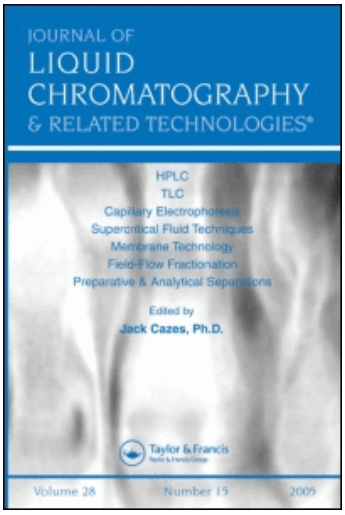

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EFFICIENT SEPARATION OF DOLAPROINE STEREOISOMERS BY OPTIMIZATION OF A THREE COMPONENT CHROMATOGRAPHIC SOLVENT SYSTEM

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ABSTRACT

A solvent system composed of *n*-hexane-chloroform-acetone was selected (by TLC) for resolving a difficult to separate mixture of protected dolaproine stereoisomers. A set of points representing solvent ratios was generated by an application of statistical methods. From thin layer chromatography data semifinal solvent ratios were selected. By applying simplex optimization, the solvent ratio was found to be 32.3 : 53.4 : 14.3 respectively for the preceding solvent system.

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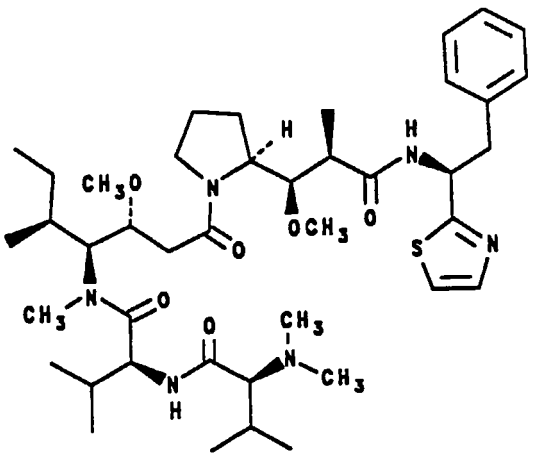
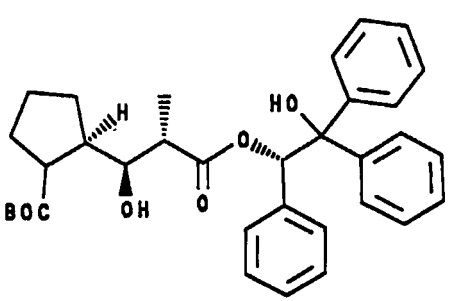


Dolastatin 10 1



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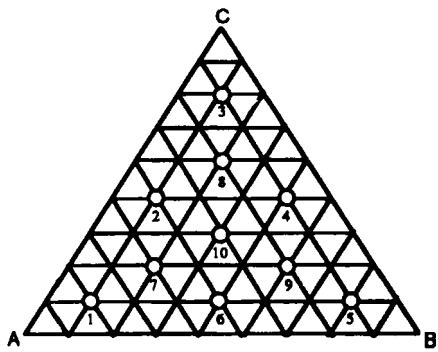
INTRODUCTION

A very challenging investigation of *Dolabella auricularia* (an Indian Ocean sea hare) antineoplastic constituents resulted in our discovery,[2] structural elucidation and total synthesis of the remarkably potent dolastatin 10 (**1**). Presently this unprecedented peptide is undergoing preclinical development and meeting supply objectives required an effective total synthesis.[3] Separation of the required S,S,R isomer from three other stereoisomers produced during synthesis of the protected dolaproine unit (**2**) proved to be the most time-intensive step. Previous experience with difficult to separate mixtures[4-6] of closely related substances on silica gel columns and guided by results of thin layer chromatographic comparisons on silica gel led us to the three component eluent *n*-hexane-chloroform-acetone. Selection of the presumed optional solvent ratio was derived by a statistical analysis treatment utilizing simplex optimization.[8,9]

Spiegeler[10] introduced a new response criterion for the evaluation of high-performance TLC data. His method takes into account all components of a complex mixture and applies an elaborate mathematical function as the criterion for resolution. Sabaté[11] considers the distance between the lowest and highest spot regardless of their position in the chromatogram. However, Sabaté's expansion of the original Simplex method generates a second degree polynomial which is too complex for every day application.

EXPERIMENTAL

All solvents were redistilled. The *N-tert*-butyloxycarbonyl dolaproine 2-(S)-(1-hydroxy-1,1,2-triphenyl) ethyl ester isomers were prepared by an aldol condensation.[3] Small aliquots of the mixture of aldol condensation products in 50μL of ethyl acetate were chromatographed on "uniplate"-



Mixture No.	factor level (%)		
	A	B	C
1	77.7	11.1	11.1
2	44.4	11.1	44.4
3	11.1	11.1	77.7
4	11.1	44.4	44.4
5	11.1	77.7	11.1
6	44.4	44.4	11.1
7	55.5	22.2	22.2
8	22.2	22.2	55.5
9	22.2	55.5	22.2
10	33.3	33.3	33.3

Figure 1. Distribution of a three solvent system

type 5 x 10 cm HPTLC plates supplied by Analtech, Inc. Positions of the components were visualized by UV and by a 3% ceric sulfate solution (developed at 100°C). The silica gel column (9.5 cm diameter, 57 cm bed) was filled with a slurry of Kieselgel 60 (<0.063mm). The reaction mixture (80 g) was dissolved in the solvent mixture 32.3 : 53.4 : 14.3 *n*-hexane-chloroform-acetone and applied to the column. Fractions eluted between volumes 2200-3100 mL afforded 30.5 g of the pure S,S,R isomer.

METHODS AND DISCUSSION

When three component solvent (A,B,C) systems are considered for elution, possible initial distribution percentages of each in the mixture can be represented as illustrated in Fig. 1. Analogously, a more complex four solvent (A,B,C,D) system initial distribution can be represented as in Fig. 2.

The points in the diagrams are selected by the following criterion: a. With three solvents, parallel lines must be drawn separating each side of the figure into nine grids; b. in the case

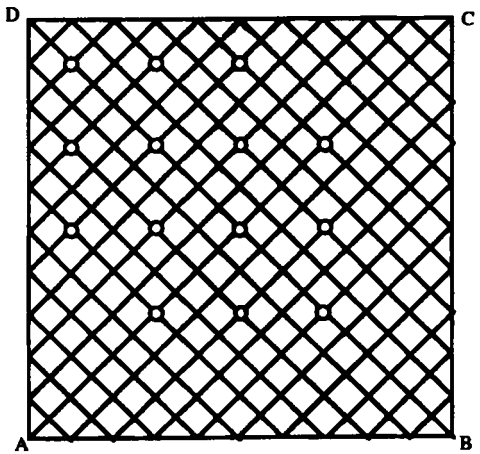


Figure 2. Distribution of a four component solvent system

of four solvents, parallel lines must be drawn separating each side of the figure into ten grids; points must be equal distances from each other and points must cover the entire diagram. For simplicity when selecting a four solvent system, it is better to predict a smaller area of the diagram and thereby reduce the solvent mixture to reasonable composition numbers. Each point on the diagram represents a specific solvent mixture. TLC data is obtained using each of these solvent mixtures. For column chromatography it is advantagous to have a less polar solvent mixture than for thin-layer chromatography. In the case of only one compound in the mixture, an optimal solvent system produces a chromatogram where all spots lie equi-distant from each other and their boundaries (Fig. 3). The position of the compound of interest must be in the middle of the chromatogram with the impurities as far away as possible. Since this was the practical problem at hand, we derived

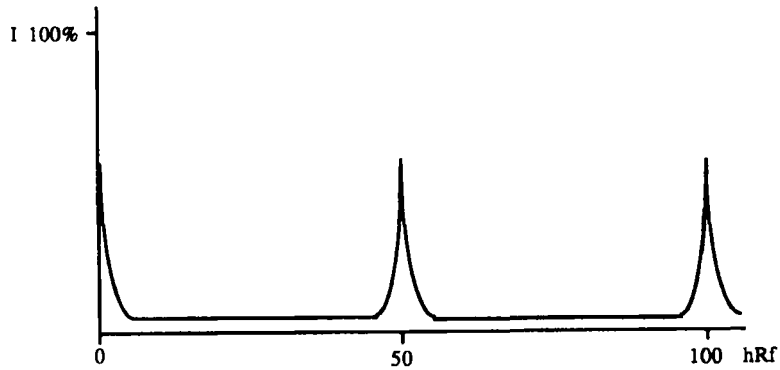


Figure 3. Model of maximal distribution between three spots.

response criterion from Spiegeleer's[10] equation employing extended Sabaté criterion:[11]

$$R = \frac{\prod_{i=1}^{n-1} (d_{(i+1)}-d_{(i)})]}{[ds/(n-1)]^{(n-1)}} \times 100 \quad [\%] \qquad (1)$$

In equation 1, n is the number of spots of interest; $d(i)$ is the distance between spots; and ds is the distance of the solvent front. This response (R) is expressed as a percentage. Once the TLC data is obtained, the three solvent system providing the best resolution according to the above criterion are graphically separated from the other points. Applying this method to the dolaproine isomer problem generated the set of points recorded in Figures 1 and 4.

After selection of the three best solvent mixtures, the simplex optimization method was used to estimate a better

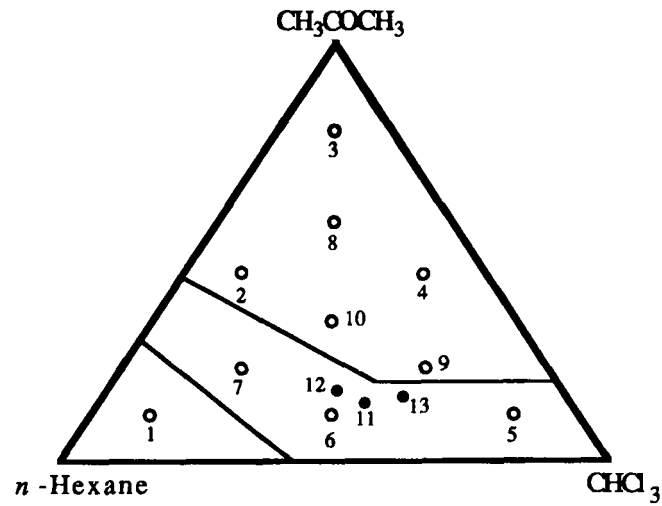


Figure 4. Results for solvent optimization
(o: initial search; ●: sipmlex optimization)

solvent mixture. The same method can be applied in searching for solvent mixtures for high performance liquid chromatography where retention times are measured. The only prerequisite is an initial TLC evaluation. The optimization was terminated at vertex 13 where a maximum response was obtained.* The factors used for optimization were the volume fractions of *n*-hexane (A) and chloroform (B). The fraction of acetone (C) required was defined by difference. The preceding experimental and mathematical approximation based approach to ascertaining the optimum solvent ratio with multicomponent solvent systems for column chromatography should prove useful in a broad variety of future applications.

* Vertex 5 gave the same response as vertex 13. However, the less polar vertex 13 was used for column purification.

	Factor Level (%)			R
Vertex No	*n*-Hexane	$CHCl_3$	CH_3COCH_3	[%]
6	44.4	44.4	11.1	1.18
7	55.5	22.2	22.2	1.48
5	11.1	77.7	11.1	3.70
11	38.9	47.2	13.9	2.17
12	40.3	42.3	17.4	3.16
13	32.3	53.4	14.3	3.70

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