues including *N*-methyldaunorubicin, *N*,*N*-dimethyldaunorubicin, 3′-hydroxydaunorubicin, desacetyladriamycin (**8**), and 4-phenylmethoxydaunorubicin. Of these only desacetyladriamycin proved to be catalytically competent in assays with the purified, recombinant DoxA. Samples of the anthracycline analogues were also incubated in vitro with purified, recombinant DoxA using a NADPH-regeneration system and analyzed by TLC and HPLC. Only in the case of desacetyladriamycin (**8**) was a new compound present that was absent from control reactions. Conversion of the desacetyladriamycin was fairly rapid in vitro; 3 mg of the recombinant DoxA afforded 100% conversion of 1 mg of the anthracycline in 45 min. As an alternate strategy we used an in vivo technique; the anthracycline was added to a shake flask culture of *S. lividans* TK24(pANT195), which contains the *doxA* gene in a plasmid under control of the SnpR-activated *snpA*-promoter.[4,6] Approximately 0.1 mg of the oxidation product was recovered 24 h after adding 1 mg of desacetyladriamycin to the culture. The new anthracycline was recovered and its structure elucidated by standard HRMS and NMR techniques (Table 1). Unequivocal assignment of the positions in the A-ring [CHOR-CH_2-CHOH-CHOH] came from a COSY experiment. The substitution pattern is only consistent with hydroxylation at C10. The relative stereochemistry at C10 was deduced to be cis on the basis of the 2.3 Hz coupling constant of H10 to H9. Dihedral angles for H10−C10−C9−H9 were estimated from models minimized with the MM2 force field to be approximately 45° for the cis stereochemistry and 170° for the trans stereochemistry. These values led to predicted[9] coupling constants of 3.6 (cis) and 7.6 (trans) Hz compared with the

Table 1. NMR Data for 10-Hydroxydesacetyladriamycin

carbon no.	δ_C (100 MHz, CD_3OD)	δ_H (400 MHz, CD_3OD) (multiplicity, *J*/Hz)
1	120.6	7.93 (d, 8.0)
2	137.5	7.77 (t, 8.4)
3	120.4	7.50 (d, 8.8)
4	162.8	
4-OMe	57.2	3.93 (s)
4a	121.8	
5	188.8	
5a	113.8	
6	157.5	
6a	136.7	
7	70.8	4.89 (m)
8	30.8	2.20 (m)
9	69.2	4.07 (br d, 3.0)
10	65.0	4.93 (d, 2.3)
10a	136.4	
11	157.8	
11a	112.9	
12	188.2	
12a	137.3	
1′	101.4	5.38 (br d, 3.0)
2′	29.6	1.74 (br d, 11.2) 1.90 (m)
3′	47.6	3.43 (m)
4′	68.0	3.51 (m)
5′	67.8	4.16 (q, 6.7)
5′-Me	16.9	1.17 (d, 7.0)

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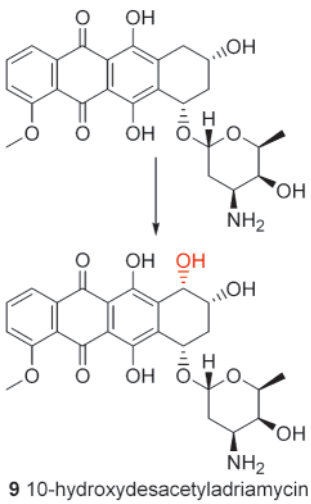
observed value of 2.3 Hz. Oxidation at the C-10 position had undoubtedly occurred, leading to the new anthracycline, 10-hydroxydesacetyladriamycin (Figure 2).



9 10-hydroxydesacetyladriamycin

Figure 2. New function for DoxA; C-10-hydroxylation.

Using our in vitro assay for DoxA activity, we were able to measure kinetic parameters for the oxidation of desacetyladriamycin (k_{cat} 0.0044 s^{-1}, K_m 6 × 10^{-7} M). The oxidation is over 50-fold more efficient than the C-14 hydroxylation of daunorubicin (**1**), one of the known physiological substrates for DoxA, and only 3-fold slower than the most efficient physiologically significant conversion, that is, hydroxylation at the C-13-position of 13-deoxydaunorubicin (**5**).[7]

In this work we have characterized a new function for DoxA, that is, C-10-hydroxylation of the anthracycline ring. DoxA is capable of oxidation at both exocyclic positions, C-13 and C-14, in the propionate-initiated anthracycline ring system and also at C-10 in an anthracycline lacking the side chain. We have also shown that it is possible to produce new anthracyclines through biochemical modification using purified, recombinant enzymes from secondary metabolic pathways. The latter approach is significant as enzyme-catalyzed modification may be a highly efficient method to selectively and mildly alter sensitive, complex natural product structures to generate new compounds.

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