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Journal of Chromatography A, 830 (1999) 191–195

JOURNAL OF CHROMATOGRAPHY A

Short communication

Enantioselective high-performance liquid chromatography of chiral intermediates in the total synthesis of 4-demethoxydaunomycinone

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Received 9 June 1998; received in revised form 3 September 1998; accepted 20 October 1998

Abstract

Ten pairs of chiral intermediates from the total synthesis of 4-demethoxydaunomycinone (aglycone of important anticancer drug Idarubicine and some new drugs under development) have been separated on the same chiral stationary phase, offering an excellent method for rigorous in-process monitoring of enantiomeric purity. Stereoselective HPLC of these intermediates have not been reported before, even for diastereoisomeric imines at the crucial step of synthesis, resulting in enantiomeric resolution.

Keywords: Enantiomer separation; 4-Demethoxydaunomycinone

1. Introduction

Anthracycline antibiotics constitute a class of valuable antitumor therapeutics of natural origin, which have found wide clinical application. The prototype member of the group: daunorubicine **1**, exhibits, apart from side effects typical for cytotoxic agents, cumulative cardiotoxicity and the tendency to induce multidrug resistance. Continuous efforts to develop more effective and less toxic drugs by chemical synthesis of new analogs of **1** involve application of unnatural aglycones, which in turn calls for availability of relevant building blocks in high enantiomeric purity (Fig. 1).

Total synthesis of daunomycinone **2**, elaborated by Swenton [1] has, despite its length, positive characteristics of a sound laboratory method amenable to process development. In fact, its generality, allowing for some structural variation, can provide access to natural (e.g., **2** and **3**) as well as synthetic (**4** and **5**) aglycones. Required enantiopurity of the final compound is achieved through separation of diastereoisomeric imines (**8**) at advanced stage of synthesis (Fig. 2), which makes it a crucial point in terms of process efficiency. Working on a scale-up of this step we were unable to find a simple test for control of separation of **8a** and **8b**, since both TLC and HPLC on normal as well as reversed phases have failed. Although diastereoisomeric content in mixtures of the imines could be quantified by analysis of their ^{1}H NMR spectra, we have continued our search for a method more appropriate for in-process control and eventually turned our attention to HPLC on chiral phases. Wide application of the carbohydrate CSP is

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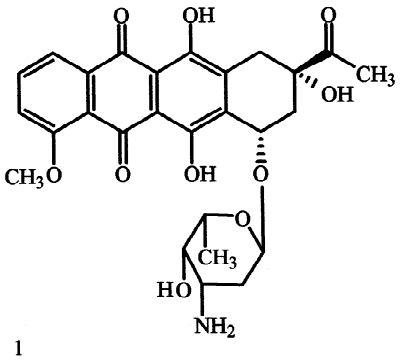
0021-9673/99/$ – see front matter
PII: S0021-9673(98)00885-1



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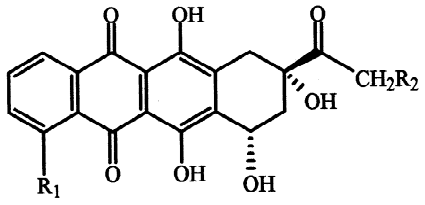

2 $R_1 = OCH_3$, $R_2 = H$

3 $R_1 = OCH_3$, $R_2 = OH$

4 $R_1 = H$, $R_2 = H$

5 $R_1 = H$, $R_2 = OH$

Fig. 1. Structures of daunorubicine and related compounds.

well documented and extensively reviewed [2–4], which inspired our column selection. We have found that cellulose carbamates (but not amylose carbamates!) separate not only imines **8** but also structurally related chiral synthons depicted in Fig. 2.

2. Experimental

2.1. Chemicals

Compounds **6**–**14** were synthesised according to published procedure [1] in the authors' laboratory and identified by standard analytical and spectral (HPLC, TLC, ^{1}H NMR, MS, IR) methods. Diastereoisomeric imines **8** were completely resolved by multiple crystallization and further synthesis (**9**–**14**) was carried out starting from enantiopure precursors (e.e.>95). Reagents, solvents and auxiliary materials used in the synthesis were at least of 'pure' grade. All solvents used in analytical chromatographic separation experiments were of HPLC grade.

2.2. Apparatus

The HPLC equipment used was manufactured by Waters Assoc., Milford, MA, USA. It consisted of a Multisolvent Delivery System 600 E, a Photodiode Array Detector 996, a Rheodyne Model 7725i injector and a Chromatography Manager Millenium version 2.15.01 software for PC computations.

2.3. Chromatographic conditions

Samples were dissolved in ethyl acetate, followed by dilution with mobile phase, to a concentration 0.2 mg/ml. Twenty-μl samples were injected on the column and analyses were carried out at ambient temperature by elution with hexane–2-propanol mixtures, using a UV absorption detector. Although analysed compounds exhibit maximum UV absorption in the range 205–230 nm, detection wavelengths 295 and 254 were used to avoid interference with ethyl acetate. Detailed analytical conditions are specified for each mixture in the Table 1.

2.4. Chiral columns

Chiracel OD columns with 10-μm particles (Daicel Chemical Industries, Ltd., Japan), 50×4.6 mm precolumn and 250×4.6 mm main column, were used for enantiomer separations.

2.5. Mobile phases

A mixture of hexane and 2-propanol (LAB-SCAN Analytical Sciences, Ireland) in a 7:3 (v/v) ratio, pumped at a rate of 1 ml/min, afforded satisfactory separations in most cases. HPLC-grade ethyl acetate (J.T. Baker, Holland) was used for dissolving samples.

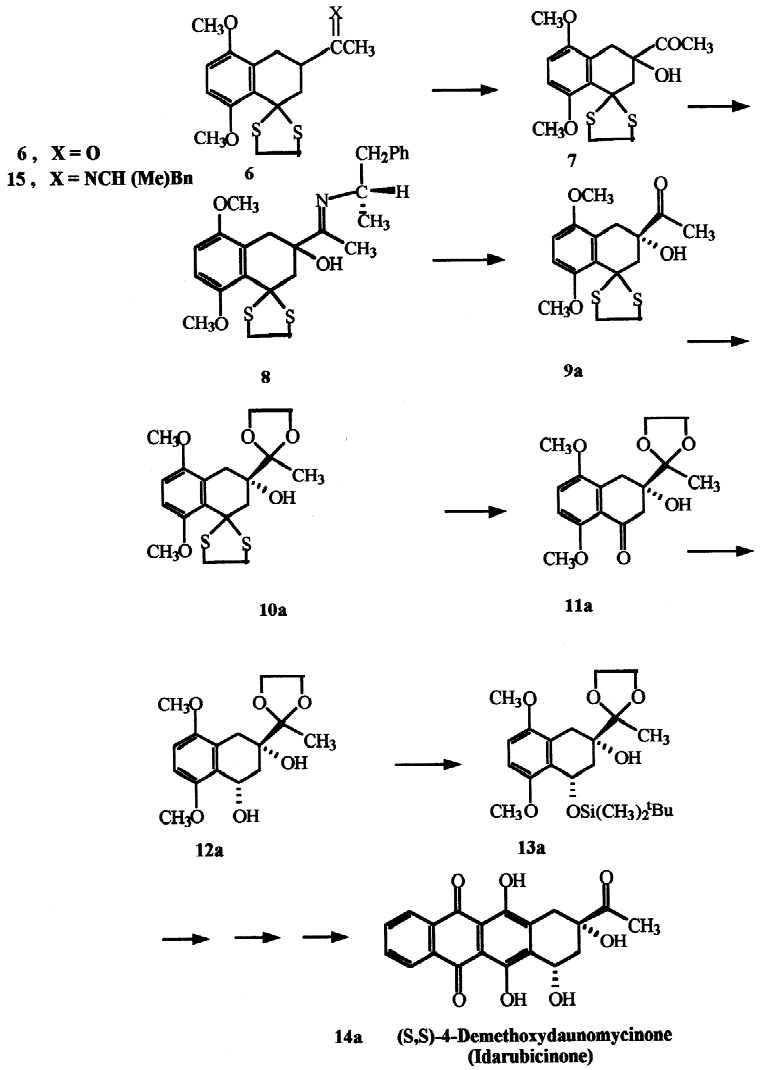

Fig. 2. Synthesised compounds and the final product Idarubicinone.

3. Results and discussion

Part of the scheme of the total synthesis of 4-demethoxydaunomycinone, which is essential for control of enantiopurity of the final product **14**, is shown in Fig. 2. All intermediates are chiral and execution of the synthesis in nonstereoselective manner would result in a mixture of four diastereoisomeric aglycones. To our knowledge the question of quantitative determination of enantiomers in enantio-enriched mixtures resulting from separation of diastereoisomeric **8** by crystallization have never before been addressed. Likewise, there are no reports of chromatographic separation, analytical or preparative, of **8** on achiral or even chiral phases. Results of our study are summarized in Table 1. As can be seen, application of one chiral column and a common eluting system secured baseline separations for all pairs of enantiomeric intermediates. Similarly, (*R*,*S*) and (*S*,*S*) imines obtained from enantiomeric hydroxyketones **7** in reaction with enantiopure benzylmethylamine, which could not be separated on

Table 1
Analytical conditions

Compound no.	k'	Resolution, R_s	Selectivity, α	Asymmetry (tailing factor)	Plate count (tangent method)
6$_{(1)}$	10.63	1.72	1.09	1.30	7800
	11.59			1.44	7200
7	14.60	12.08	2.01	1.25	7300
	29.40			1.21	5600
8	10.55	11.59	2.19	1.24	4700
	23.13			1.18	4200
8a	22.57			1.21	4100
8b	10.42			1.29	4700
9a	31.17			1.24	5500
9b	16.72			1.30	5500
9a+9b		9.45	1.16		
10a	25.15			1.26	5400
10b	14.45			1.38	5400
10a+10b		9.52	1.78		
11a$_{(2)}$	16.22			1.73	3400
11b$_{(2)}$	26.46			1.83	2900
11a+11b		6.39	1.60		
12a	17.03			1.35	4700
12b	19.39			1.36	4500
12a+12b		2.08	2.35		
13a$_{(3)}$	21.90				
13	11.00	13.44	2.19	1.83	5100
	21.90			1.48	6300
14a$_{(2)}$	30.00			2.15	1600
14b$_{(2)}$	34.13			2.19	1800
14a+14b		1.27	1.40		
15$_{(4)}$	13.68	1.09	1.06		6700
	14.49				6400

Mobile phase, hexane–propan-2-ol (7:3, v/v); λ=295 nm; flow rate, 1.0 ml/min for all experiments except following cases: (1) flow rate, 0.7 ml/min; (2) λ=254 nm; (3) mobile phase, 99:1 (v/v); (4) mobile phase 9:1 (v/v) and flow rate 0.5 ml/min. Capacity factor $k'=R_t/V_0-1.0$, where R_t is the retention time and V_t is the void volume time resolution $R_s=(R_{t2}-R_{t1})/(W_2+W_1)$, where W is the peak width at baseline using tangent lines selectivity $\alpha=(R_{t2}-V_0)/(R_{t1}-V_0)$ asymmetry USP tailing factor $T=W/2\cdot F$, where W is the peak width at 5% of peak height and F is the width of line from peak start to R_t at 5% of peak height plate count (tangent method) $N=16\cdot(R_t/W)^2$.

achiral columns, are easily resolved under selected conditions. Detection limits for enantiomeric admixtures are in all cases better then 0.5%. Although bicyclic ketone **6** is used in Swenton's synthesis as a racemic mixture and the following transformation of introducing tertiary hydroxyl group is carried out in a nonstereoselective manner, we consider this point in synthesis as important for further studies on possible enantioselective transformations. Consequently, compound **6** and the pair of corresponding (*R*,*S*) and (*S*,*S*) imines **15** were included in our study. Parameters characterizing the column and particular separations, calculated with use of Waters software, are presented in Table 1. Apparently, for the employed Chiracel column there is no correlation between an absolute configuration (*R*,*S*) descriptor of chromatographed compound and the order of elution.

The title compound constitutes an important pharmaceutical intermediate used in manufacturing of the valuable anticancer drug Idarubicin and some new drugs under development (e.g., Annamycin, MEN-1075), presently undergoing clinical trials. In each case, rigorous control of enantiomeric purity of the intermediate aglycone **14** constitutes an important part of pharmaceutical quality assurance. Anthracyclines are notorious for difficulties with reproducibility of specific rotation measurements, which are traditionally employed for determination of enantiomeric purity [5]. In particular, 4-demethoxydaunomycinone (**14**) and its derivatives have been

cited [6–8] for inconsistencies concerning results of chiroptical analyses of seemingly enantiopure samples, whereas resulting controversies had to be resolved by support of ^{1}H NMR chiral shift reagent experiments [9]. The results of this study extended efficient control of enantiomeric purity over the entire course of the aglycone synthesis, which in turn greatly assisted elaboration of the process scale up.

Acknowledgements

Partial support of this work from II Maria Skłodowska-Curie Fund is gratefuly acknowledged. The authors are grateful to Drs Lukasz Kaczmarek and Izabela Fokt for furnishing us with samples from their independent syntheses.

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