

MALDI MS study of complexed alkaloid from *Colchicum* species

Abstract

The colchicine complexes with Li^+ , Na^+ , K^+ cations of perchlorates and iodides have been synthesized and studied in details by MALDI MS mass spectra.

1. INTRODUCTION

Properly designed metal complexes are interesting materials mainly because of their many applications due to their catalytic or many biological activities as anticancer, antifungal, antiviral and antibacterial. One of the known biologically active compound is colchicine. Colchicine **1** (Fig.1.) is a tropolone alkaloid of *Colchicum autumnale*. It shows antimitotic, antifibrotic, anti-inflammatory activity [1] and can efficiently exacerbate the symptoms during gout attack. More recently it has been introduced in the treatment of familiar Mediterranean fever [2]. Moreover, colchicine **1** is a potent anti-mitotic agent and shows carcinogenic activity [4-6]. Similarly to other alkaloids, colchicine **1** can act through blocking or activating of specific receptors or ion channels in living organisms. Its activity depends on the ability of formation of noncovalent complexes with macromolecules such as tubulin in microtubules [7].

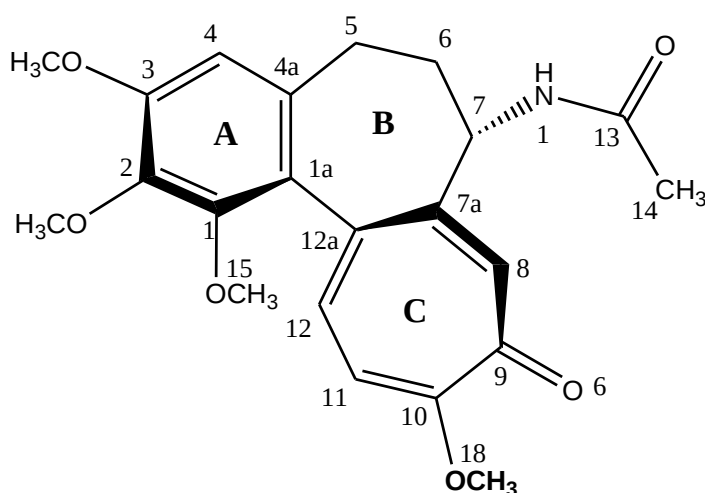


Figure 1. Structure of colchicine **1**

Not much research has been made on the formation of complexes between colchicines and cations. Only Mackay et al. in 1998 obtained a derivative of colchicine -hydrated crystals of copper(II) colchiceine that belongs to the tetragonal space group [8].

In the previous work we reported results of a study on coordination process of colchicine **1** with iodides and perchlorates of monovalent metal ions (lithium, sodium and potassium salts). Colchicine complexes with Li^+ , Na^+ and K^+ **2-7** have been characterized by spectral methods such as FT IR, UV vis, ESI MS mass spectra, ^1H NMR and ^{13}C NMR. Moreover theoretical studies of complexation process of colchicine have been made by semiempirical PM5 method and DFT studies [9,10]. Some other theoretical study also has been made on coordination process of colchicine - Na^+ cation. [11] Also for colchiceine (derivative of colchicine) the ability to form stable complexes has been studied [12].

More recently, new series of colchicine complexes with human body fluid ions such as Na^+ , K^+ , Mg^{2+} , Ca^{2+} , CO_3^{2-} and SO_4^{2-} have been obtained and characterized by spectral methods FT IR, ESI MS mass spectra, MALDI MS mass spectra, UV vis, ^1H NMR and ^{13}C NMR. Complexation capacity of these complexes has been also studied by DFT theoretical calculation [13].

The aim of this study was examination of colchicine coordinative compound with inorganic salts: perchlorates and iodides (lithium, sodium and potassium) by MALDI MS [9, 11]. On the basis of experiments performed and the analysis of MALDI MS spectra studies of these compounds, colchicine was demonstrated to have complexing capacity of the ions mentioned.

2. MATERIAL AND METHODS

Colchicine **1** is commercially available on ApplyChem. For complexation reaction was used natural isomer of colchicine (-)-(aR, 7S) colchicine. Salts LiI, NaI, KI, LiClO₄, NaClO₄, KSCN from Sigma-Aldrich were obtained commercially and used without any purification. Solvents used for the synthesis were purified by standard methods.[9] Colchicine complexes **2-7** were obtained by dissolving of the respective salts and colchicine in the 1:1 ratio in methanol.

2.1. Measurements

The matrix-assisted laser desorption/ionization measurements, including MS/MS experiments, have been accomplished on Waters Q-TOF Premier instrument, equipped with nitrogen laser MALDI source and MassLynx™ software. MALDI measurements have been performed in the positive ion mode. In order to prepare the target spots, the methanolic solution containing matrix (1 µl, concentration 0.5 mol/dm³) has been deposited on the spot and allowed to dry at room temperature. After a few minutes 1 µl of solution containing colchicine derivative (the concentration about 1 mmol/dm³) has been placed as a next layer over the dried matrix and left to cocrystallize. The MS/MS experiment with dithranol as a matrix and argon as colliding neutral gas (at flow rate of 0.5 mL/min) has been performed and the collision-induced fragmentation of protonated molecules [M+H]⁺ has been analyzed,

depending on collision energy CE (Table 4). The product ion MS/MS spectra were collected at four collision energy values, i. e. 10, 20, 30 and 40 eV. It should be noticed the nanospray MS of colchicine and MS² of the m/z 400±0.5 ion [M+H]⁺ have already been reported and representative fragment ions, which originate from m/z 400, are given, i.e. m/z 382, 368, 358, 341, 326, 310, 298 and 282. MALDI MS/MS fully confirms this fragmentation pattern, since the same m/z values appear in collisionally induced dissociation as well as in previously published papers. [14]

3. RESULTS AND DISCUSSION

The colchicine complexes with salts (LiClO₄, LiI, NaClO₄, NaI, KI, KSCN) were obtained as yellow to dark orange crystalline products with very good yields by dissolving of the respective salts and colchicine in the 1:1 ratio [9]. The complexes previously have been studied by spectral analysis: UV-Vis, ¹H and ¹³C NMR, FT IR ESI MS, MS FAB and semiempirical calculation [9].

MALDI studies

A six of colchicine complexes with monovalent Li⁺, Na⁺ and K⁺ cations **2-7** has been formed and investigated through MALDI mass spectrometry. The salts: LiClO₄, NaClO₄, KSCN, LiI, NaI, KI complexes **2-7** have been tested in order to establish the cation and anion importance for complexing process. It should be noticed the nanospray MS of colchicine and MS² of the m/z 400±0.5 ion [**1**+H]⁺ have already been reported and representative fragment ions, which originate from m/z 400, are given, i.e. m/z 382, 368, 358, 341, 326, 310, 298 and 282. MALDI MS/MS fully confirms this fragmentation pattern, since the same m/z values appear in collisionally induced dissociation as well as in previously published papers.[14,15,16]

The obtained colchicine complexes with lithium, sodium and potassium iodides, lithium and sodium perchlorates and potassium thiocyanate [9] were submitted to analysis using MALDI TOF mass spectrometry to check what kind of complexes exist or prevail in the gas phase and how their dissociation proceeds in collision-induced conditions. For the tested samples, in the MALDI TOF MS spectra, complexes colchicine-metal cation in a ratio of 1: 1, i. e. $[\mathbf{1} + \text{Li}]^+$ for **2** and **3**, $[\mathbf{1} + \text{Na}]^+$ for **4** and **5**, $[\mathbf{1} + \text{K}]^+$ for **6** and **7** were observed. The presence of the complexes of stoichiometry 2:1, i.e. $[\mathbf{2} \cdot \mathbf{1} + \text{Met}]^+$ (where Met is metal ion) was seen as well, but the latter ions have a smaller abundance than $[\mathbf{1} + \text{Na}]^+$.

In the MALDI TOF MS spectra for colchicine and its complexes with alkali metal ions (**2-7**) with dithranol as a matrix: ions $[\mathbf{1} + \text{Li}]^+$, $[\mathbf{1} + \text{Na}]^+$ and $[\mathbf{1} + \text{K}]^+$ respectively, have been observed, the data (elemental composition and relative abundance) are given in the Table.1..

Table. 1. MALDI TOF MS spectra of colchicine complexes **2-7**

Ion	complex	Elemental composition	m/z	Relative Abundance (%)
$\mathbf{1} + \text{Li}^+$	$\text{C}_{22}\text{H}_{25}\text{NO LiI}$ (2)	$\text{C}_{22}\text{H}_{25}\text{NO Li}^+$	406	50
	$\text{C}_{22}\text{H}_{25}\text{NO LiClO}_4$ (3)	$\text{C}_{22}\text{H}_{25}\text{NO Li}^+$	406	100
$\mathbf{1} + \text{Na}^+$	$\text{C}_{22}\text{H}_{25}\text{NO NaI}$ (4)	$\text{C}_{22}\text{H}_{25}\text{NO Na}^+$	422	85
	$\text{C}_{22}\text{H}_{25}\text{NO NaClO}_4$ (5)	$\text{C}_{22}\text{H}_{25}\text{NO Na}^+$	422	70
$\mathbf{1} + \text{K}^+$	$\text{C}_{22}\text{H}_{25}\text{NO KSCN}$ (7)	$\text{C}_{22}\text{H}_{25}\text{NO K}^+$	438	30
	$\text{C}_{22}\text{H}_{25}\text{NO KI}$ (6)	$\text{C}_{22}\text{H}_{25}\text{NO K}^+$	438	25
$\mathbf{2} \cdot \mathbf{1} + \text{Li}^+$	$\text{C}_{22}\text{H}_{25}\text{NO LiI}$ (2)	$\text{C}_{44}\text{H}_{50}\text{N}_2\text{O Li}^+$	805	10
	$\text{C}_{22}\text{H}_{25}\text{NO LiClO}_4$ (3)	$\text{C}_{44}\text{H}_{50}\text{N}_2\text{O Li}^+$	805	1
$\mathbf{2} \cdot \mathbf{1} + \text{Na}^+$	$\text{C}_{22}\text{H}_{25}\text{NO NaI}$ (4)	$\text{C}_{44}\text{H}_{50}\text{N}_2\text{O Na}^+$	821	5

	$\text{C}_{22}\text{H}_{25}\text{NO}_6\text{NaClO}_4$ (5)	$\text{C}_{44}\text{H}_{50}\text{N}_2\text{O}_{12}\text{Na}^+$	821	7
$2\cdot\mathbf{1}+\text{K}^+$	$\text{C}_{22}\text{H}_{25}\text{NO}_6\text{KI}$ (6)	$\text{C}_{44}\text{H}_{50}\text{N}_2\text{O}_{12}\text{K}^+$	837	10

In the TOF MS LD⁺ mass spectra of colchicine complexes with potassium salts some characteristic fragment ions for colchicine itself are observed, like m/z= 341, m/z=282. Since in MALDI measurements a great excess of matrix (here – dithranol, DIT) in relation to the analyte is used and matrix is much more prone to deprotonation than colchicine, in the negative ion mode prevail matrix ions (deprotonated dithranol, its dimer, trimer and tetramer, Fig. 2.).

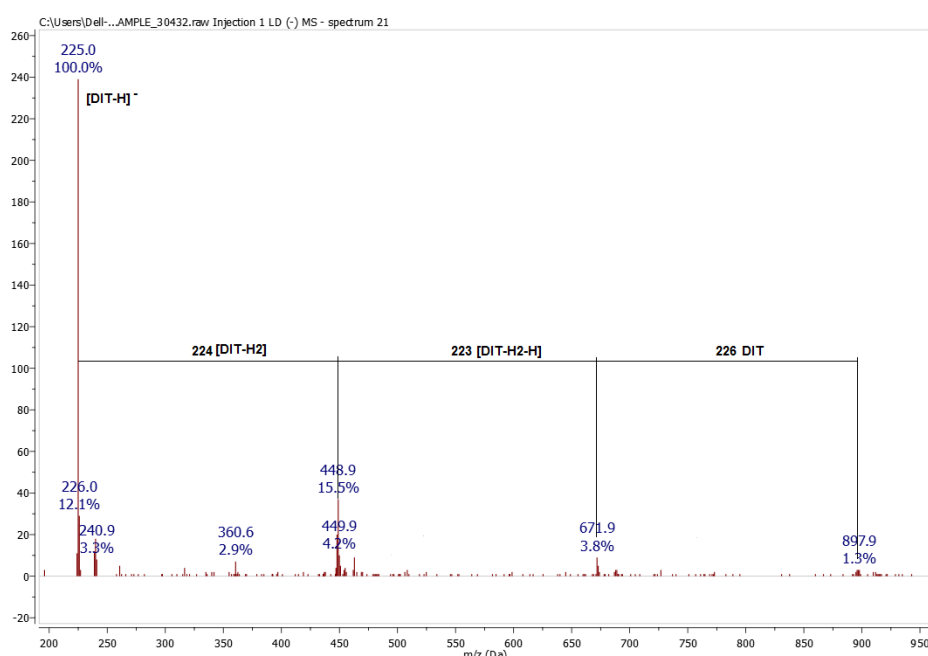


Figure 2. MALDI TOF mass spectrum of complex 5 (colchicine with NaClO₄) in the negative ion mode.

As precursors for MSMS experiment, the ions $[\mathbf{1}+\text{Li}]^+$, $[\mathbf{1}+\text{Na}]^+$ and $[\mathbf{1}+\text{K}]^+$ were chosen and the decay of these ions under collision with argon atoms conditions, using collision energy 20, 30, 40, 50 and 60 eV, respectively, were examined (Table 2).

Table 2. MALDI MS/MS LD⁺ spectral data for complexes 2 ($\mathbf{1}+\text{LiI}$) and 3 ($\mathbf{1}+\text{LiClO}_4$) and precursor ion $[\mathbf{1}+\text{Li}]^+$ m/z 406, depending on collision energy (Ce).

m/z	% of relative abundance (% r.a.)									
	Ce = 20		Ce = 30		Ce = 40		Ce = 50		Ce = 60	
	3	2	3	2	3	2	3	2	3	2
406	100	100	100	100	10					
378			9	51						
376					13	13	3			
365			5							
363				23		10				
347			20	29	9		3			
343									16	
341		3	84	88	62	39	5			
332			5	13	18	16			5	9
317						32	13			
313			8	10	26	23	3			
311							15			
302							10			
298			2		20		16			
295				3			15			
289				9		71	18	46	16	30
285			5		47		26			
282			10	19	100	100	69		16	
267					30	55	100	100	74	65
255						19	34	54	79	
252								54	95	
251					11		35	38		57
239					9		26	46	74	

231		4	6	13	13	11	23		
224						19	38	100	100
209								68	61
207					7	10	38	42	
195						8			57
194								47	
190						11	15		
181						7		21	35
165								32	26
160						5			13
154								11	
145				3		5		11	
141								5	9
128								11	

118

119 **Table 3.** MS MS LD⁺ (**1** + NaClO₄) for the precursor ion [**1**+Na]⁺ m/z 422 on different
120 collision energy (Ce).

% of relative abundance (% r.a.)										
m/z	Ce = 20		Ce = 30		Ce = 40		Ce = 50		Ce = 60	
	4	5	4	5	4	5	4	5	4	5
422	100	100	100	100	9	21				
394			11							
392				11	25	29	2			
374					4					
363			11	13		7				
341			33	38	41	36				
333					11		3			

325			3				
313		4	6	17	21	3	
298				15			
294							6
285				46			
283							17
282		6	6	100	100	32	43
270				18		20	
267				40		100	100
255						47	36
252							54
239				9		49	42
231		6	10	29			
224				3		31	32
211					14	13	
205				3			
195						14	11
181						11	10
178							42
169							21
152							21

From the obtained results (Table 2 and 3) one can conclude that the cations of alkali metals bind quite strongly to the colchicine molecule, since no decomposition with elimination of a metal cation or metal cation exchange on a proton, ie. $[\mathbf{1}+\text{Met}]^+ + \text{H}^+ \rightarrow [\mathbf{1} + \text{H}]^+ + \text{Met}^+$, had been observed in the MS or MALDI MSMS and no ion at m/z 400, (protonated colchicine molecule) had been recorded. On the other hand, a characteristic fragment ion of high

abundance in MSMS spectra of the above complex ions is an ion at m/z 341, indicating a decomposition of the complex with the loss of the NH_2COCH_3 (acetamide group) together with the metal cation, which implies the binding of Li^+ and Na^+ by the oxygen atom of the amide group.

Table 4. MSMS dissociation of the precursor ion at m/z 821, $[\mathbf{2} \cdot \mathbf{1} + \text{Na}]^+$, obtained from the colchicine complex with NaClO_4 on collision energy (Ce).

m/z	Ce	20	30	40	50	60
821		100	40	40	23	5
422		100	100	100	100	100
363					17	
341						48
326			20			
324				5		
282						17
231						22

The other important fragments in the MSMS spectra of the complexes $[\mathbf{1} + \text{Li}]^+$, m/z 406, are these at m/z 378 and 376 and m/z 347 corresponding to the elimination from the complex masses -28 (CO) at -30 (CH_2O) and (-59u), while in the case of $[\mathbf{1} + \text{Na}]^+$, m/z 422, respective resulting ions are m/z 394 (422-28u), 392 (422-30u) and 363 (422-59u) (Table 4). All of these fragment ions contain in their structure an alkali metal cation, which has not detached from the colchicine molecular backbone.

However, in the MSMS spectra of complexes, some ions characteristic for the MS dissociation of $[\mathbf{1} + \text{H}]^+$ ion, for example m/z 382 (water loss), 368 (MeOH loss), 358 (ketene $\text{CH}_2=\text{C}=\text{O}$ loss) or 310 are lacking. Frequently observed ion (but with a small abundance) is an ion at m/z 313. At $\text{ce} = 40$ eV most abundant ion in the MSMS spectra is that at m/z 282

(100% of relative abundance), $C_{18}H_{18}O_3$, containing no amide group $-NHCOCH_3$, known also from MALDI MSMS fragmentation pattern of colchicine and alkylthiocolchicines.[14] For $ce = 50$ eV, the one of 100% of relative abundance is the ion at m/z 267, $C_{17}H_{15}O_3$, [14] and for $ce = 60$ eV, m/z 224 (100% r.a.), ion coming from deep decay of colchicine molecule, which is also seen in MALDI TOF MSMS spectra of alkylthiocolchicines at $ce = 40$ eV [14, Fig.3.] These data indicate that alkali metal ion complexation by colchicine molecule involved is mainly acetamide group located on carbon C7. This conclusion is in agreement with calculations Bodoki et al. [11], who showed theoretically that the most favorable position of the sodium cation is between the oxygen atom of the amide group and the oxygen atom of CH_3O- , located at the C1 carbon atom.

In the case of ions $[1+K]^+$ fragmentation in collision conditions is in general very poor, the only daughter ions of significant abundance is the ion at m/z 149. Similarly proceeded decay of the complexes $[1+K]^+$, formed by colchicine with K_2CO_3 and K_2SO_4 [13], which indicates that the anion has limited effect on the structure and stability of the complex **1**-Met in the gas phase.

4. CONCLUSIONS

Series of colchicine complexes with monovalent metal cations of perchlorates and iodides were described by MALDI MS mass spectra. The MALDI experiments suggest that amide group of colchicine plays important role in coordinating the metal s of the first group.

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