MICROBIAL N-ACETYLATION OF DAUNORUBICIN AND DAUNORUBICINOL*

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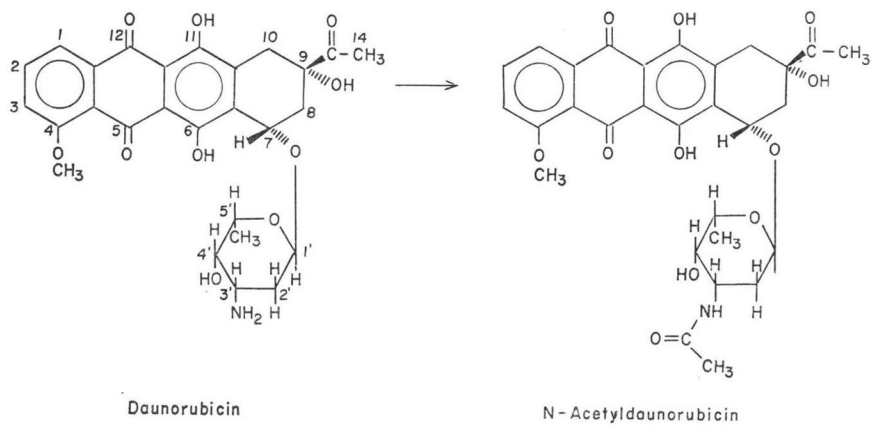
Microbial transformation of anthracyclines, particularly daunorubicin, has recently received attention as a technique for preparing modified anthracyclines for antitumor testing. Daunorubicin transformation products which have been observed in microbial systems include daunorubicinol[1,2], 7-deoxydaunomycinone[3~5], and 7-deoxydaunorubicinol aglycone[5]. In addition, identical daunorubicin biotransformation products have been noted in animal systems[6,7]. Moreover, microbial transformation of daunorubicinone to daunorubicinol aglycone has also been observed[8]. We report in this note the observation of N-acetyldaunorubicin as a microbial transformation product of daunorubicin (see Fig. 1) and N-acetyldaunorubicinol as a transformation product of daunorubicinol. To our knowledge, N-acetylation of daunorubicins has not been reported by others for either microbial or animal systems.

A microorganism which we isolated in our laboratory (assigned number FCRC 1321 and identified as *Bacillus cereus* var. *mycoides* by the American Type Culture Collection) was grown for two days at 28°C in trypticase-soy broth (BBL) contained in baffled shaken flasks (200 ml broth per liter flask). Filter-sterilized aqueous daunorubicin-HCl (kindly supplied by Dr. J. D. DOUROS, Division of Cancer Treatment, NCI) was then added to yield a concentration of 0.12 mg/ml in the broth, and incubation with shaking was continued for one day. The fermentation broth (4×200 ml) was adjusted to pH 10 using 4% Na_2CO_3, and extracted with chloroform. The chloroform extract was vacuum evaporated to dryness and the residue was purified by preparative TLC on silica gel in the dark with $CHCl_3$-MeOH - H_2O (120: 20: 1) as developing solvent. The band at Rf=0.6 was scraped off the TLC plates and eluted with $CHCl_3$ - MeOH - H_2O (80: 30: 3). The eluate was evaporated to dryness, redissolved in 15 ml chloroform, washed with dilute aqueous HCl (pH 2, 2×20 ml) and then with distilled water, and finally the chloroform phase was evaporated to dryness to obtain 21.5 mg of pure material. The pure material was characterized by TLC, ^{1}H-NMR and IR,

Fig. 1. Microbial N-acetylation of daunorubicin



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and shown to be identical with synthetically prepared N-acetyldaunorubicin: TLC with solvent systems $CHCl_3$ - MeOH - H_2O (120: 20: 1), Rf= 0.60; 1-propanol - H_2O - AcOH - pyridine (8: 2: 1: 1), Rf=0.80; methanol, Rf=0.78; IR (KBr): amide at 1630 cm^{-1}; ketone at 1710 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 2.00 (s, N-Ac); δ 2.41 (s, -CO-Me). The ^{1}H-NMR ($CDCl_3$) spectrum corresponds to that already published[9] for chemically synthesized N-acetyldaunorubicin.

We also found that the same microorganism transformed daunorubicinol (kindly supplied by Dr. G. JOLLES, Rhône-Poulenc). The transformation product appeared to be identical to N-acetyldaunorubicinol (prepared as described previously[2]) based on TLC results (Rf=0.44 with $CHCl_3$ - MeOH - H_2O (120: 20: 1); Rf= 0.73 with $CHCl_3$ - MeOH - H_2O (80: 30: 3)).

The antitumor properties of N-acetyldaunorubicin prepared by chemical synthesis have already been studied[10]. In a previous paper[2], we first reported synthesis of N-acetyldaunorubicinol by a route different from that described here, and antitumor testing of this compound is in progress. The results given here, however, show that when a microbial transformation screen is executed in the search for modified anthracyclines for antitumor testing, N-acetylated products can well appear.

References

1) NINET L.; J. FLORENT, J. LUNEL, J. RENAUT, A. ABRAHAM, B. LOMBARDI & R. TISSIER: Duborimycin (20 798 R.P.)—preparation by biochemical reduction of daunorubicin. Abstracts of the Fifth International Fermentation Symposium-Berlin p. 320, 1976

2) ASZALOS, A. A.; N. R. BACHUR, B. K. HAMILTON, A. F. LANGLYKKE, P. P. ROLLER, M. Y. SHEIKH, M. S. SUTPHIN, M. C. THOMAS, D. A. WAREHEIM & L. H. WRIGHT: Microbial reduction of the side-chain carbonyl of daunorubicin and N-acetyldaunorubicin. J. Antibiotics 30: 50~58, 1977

3) WILEY, P. F. & V. P. MARSHALL: Microbial conversion of anthracycline antibiotics. J. Antibiotics 28: 838~840, 1975

4) MARSHALL, V. P.; E. A. REISENDER, L. M. REINEKE, J. H. JOHNSON & P. F. WILEY: Reductive microbial conversion of anthracycline antibiotics. Biochemistry 15: 4139~4145, 1976

5) MARSHALL, V. P.; E. A. REISENDER & P. F. WILEY: Bacterial metabolism of daunomycin. J. Antibiotics 29: 966~968, 1976

6) TAKANASKI, S. & N. R. BACHUR: Daunorubicin metabolites in human urine. J. Pharmacol. & Exp. Therapeutics 195: 41~49, 1975

7) BULLOCK, F. J.; R. J. BRUNI & M. A. ASBELL: Identification of new metabolites of daunomycin and adriamycin. J. Pharmacol. & Exp. Therapeutics 182: 70~76, 1972

8) KARNETOVA, J.; J. MATEJU, P. SEDMERA, J. VOKOUN & Z. VANIK: Microbial transformation of daunomycinone by *Streptomyces aureofaciens* B-96. J. Antibiotics 29: 1199~1202, 1976

9) ARCAMONE, F.; G. CASSINELLI, G. FRANCESCHI, P. OREZZI & R. MONDELLI: The total absolute configuration of daunomycin. Tetrahedron Letters 1968-30: 3353~3356, 1968

10) YAMAMOTO, K.; E. M. ACTON & D. W. HENRY: Antitumor activity of some derivatives of daunorubicin at the amino and methyl ketone functions. J. Med. Chem. 15: 872~875, 1972