Journal of Chromatography, 205 (1981) 482–485
Elsevier Scientific Publishing Company, Amsterdam — Printed in The Netherlands

CHROM. 13,338

Note

Isolation of daunorubicin derivatives by counter-current chromatography with the horizontal flow-through coil planet centrifuge

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(Received September 15th, 1980)

In basic studies on metabolism of anthracycline quinone drugs and the mechanism of their action, separation and purification techniques play an essential role[1–3]. It has been reported[4] that one of the metabolites of daunorubicin, 7-deoxydaunorubicin aglycone, was produced by sodium dithionite reduction. Our preliminary experiments have shown that sodium dithionite reduction of daunorubicin yields one additional aglycone as a major product. However, isolation of this aglycone with a liquid chromatographic method produced a problem in the subsequent mass spectrometric analysis due to contamination of the solid support materials in the fraction. Although the counter-current distribution method can be alternatively used, the separation is time-consuming and the apparatus takes up a large space in the laboratory. We considered[5–13] that counter-current chromatography would be the best choice since this new partition method uses no solid supports and yields a high partition efficiency comparable to liquid chromatography.

This paper describes the application of the horizontal flow-through coil planet centrifuge[10–13] to counter-current chromatographic separations of daunorubicin aglycones produced by sodium dithionite reduction. This application can easily be extended to the purification and isolation of other drugs and drug metabolites.

EXPERIMENTAL

Materials

Daunorubicin was obtained from the Cancer Treatment Division of the National Cancer Institute, Bethesda, MD and further purified as described previously[1]. Daunorubicinol was provided by Dr. R. L. Felsted of the National Cancer Institute. Sodium dithionite ($Na_2S_2O_4$) was purchased from Fisher Scientific (Fair Lawn, NJ, U.S.A.). Chloroform, ethylene chloride, hexane and methanol used for counter-current chromatography were of a chromatographic grade. The other chemicals were analytical grade.

0021-9673/81/0000–0000/$02.50

Reaction of sodium dithionite with daunorubicin

In a typical experiment, 0.014 mmol of daunorubicin was dissolved in 20 ml of a solvent mixture composed of water–tetrahydrofuran–methanol (3:1:1) in a 125-ml flask equipped with a glass stopper. Then 13 mmol of sodium dithionite were added. The flask was stoppered and the contents were stirred with a PTFE-coated magnetic stirrer bar for 18 h at room temperature. The contents were then transferred to a 250-ml separatory funnel and two 30-ml portions of chloroform were added to extract the aglycones into the lower phase. The pooled extract was evaporated to dryness under vacuum. During the reaction, aglycone formation was monitored by thin-layer chromatography (TLC) (silica gel 60, E. Merck, Darmstadt, G.F.R.) with a solvent mixture composed of chloroform–methanol–acetic acid (100:2:5) which gives R_F values of 0.37 and 0 for 7-deoxydaunorubicin and daunorubicin, respectively.

Counter-current chromatography with the horizontal flow-through coil planet centrifuge

The design and principle of the horizontal flow-through coil planet centrifuge were described earlier[11,12]. The apparatus holds two types of coiled separation columns, each subjected to a particular mode of synchronous planetary motion. One column enables analytical-scale separations and the other preparative-scale separations, both with high partition efficiency. We used the preparative column which consists of 1000 helical turns of a 2.6 mm I.D. PTFE tube with a total capacity of about 270 ml. A two-phase solvent system composed of chloroform–ethylene chloride–hexane–methanol–water (1:1:1:3.5:1) was selected because it provides high solubility and suitable partition coefficients for the aglycones. The sample solution was prepared by dissolving the dried reduction products in 5 ml of the above solvent system consisting of equal amounts of the aqueous and nonaqueous phases.

In our typical experiments, the column was first filled with the lower non-aqueous stationary phase and then the sample solution was injected through the sample port. The aqueous mobile phase was pumped into the column at a flow-rate of 24 ml/h with a Chromatronix Cheminert pump while the apparatus was run at 400 rpm. The elution profile was obtained by monitoring UV absorption of the eluate at 280 nm by a 3-mm light path flow cell with an LKB Uvicord S and LKB recorder. An LKB fraction collector was used for fractionation of the sample at a rate of 6 ml per tube.

Mass spectrometry

Electron impact mass spectra were obtained by direct probe insertion on a VG 30F mass spectrometer (VG Micromass, Winsford, Great Britain) operated under computer control (VG 2040 data system). Mass spectrometer operational conditions were the following: ion source, 200°C; electron energy, 70 eV; trap current, 170 μA; and scan speed 3 sec per decade.

RESULTS AND DISCUSSION

On processing the reaction mixture through the coil planet centrifuge, three major peaks, fractions 26, 36, and 48, were well resolved and eluted within 13 h (Fig. 1). The partition efficiency calculated according to the standard gas chromatographic formula gives 1565 theoretical plates (TP) for the second peak and 915 TP for the third peak.

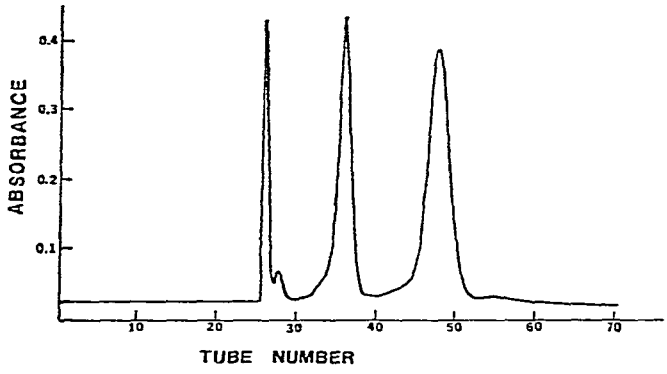

Fig. 1. Separation of daunorubicin reduction products with counter-current chromatography.

TLC with chloroform–methanol–acetic acid–water (80:20:40:6) showed that fraction 26 co-chromatographs with daunorubicin. With a different solvent system of chloroform–methanol–acetic acid (100:2:2.5), fractions 36 and 48 co-chromatographed with 7-deoxydaunorubicinol aglycone and 7-deoxydaunorubicin aglycone, respectively. The mass spectrometric analysis also showed that fraction 36 was identical to 7-deoxydaunorubicinol; *m/e* 384 (M^+), 339($-HCOHCH_3$), 321($-HCOHCH_3$, $-H_2O$), and that fraction 48 was identical to 7-deoxydaunorubicin aglycone standards; *m/e* 382 (M^+), 364($-H_2O$), 339($-COCH_3$), 321($-H_2O$, $-COCH_3$).

These results indicate that the reductive cleavage of daunorubicin with an excess dose of sodium dithionite produces 7-deoxydaunorubicin aglycone[4] and 7-deoxydaunorubicinol aglycone (Fig. 2). In order to confirm the identification of the

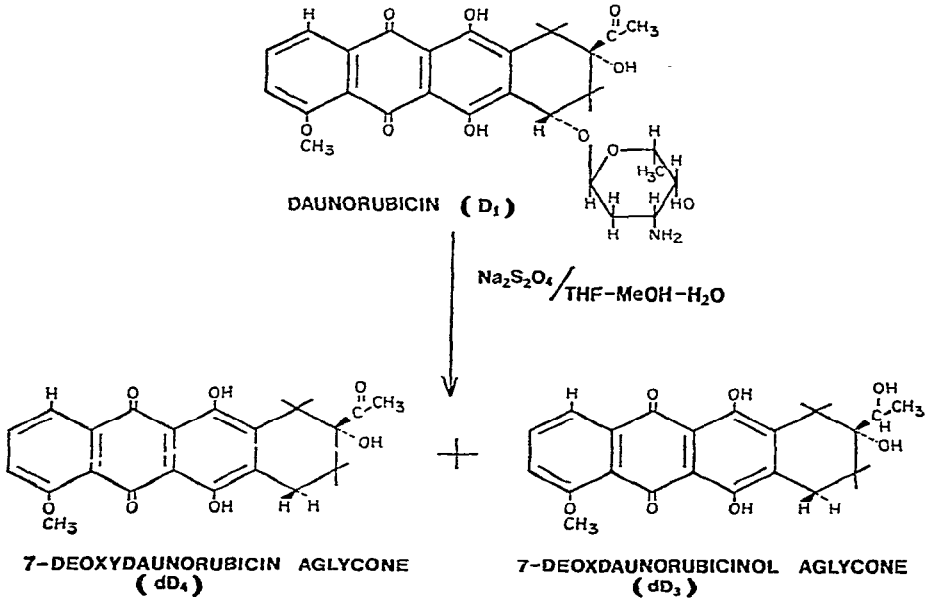

Fig. 2. Aglycone formation from daunorubicin by sodium dithionite reduction.

latter compound, daunorubicinol was similarly treated with an excess amount of sodium dithionite. The TLC studies of this product were in good agreement with the spot of fraction 36.

Our experiments clearly show that counter-current chromatography offers a great advantage over liquid chromatography in isolation of drugs and drug metabolites. Counter-current chromatography yields high-purity fractions which facilitate further instrumental analysis without interference caused by contamination with the solid supports. Compared with the counter-current distribution method, the present system is non-destructive and allows small amounts of solvents to yield highly concentrated fractions at high partition efficiencies. Further applications of this counter-current chromatographic technique for investigation of anticancer agents are now in progress in our laboratory.

ACKNOWLEDGEMENT

The authors are deeply indebted to Dr. R. L. Felsted for his generous gift of daunorubicinol.

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