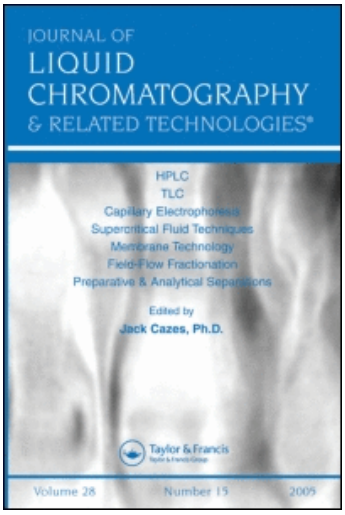

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HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY OF NEW SEMISYNTHETIC DAUNOMYCINONE DERIVATIVES

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ABSTRACT

Reversed-phase HPLC utilizing LiChrosorb RP-8 was used to separate reaction mixtures of new semisynthetic daunomycinone derivatives and determine their relative occurrence.

Chromatographic behaviour of the following compounds was studied: daunomycinone (I), 7(S) and 7(R)-O-(2-hydroxyethyl)-13-ethyleneacetal daunomycinone (II and III), 13-ethyleneacetal daunomycinone (IV), 13-ethyleneacetal bisanhydrodaunomycinone (V), 7(S) and 7(R)-O-(3-hydroxypropyl)-13-propyleneacetal daunomycinone (VI and VII), 13-propyleneacetal daunomycinone (VIII), 13-propyleneacetal bisanhydrodaunomycinone (IX), 7(S) and 7(R)-O--(4-hydroxybutyl) daunomycinone (X and XI), 4-toluenesulfonylhydrazone daunomycinone (XII).

INTRODUCTION

Different stationary phases and different detection methods are used in the HPLC of known anthracyclines and anthracyclinones. The above compounds are separated on

silica gels (1, 2), normal bonded-phases (3, 4) and reversed-phases (5, 6, 7). The use of the HPLC method in the analysis of new anthracyclines and anthracyclinones requires modifications of the experimental conditions with respect to the optimum separation of mixtures of these compounds.

In the present paper HPLC was used for the separation of new semisynthetic derivatives of daunomycinone (Figure 1) and determination of relative representation of components of the reactions yielding these new derivatives. Solutes were identified by comparing their capacity ratios k' with those of standards.

MATERIAL AND METHODS

Chemicals. Daunomycinone (I) was obtained from Medexport (U.S.S.R), derivatives (II-XI) were prepared according to Jizba et al. (8), compound (XII) according to Smith et al. (9). UV-Grade solvents (methanol, acetone, acetonitrile and redistilled water) were used.

Apparatus. The high pressure liquid chromatograph SP-8000 (Spectra Physics Corp., Santa Clara, CA, U.S.A) equipped with a 250 x 4.6 mm column (LiChrosorb RP-8, 10 μm) and a detector SP-770 was used. Experimental conditions were as follows: flow 1.5 mL/min, pressure 9.1 - 11.2 MPa, temperature 25 ^{o}C, detection at 480 nm. Dead time for calculation of the capacity ratio k' was determined by injecting redistilled water.

Procedure. The following mixtures of reaction components (after a reaction time of 8 - 12 h), and, in parallel, corresponding mixtures of standards were subjected to the chromatographic analysis (see Table 1):

A. Daunomycinone (I), 7(S) and 7(R)-O-(2-hydroxyethyl)-13-ethyleneacetal daunomycinone (II and III), 13-ethyleneacetal daunomycinone (IV), 13-ethyleneacetal bisanhydrodaunomycinone (V).

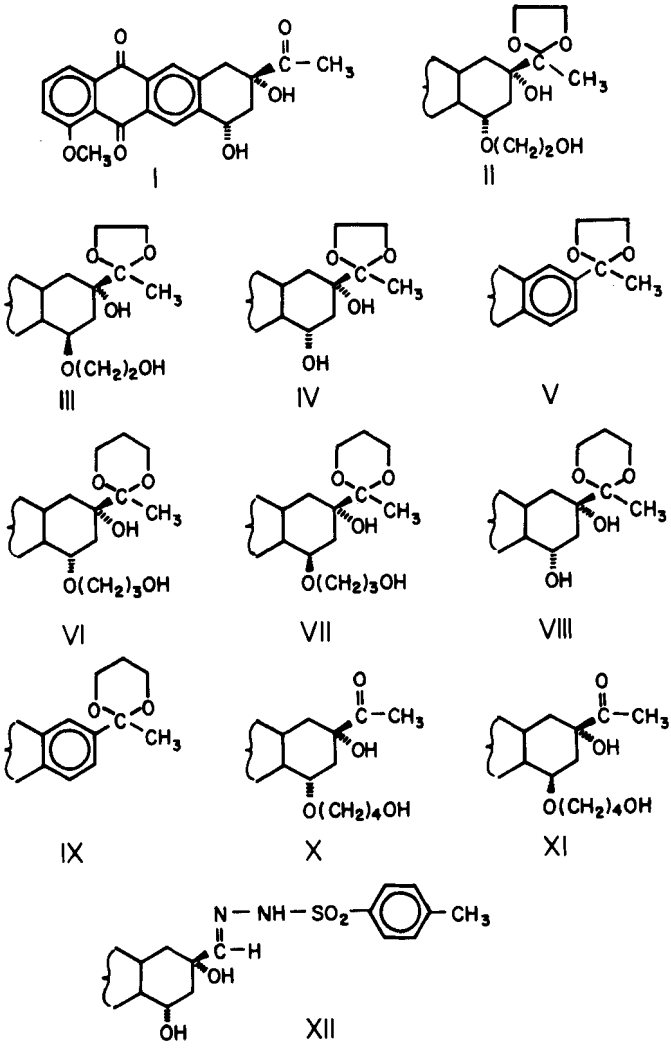

FIGURE 1

Chemical structures of daunomycinone derivatives

TABLE 1

Capacity ratios k´ of the solutes and their relative occurence in reaction mixtures

Compound	k´		%	Conditions
	A	B		
III	3.2	3.2	16	methanol-water (55:45) (20 min) linear gradient to acetone (30 min)
I	5.1	5.1	5	
IV	7.5	7.5	9	
II	10.8	11.0	60	
V	20.2	20.0	10	
	C	D		
VII	1.1	1.1	31	methanol-water (70:30) (10 min) linear gradient to acetone (30 min)
I	1.1	1.1		
VIII	1.9	2.0	48	
VI	3.1	3.2	18	
IX	16.5	16.7	3	
	E	F		
XI	1.5	1.5	7	acetonitrile-water (40:60) (12 min)
I	2.1	2.1	48	
X	4.0	4.1	45	
	G	H		
I	4.0	4.0	10	methanol-water (60:40) (15 min)
XII	5.5	5.5	90	

A, C, E, G - mixture of standards

B, D, F, H - reaction mixtures

C. Daunomycinone (I), 7(S) and 7(R)-O-(3-hydroxypropyl)-13-propyleneacetal daunomycinone (VI and VII), 13-propylene daunomycinone (VIII), 13-propyleneacetal bisanhydrodaunomycinone (IX).

E. Daunomycinone (I), 7(S) and 7(R)-O-(4-hydroxybutyl) daunomycinone (X and XI).

G. Daunomycinone (I), 4-toluenesulfonylhydrazone daunomycinone (XII).

Evaporates of reaction mixtures B and D and corresponding standard mixtures A and C were dissolved in acetone. Evaporates of the reaction mixtures F and H and corresponding standard mixtures E and G were dissolved in methanol. The samples (0.8 - 1.5 μg) were injected to a 10 μL loop. Concentrations of individual reaction components were determined according to calibration relationships and their relative occurrence in the reaction mixtures was calculated.

RESULTS AND DISCUSSION

Table 1 summarizes capacity ratios k' of solutes of individual reactions and corresponding standards. The relative representation of individual components after the reaction is also presented. Under the experimental conditions used the baseline separation of the solutes was reached. Only in the reaction mixture D it was not possible to separate daunomycinone (I), the starting compound of the reaction, from the reaction product (VII), even when using various modifications of the mobile phase.

The separation of configuration isomers of individual pairs (II and III, VI and VII, X and XI) was remarkably good. The different retention in individual pairs might be due to formation of the intramolecular hydrogen bridge between the hydroxyl group on carbon C-9 and hydroxyl group on 7(S)-O-(n-alkyl)-13-alkyleneacetal of

daunomycinone. The formation of the hydrogen bond results in a decreased polarity of the compound and, thus, in prolongation of the retention time of these compounds on the reversed-phase. This phenomenon is not observed in the case of 7(R)-O-(n-alkyl)-13-alkyleneacetal of daunomycinone. Compounds (V) and (IX) differing chemically from other solutes were also present in the cromatographic mixtures. An acetone gradient had to be used for their elution, and, hence, the time required for their analysis was extended by 30 min. Other compounds were eluted with a retention time of up to 20 min.

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