

MICROBIAL TRANSFORMATION OF DAUNOMYCINONE BY  
*STREPTOMYCES AUREOFACIENS* B-96

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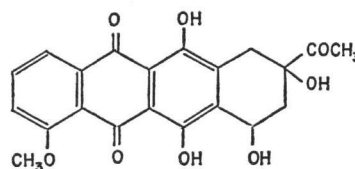
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Daunomycinone, aglycone of the anthracycline antibiotic daunomycin, was transformed by a washed mycelia of *Streptomyces aureofaciens* B-96 in a buffer solution containing sucrose; the obtained product, dihydrodaunomycinone (9-(1-hydroxyethyl)-7,8,9,10-tetrahydro-6,7,9,11-tetrahydroxy-4-methoxy-5,12-naphthacenequinone), was identified by measuring basic physico-chemical characteristics (IR, UV and visible spectra, mass spectra and NMR, optical rotation and m.p.).

The mutant strain, *Streptomyces aureofaciens* B-96 producing the glycoside aureovocin, has the ability to glucosidize certain hydroxyderivatives of anthraquinones<sup>9,10</sup>. In the present communication this strain was used to investigate transformation of daunomycinone (Fig. 1), aglycone of the antitumor antibiotic daunomycin.

Fig. 1. Daunomycinone.



### Material and Methods

#### Spectra

Ultraviolet and visible spectra were measured in a Cary 118C spectrophotometer (methanol), infrared spectra (KBr tablets) were measured in a Unicam SP-200 spectrophotometer, mass spectra with a Varian MAT-311 (energy of ionizing electrons 70 eV, current of ionizing electrons 1 mA, temperature of ion source 200°C, temperature of direct inlet system 170°C, high resolution measurement was performed with an error less than 5 ppm) and metastable transformations were studied using the DADI technique<sup>11</sup>. NMR spectra were measured in a Varian HA-100 (100 MHz) spectrometer in a mixture of CDCl<sub>3</sub>+d<sub>6</sub>-DMSO, TMS serving as internal standard. Optical rotations were determined in a Jasco-5 spectropolarimeter.

#### Strain and Cultivation

The strain, *Streptomyces aureofaciens* B-96<sup>4</sup>, was maintained on agar slants<sup>7</sup>. Spores were transferred to the inoculation medium containing soya-bean meal<sup>7</sup>.

The flasks with the fermentation medium<sup>8</sup> were inoculated with a 16-hour inoculum (5%). After a 20-hour cultivation, the mycelia of the second vegetative generation were separated by centrifugation (2°C, 10 minutes, 1,000 g), washed with water, excess water was removed by centrifugation (2°C, 10 minutes, 10,000 g) and the mycelia were maintained at -20°C.

The strain, *S. aureofaciens* B-96, was cultivated on a reciprocal shaker (1.6 Hz, amplitude 10 cm) at 28°C.

#### Hydrolysis of Daunomycin

Daunomycin (Medexport, 100 mg) was hydrolyzed with 0.35 N HCl (250 ml, 1.5 hours, 100°C). After neutralization with ammonia, the reaction mixture was extracted with chloroform. The solvent was removed by distillation and the residue was chromatographed on a silica-gel column (100 g, Kieselgel 60, particle size 0.063~0.200 mm/70~230 mesh ASTM/Merck) and eluted with a solvent series of

benzene and chloroform (7:3~1:1).

#### Microbial Transformation and Isolation of the Product

The mycelia were incubated with daunomycinone in 0.2 M Tris-HCl buffer pH 7.5 (50 ml per flask) with 4% sucrose; 100  $\mu$ l daunomycinone in dimethylformamide (25 mg/ml) was added to the flasks. After the incubation, the mycelia were separated by centrifugation and extracted with methanol. The fermentation liquid was extracted with ethylacetate. The dried extract was evaporated under reduced pressure and purified by chromatography on a silica-gel column (80 g). The column was eluted with a series of solvents benzene, benzene - chloroform (9:1~1:1), chloroform, chloroform - methanol (9.5:0.5~9:2). The final purification of the product was carried out by repeated thin-layer chromatography (Silufol, Kavalier Czechoslovakia) in the solvent mixture chloroform - methanol - water(9:1.5:0.1).

#### Results and Discussion

The microbial transformation of daunomycinone by the strain *S. aureofaciens* B-96 gave rise to a red compound (45 mg) forming needles with m.p. 183~185°C (hexane - chloroform) and an optical rotation of  $[\alpha]_D^{20}$  97° (c 0.05, CHCl<sub>3</sub>). Ultraviolet and visible spectra are identical with those of daunomycinone. In the mass spectrum (Fig. 2) this compound has a molecular ion  $m/e$  400 corresponding to C<sub>21</sub>H<sub>20</sub>O<sub>8</sub>, a molecule having two additional hydrogen atoms with respect to daunomycinone. Comparison of the fragmentation schemes of both compounds indicates that the base peak of the spectrum originates in both cases by the loss of two water molecules. A difference is observed in splitting of the M--H<sub>2</sub>O ion; in daunomycinone the side chain is lost due to splitting of C<sub>2</sub>H<sub>3</sub>O (BULLOCK *et al.*<sup>6</sup>), whereas in the case referred to here, C<sub>2</sub>H<sub>4</sub>O is eliminated (Fig. 3). The infrared spectrum reveals bands at 1565 and 1605 cm<sup>-1</sup> that belong to the quinone carbonyls and are identical with those in the spectrum of daunomycinone; however, the band at 1700 cm<sup>-1</sup> of the aliphatic carbonyl is missing.

The proton magnetic resonance spectrum contains, similarly to daunomycinone, the methoxy group signals ( $\delta$  4.06), further signals of the ABC system of three vicinal aromatic protons ( $\delta$  7.38, 7.44 and 7.95 ( $J_{AB}$ =8 Hz,  $J_{AC}$ =1.5 Hz,  $J_{BC}$ =8 Hz)), signals of the ABX system of the grouping O-CH-CH<sub>2</sub> ( $\delta$  5.25, 2.43 and 1.76 ( $J_{AX}$ =5 Hz,  $J_{BX}$ =1.5 Hz,  $J_{AB}$ =15 Hz)) and the AB system of the isolated methylene group ( $\delta$  3.13 and 2.61 ( $J_{AB}$ =18.5 Hz)). However, instead of the methylene group singlet a secondary methyl doublet ( $J$ =6.2 Hz), ( $\delta$  1.29) is present. It is coupled (as verified by a double resonance experiment) to the methine proton ( $\delta$  3.71 (quadruplet  $J$ =6.2 Hz)). The latter two signals belong to the moiety



Fig. 2. Mass spectra of dihydrodaunomycinone.

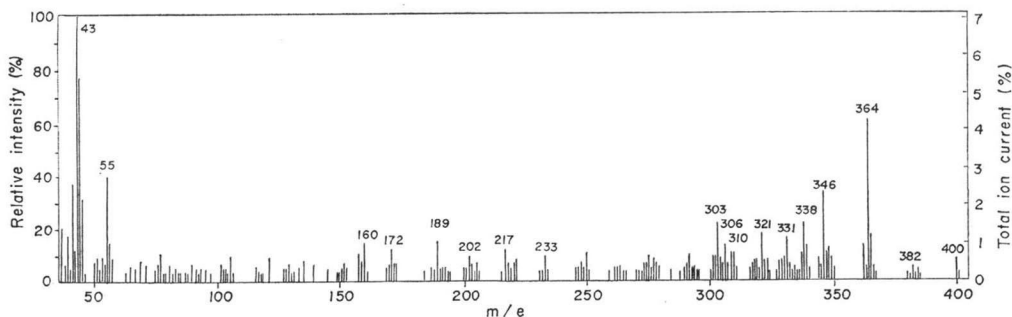
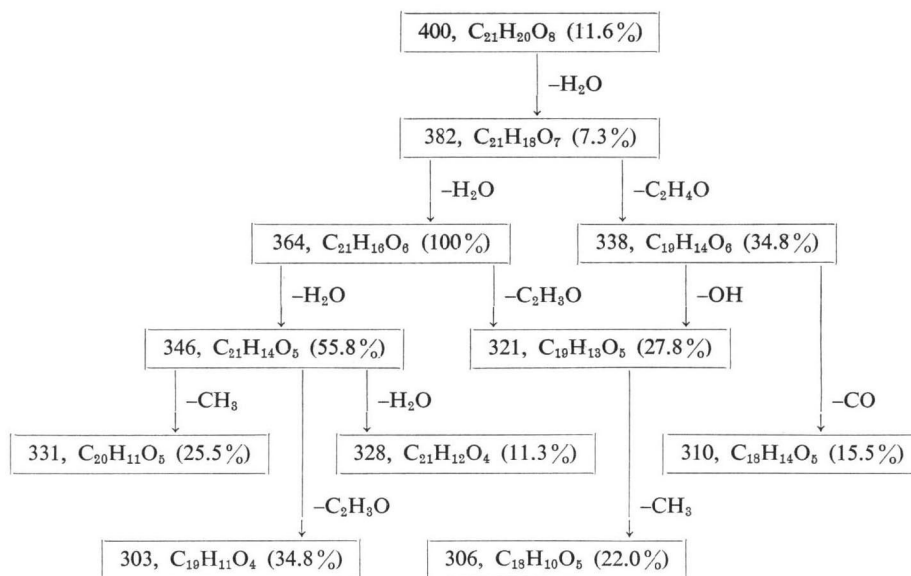


Fig. 3. Fragmentation scheme of dihydrodaunomycinone.

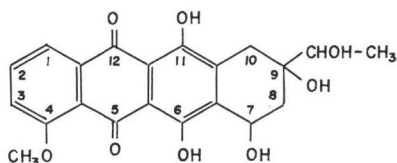


It follows from all the data presented that the transformation product is dihydrodaunomycinone (9-(1-hydroxyethyl)-7,8,9,10-tetrahydro-6,7,9,11-tetrahydroxy-4-methoxy-5,12-naphthacenequinone (Fig. 4) differing from daunomycinone by a 1-hydroxyethyl group at C<sub>9</sub> instead of the acetyl group.

The formation of the same metabolic product by reduction of the carbonyl group in the side chain of daunomycinone was described in mice, rats, and hamsters<sup>1,3</sup>). An enzyme catalysing the reduction of daunomycin was isolated from mammalian tissue homogenates<sup>2,6</sup>). The reaction is NADPH-dependent<sup>1,3,5</sup>).

It may be assumed on the basis of our results that a similar enzymic system reducing daunomycinone is present also in the strain, *Streptomyces aureofaciens* B-96.

Fig. 4. Dihydrodaunomycinone.



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