NOTES

BACTERIAL METABOLISM OF DAUNOMYCIN

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(Received for publication June 7, 1976)

As anthracycline antibiotics are almost universally antitumor agents, and several, including adriamycin and daunomycin, are useful clinically against neoplastic diseases[1,2], it is worthwhile to develop analogs. One means to this end is the use of microbial biomodification, and this communication discusses a limited application of this method to daunomycin.

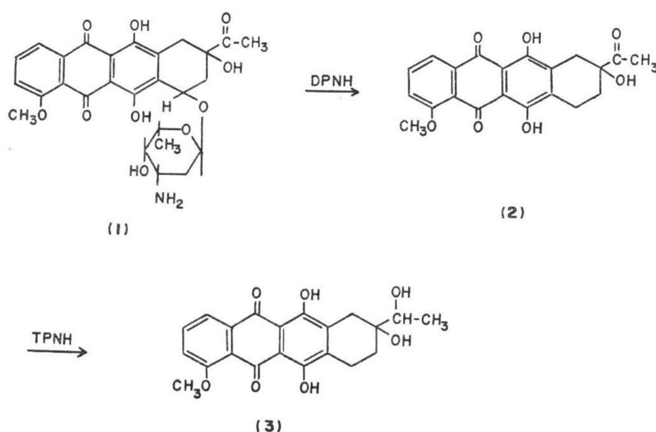
Daunomycin (**1**) was used as a substrate in a conversion in which a crude enzyme preparation of *Streptomyces steffisburgensis* was used as the biological agent. The fermentation was extracted, and the isolated material was chromatographed to give two products identified as 7-deoxydaunomycinone (**2**) and 7-deoxydaunomycinol aglycone (**3**).

The principal product **2**, which was obtained in a yield of 66%, was identified by comparison with an authentic sample by tlc in three solvent systems. The minor product **3**, which was obtained in a yield of 31%, was identified in the same manner and also by a high resolution mass spectrum. It seems probable that **2** was formed by a reductive glycosidic cleavage as was suggested previously for such reactions[3,4] as no hydrolysis products were detected.

It was found that conversion of **1** to **2** required DPNH (Fig. 1). Such a cofactor requirement has also been shown in the reductive cleavage of daunomycin by *Aeromonas hydrophila*. The further reduction of **2** to **3** involving C-13 carbonyl reduction is catalyzed by a TPNH linked keto reductase (Fig. 2). These reactions are outlined in Scheme 1. Evidence is not conclusive that **2** is the first product followed by carbonyl reduction to **3**. However, the C-13 dihydrodaunomycin arising from initial reduction at C-13 was not found as a reaction product, and it was established that **2** can be converted to **3** making the indicated sequence probable.

In these experiments a further example of microbial metabolism of anthracyclines is presented which follows the same pathway as was previously demonstrated[5~7] for mammalian metabolism.

Scheme 1.



Material and Methods

Thin-layer systems

System 1: $CHCl_3$ - MeOH(95: 5)

System 2: Cyclohexane - EtOAc - 95%EtOH (5: 3: 2)

System 3: EtOAc - EtOH - H_2O(92: 5: 3)

Cell-free Extract Preparation

Five to 10 g of *S. steffisburgensis* grown aerobically for 48 hours at 28°C on tryptone - yeast extract - glucose (5: 3: 20) medium were suspended in 15 ml of 100 mM potassium phosphate buffer (pH 7.4) containing 11.7 mg (0.15 mmoles) of β-mercaptoethanol. The suspension was sonicated in the cold using a Raytheon 10 kc sonic oscillator followed by centrifugation at $10^4 \times g$ for 15 minutes. The supernatant fluid was dialysed for 24 hours *vs.* 1 liter of the same buffer containing β-mercaptoethanol and was used as the crude enzyme source.

Cell-free Conversion of Daunomycin (1)

An undialysed cell-free extract prepared from 25 g of *S. steffisburgensis* (containing an endogenous supply of TPNH) with a final volume of 40 ml was added to a solution of 20 ml of 1 M potassium phosphate (pH 7.4) and 10 ml of water. Fifty mg of daunomycin (**1**) dissolved in 1 ml of DMF was added. The solution was allowed to stand at 37°C for 20 hours.

The reaction mixture was mixed with 10 g of filter-aid and filtered, and the filter cake was washed with 50 ml of water. The filtrate was extracted with four 50-ml portions of CH_2Cl_2, and the filter cake was extracted with four 100-ml portions of CH_2Cl_2. The combined extracts were evaporated *in vacuo*, residue 125 mg, and chromatographed on silica (125 g) using $CHCl_3$-MeOH (99: 1). The faster moving colored peak was combined as one pool and the slower moving as a second pool, and these were evaporated *in vacuo*. The less polar material gave 24 mg (66%) of material which had the same R_f as 7-deoxydaunomycinone (**2**) in tlc in three solvent systems. In solvent systems 1, 2 and 3 the R_f values were, respectively, 0.24, 0.64 and 0.68. The more polar fraction gave 11.5 mg (31%). On tlc this material showed a strong spot in three solvent systems having the same R_f values as did 7-deoxydaunomycinol aglycone (**3**). In solvent systems 1, 2 and 3 R_f values were, respectively, 0.18, 0.55 and 0.59. There was also a weak spot present attributed to **2**. The more polar material gave *m/e* 384.1199 (Required for $C_{21}H_{20}O_7$. 384.1209) *m/e* (TMS derivative) 672.

Effects of Pyridine Nucleotides

The following reaction protocols were employed.

(a) Reductive Cleavage: Reaction mixtures were made on the basis of 1 ml volumes and were run under aerobic conditions at 37°C. Each reaction mixture contained 10 mg of dialysed crude enzyme preparation measured using the LOWRY protein determination, 250 μg of daunomycin (**1**), 1 mg of DPNH, 100 μmoles of potassium phosphate solution (pH 7.4) and 10 μmoles of β-mercaptoethanol.

(b) Keto Reduction: A reaction protocol essentially identical to the one discussed above was employed with the following modifications. Dialysed crude enzyme was added at 1 mg/ml with 7-deoxydaunomycinone (**2**) and TPNH added at 250 μg/ml and 1 mg/ml, respectively. Both reactions were terminated by quick freezing in acetone-dry ice mixtures.

In the cases of both (a) and (b), control reactions were run containing all reaction components with the exception of reduced pyridine nucleotide.

The 1 ml reaction mixtures were subjected to

Fig. 1. Reductive conversion of daunomycin to 7-deoxydaunomycinone.
Reaction conditions are described in text.

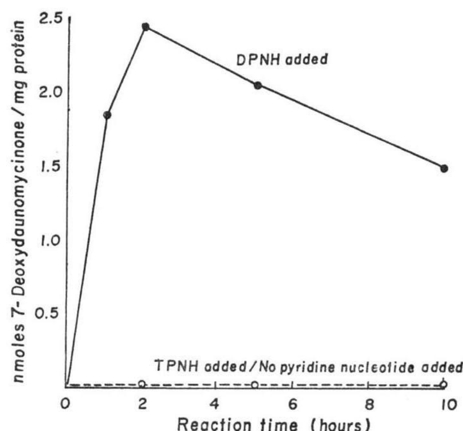
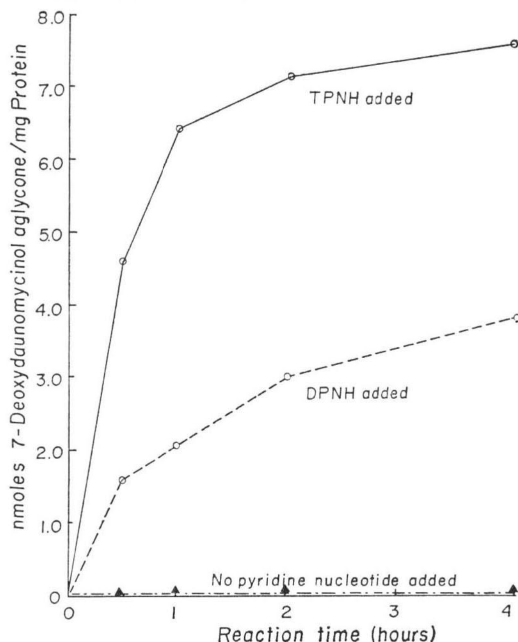


Fig. 2. Reduced pyridine nucleotide dependent conversion of 7-deoxydaunomycinone to 7-deoxydaunomycinol aglycone.
Reaction conditions are described in text.



mixing for 1 minute periods in the presence of 2 ml volumes of $CHCl_3$. The phases were separated by centrifugation for 5 minutes at $5\times10^3\times g$. The contents of these extracts were quantitated by HPLC using a Chromatec 2200 unit equipped with a Hewlett Packard-Mosely 7128A recorder and a Hewlett Packard 3370B integrator. The absorbent was Partisil 10 μ PAC (Whatman), and the solvent was $CHCl_3$ - CH_3OH - H_2O (96: 5: 1). The anthracyclines, monitored at 254 nm, were quantitated on the basis of standard curves made using authentic compounds in a range of 1～4 μg.

Acknowledgement

This work was supported in part by contract no. 1-CM-43753 from the Division of Cancer Treatment, National Cancer Institute, National Institutes of Health, Department of Health, Education and Welfare. We are grateful to Drs. N. R. BACHUR and J. DOUROS for their gifts of 7-deoxydaunomycinol aglycone and daunomycin.

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