Use of Column Chromatography for Quantitative Isolation of Mesoionic Pyrimidinium Betaines

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Abstract— Three mesoionic pyrimidinium betaines were synthesized and isolated using column chromatography. Such a technique was of a great help as far as a good isolation of the desired betaine from the synthesis crude product, being a mixture of several compounds, was not straightforward. Thin layer chromatograhy (TLC) analyses put up the better eluting system, in terms of retention factor, for a relatively quantitative separation of a pure betaine. Spectral analyses revealed the high purity of the isolated betaines.

Keywords— Betaine, column chromatography, purification, pyrimidine.

I. Introduction

THE chemistry of betaines has become an interesting subject owing to their widespread applications in biological research, particularly for their metabolic rolesin living organisms [1]. Indeed, alkylbetaines are nowadays a class of products of increasing importance in many fields. including cosmetic, medicine, pharmacy, and biology [2]-[5].

More interesting are the mesoionic betaines that bear biologically active moieties such as heterocyclic groups [6]. It was within this scope that our attention was focused on the synthesis of pyrimidine-containing mesoionic betaines.

In this present study, the latter betaines were achieved by condensation of *α*-aminopyridine or disubstituted amidine with malonic esters [7]-[11]. The applied synthetic method [10] led to a crude product with several components that defied a facile separation. To resolve such a complex mixture, column chromatography was employed, providing an adequate eluting system from TLC analysis and a good stationary phase.

II. Materials And Methods

A. Chemicals

The chemicals used in this work were purchased from Merck, Prolabo and Biochem. They were used without prior purification.

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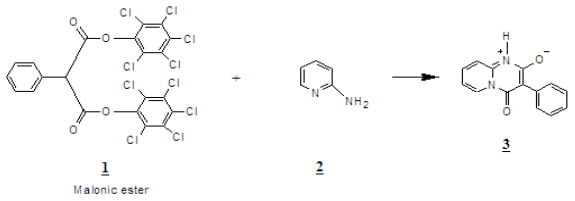
The solvents were dried using conventional techniques and stored over molecular sieves (4 Å) before use.

B. Synthesis of Pyrimidinium Betaines

*Bicyclic Betaine **3***

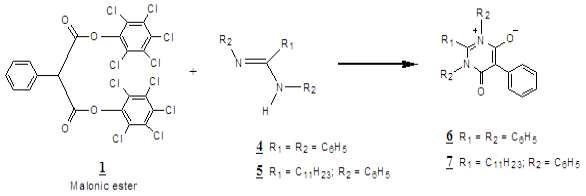
The bicyclic betaine **3** was synthesized via condensation of *α*-aminopyridine **2** with di-pentachlorophenyl phenylmalonate **1**, a malonic ester [10]. Refer to "(1)" .The reaction mixture was stirred for 30 min at room temperature. A yellow solid precipitated during the reaction and was isolated by filtration.



(1)

*Monocyclic Betaines **6** and **7***

Monocyclic betaines **6** and **7** were prepared under identical conditions as for **3** using *N*, *N'*-diphenylbenzamidine **4** and et dodecamidine **5** (a fatty amidine), respectively[10, 11]. Refer to "(2)". The crude products were in form of yellow and white solids, respectively, that precipitated during the reaction course.



(2)

C. Spectral Analyses

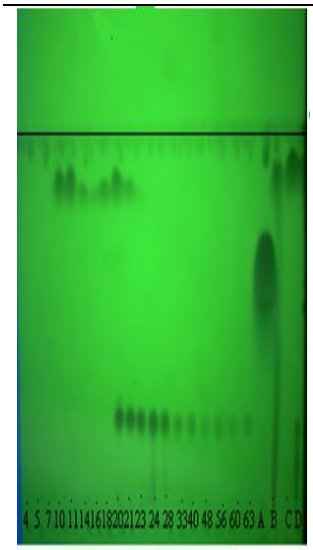
UV-visible spectra were recorded on a double beam Spectro-pho-tometer of type SHIMADZU 160, using dioxane as solvent. ^{1}H NMR spectra were taken on AVANCE 300a, using deuterated solvents (DMSO-d_6, $CDCl_3$) and tetramethylsilane (TMS) was employed as internal standard. IR spectra were recorded on JASCO 4100, samples in KBr pellets. Mass spectra were recorded on API 365 (Perkin Elmer Sciex) and Q-TRAP (Applied Biosystems).

http://dx.doi.org/10.15242/IJRCMCE.E0915003

All reaction products were analyzed by thin layer chromatography using TLC plates coated with Silica gel 60 F254 (Merck). The different spots were visualized with UV lamp (254 nm). Column chromatography was prepared under standard methods.

III. Results and Discussion

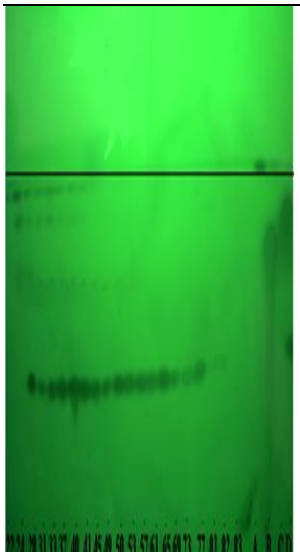
As can be noticed in Fig. 1, TLC analysis of the crude product of the reaction shown in Eq.1 revealed four spots, suggesting the presence of four components: malonic ester **1**, *α*-aminopyridine **2**, bicyclic betaine **3**, and pentachrophenol, a side product. The separation of components through column chromatography was as follows: component **2** was eluted with cyclohexane/ethyl acetate (8/2 v:v), pentachlorophenol with ethyl acetate/methanol (9/0.25 v/v), component **1** with methylene chloride/methanol (10/0.5 v/v), and component **3** with methanol.

TLC plate	Fractions	Detected compounds
	4-5	*α* -Amnopyridine **2**
	6-9	*α*-Amnopyridine **2** + Pentachlorophenol
	10-11	Pentachlorophenol
	12-18	Pentachlorophenol + Malonic ester **1**
	19	Malonic ester **1** + Betaine **3**
	20-23	Malonic ester **1**
	24-63	Bicyclic betaine **3**

A: *α*-Aminopyridine **2**
B: Malonic ester **1**
C: Pentachlorophenol
D: Betaine **3**

Fig. 1: Developed and visualized TLC plate for the crude productfrom (1). Eluent used was ethyl acetate/ methanol (9/0.5 v/v).

The results of TLC analysis of the crude product from the reaction of the malonic ester and *N*, *N'*-diphenylbenzamidine in (2) were four spots corresponding to the unreacted benzamidine **4** and malonic ester **1**, the desired betaine **6**, and the pentachlorophenol as a side product (Fig. 2).

TLC plate	Fractions	Detected compounds
	15-21	Benzamidine **4**
	22-24	Benzamidine **4** + Pentachlorophenol + Malonic ester **1**
	28-40	Benzamidine **4** + Pentachlorophenol + Malonic ester **1** + Monocylic betaine **6**
	41-50	Malonic ester **1** + Monocylic betaine **6**
	53-83	Monocylic betaine **6**

A: Benzamidine **4**
B: Malonic ester **1**
C: Pentachlorophenol
D: Monocylic betaine **6**

Fig. 2: Developed and visualized TLC plate for the crude product from the reaction of **1** with **4** as in (2). Eluent used was cyclohexane/ ethyl acetate/ methanol (20/9/1 v/v/v).

In the colomun chromatography operation, the eluting systems cyclohexane/ethylacetate (8/2, v/v), chloroform/methanol (9/1, v/v), and methanol served to isolate the unreacted benzamidine, the pentachlorophenol/malonic ester mixture, and the monocyclic betaine **6**, respectively.

As to monocyclic betaine **7**, a betaine with a fatty alkyl chain, its isolation by column chromatography was not completely successful. TLC results of the crude product from the reaction of the malonic ester **1** and the dodecamidine **5** hinted at the existence of four constituents (Fig.3): the unreacted dodecamidine **5** and malonic ester **1**, the pentachlorophenol, and the monocylic betaine **7**. In a first attempt, the dodecamidine **5** and the mixture malonic ester **1**/pentachlorophenol/monocylic betaine **7** were both eluted with cyclohexane/ethyl acetate (8/2 v/v). The use of only ethyl acetate in the tentative separation of the latter mixture favored the elution of only malonic ester and the betaine **7** could not be isolated. In further attempts, varying the volume ratio of the eluting system cyclohexane/ethyl acetate failed to separate the desired betaine **7**. However, liquid-liquid extraction allowed the isolation of the betaine in 37% yield [12].

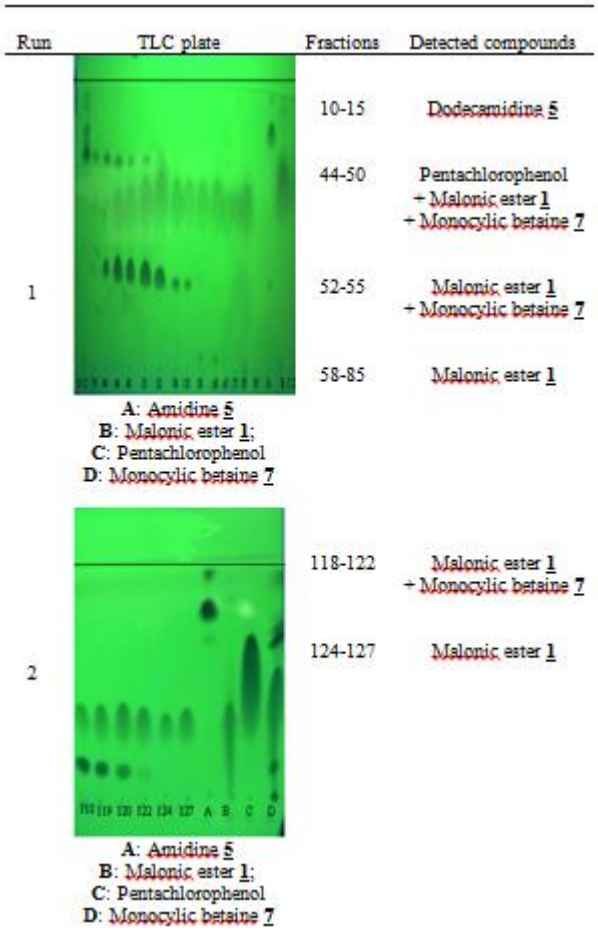


Fig. 3: Developed and visualized TLC plate for the crude product from the reaction of **1** with **5** in (2). Eluent used was cyclohexane/ ethyl acetate/ methanol (20/9/1 v/v/v).

From TLC plate depicted in Fig. 1, the retention factors R_f of the mesoionic pyrimidinium betaine **3** were deduced and are given in Table I. Thus, the binary system "ethyl acetate/methanol" in 9/0.5 v/v would elute the mixture better than the ternary one cyclohexane/ethyl acetate /methanol (20/9/1 v/v/v). Being ionic, the polarity of betaine would be eluted better in polar system; ethyl acetate/methanol is more polar than cyclohexane/ethyl acetate /methanol having a higher fraction in cyclohexane.

TABLE I
R_F VALUES FOR THE BETAINES **3** AND **6**

Betaines	Eluting system	R_f
Bicyclic betaine **3**	Ethyl acetate/methanol (9/0.5 v/v)	0.23
	Cyclohexane/ ethyl acetate/methanol (20/9/1 v/v/v)	0.10
Monocyclique betaine **6**	Cyclohexane/ ethyl acetate/methanol (10/9/0.5 v/v/v)	0.25
	Cyclohexane/ ethyl acetate/methanol (20/9/1 v/v/v)	0.21
	Cyclohexane/ ethyl acetate/methanol (20/9/0.25 v/v/v)	0.13

Also the R_f values for monocyclic betaine **6** (see Table 1) endorsed that fact that the polarity of the eluting system is pivotal for a good separation, hence a quantitative isolation, of the betaine; higher methanol fraction in the eluting system favored better resolution.

The physical and spectral characterizations of the betaines **3**, **6**, and **7** are gathered in Tables II and III.

TABLE II
PHYSICAL CHARACTERISTICS AND YIELDS OF BETAINES **3**, **6**, **7**

Betaine	Physical appearance	m.p. (°C)	Yield (%)
3	Yellow crystals	310-312*	71
6	Yellow crystals	317-319*	65
7	White needles	124-128	37

IV. CONCLUSION

Under the above-cited conditions, the separation and isolation of bicyclic betaine **3** and monocyclic betaine **6** by column chromatography were conducted without any major problem.However those of monocyclic betaine **7**, with a fatty side chain, were hampered, probably because of the closer polarities of some mixture components. The different spectral and physical characteristics confirmed the high purity of the isolated betaines.

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TABLE III
SPECTRAL CHARACTERIZATION OF BETAINES **3**, **6**, **7**

Betaine	UV-Visible (dioxane)		IR (KBr)	^{1}H NMR		MS (EI)
	λ_{max} [nm]	(logε)	$\bar{\upsilon}$ [cm^{-1}]	δ [ppm]	Solvent	
3	355	(3.68)	3112 (C-H, arom.); 2657 (NH); 1678 (C=O); 1527 (C=C, arom.)	7.13-7.46 (m, 5H, arom.); 7.66-7.72 (d, 2H, 8,10); 8.10-8.14 (t, 1H, 9); 9.03-9.05 (d, 1H, 7); 12.27-12.42 (s, 1H, NH)	DMSO-d_6	238[M]$^+$
6	357	(3.07)	3051 (C-H, arom.); 1651(C=O); 1593 (C=C, arom.)	6.98-7.82 (m, 20H, arom.)	DMSO-d_6	416[M]$^+$
7	352	(3.43)	3055 (C-H, arom.); 1647 (C=O); 1596 (C=C, arom.)	0.78-2.41 (25H, aliph.); 7.14-7.91 (15H, arom.)	$CDCl_3$	495[M+1]$^+$