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Colchiceine Complexes with Lithium, Sodium and Potassium Salts – Spectroscopic Studies

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Abstract: Colchiceine complexes with Li^+, Na^+ and K^+ cations have been synthesized and studied by ^1H and ^{13}C NMR, FT-IR, FAB MS and UV-Vis. It has been shown that colchiceine forms stable complexes especially with lithium cation and the most stable structures of the complexes are those in which the acetamide groups are involved in the coordination process. The structures of the colchiceine complexes with Li^+, Na^+ and K^+ cations are discussed in details.

Keywords: colchiceine, complexes of colchiceine, NMR spectra, FT-IR, FAB MS spectra, UV-Vis spectra.

INTRODUCTION

COLCHICINE **1**, in Figure 1, is a tropolone alkaloid of *Colchicum autumnale*. It shows antimitotic, anti-fibrotic and anti-inflammatory activity[1] and can efficiently alleviate the symptoms of gout attack when applied in the early phase. More recently, it has been introduced in the treatment of Mediterranean fever.[2] Compound **1** is known to be sensitive to light and hydrolysis.[3] Colchiceine **2** (10-demetoxy-10-hydroxy-colchicine) in Figure 1 and β- and γ-lumicolchicine are the main degradation products of colchicine.

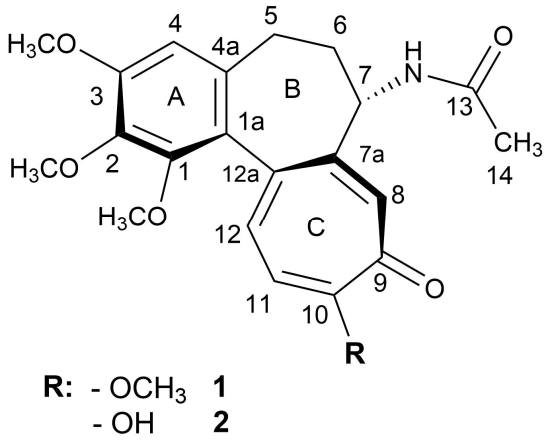

Figure 1. Structure and carbon atom numbering of Colchicine **1** and Colchiceine **2**.

Compound **2,** an important intermediate in the synthesis of colchicine C-10 derivatives, can be obtained from colchicine **1** by a gentle hydrolysis with hydrochloric acid.[4] Colchiceine **2** is also a useful reagent for many chemical reactions on ring C, for example tosylation or mesylation to obtain known derivatives or to produce new compounds. Upon tosylation or mesylation, **2** is a mixture of two products with substituent at C-9 (biologically-inactive isocolchicinoids) and C-10 (biologically-active colchicinoids) carbon atom, is caused by tosyl group migration.[5–7]

It is necessary for biologically active compounds to be easily analysed and detected by analytical methods, for example TLC and HPLC. HPLC analyses of **2** turned out to be more difficult than of **1** and Colchicum plant extracts. Colchiceine can be present in plant extracts besides colchicine, or can appear as a degradation product on storage of **1**. [8–11] Colchiceine can easily form complexes with most of silica based phases, which can be explained by the properties of its ring system especially ring C with a

hydroxyl group at C-10 position and neighbouring carbonyl group at carbon atom C-9. It is difficult to detect and separate colchiceine from plant extracts because of its chelating properties. To extensively recovery of colchiceine from silica column matrixes in a HPLC analysis a suitable metal cation in salt form, which can form complexes with **2** was added. For better separation of **2** from a mixture of alkaloids in plant extracts, usually inorganic salts are added. For instance to aqueous solution of **2,** ferric chloride is added to get a coloured complex, then the solution is extracted with chloroform. In the HPLC analysis copper sulphate usually was added to the mobile phase to form coloured complexes of metal-colchiceine. [3,12,13]

After administration of colchicine, colchiceine has been reported as a metabolite in rats,[14] but it has not been demonstrated to occur in humans.[15] Mourelle et al. have proposed that colchiceine acts as an antioxidant and protective agent against lipid peroxidation in a rat model of liver injury.[16] 10-Demetoxy-10-hydroxy-colchicine (colchiceine) has not been confirmed to occur in humans, but is produced in rodents by the same CYP homolog.[17,18] In a biochemical model of liver injury, **2** is the metabolite, suggested to have beneficial effects.[19] It has been shown that **2** prevents acute and chronic CCl_4 injury in rats.[15,20] Furthermore in an earlier study, **2** has been shown to prevent lipid peroxidation the centerpiece of CCl_4-induced injury. Protection against lipid peroxidation has also been observed in a rat model of biliary obstruction in which **2** was compared with vitamin E.[21] High concentrations of colchiceine has also been shown to protect reduced glutathione (GSH) against oxidation by atmospheric oxygen and to prevent oxygen-induced inactivation of enzymes with catalytically essential sulfhydryl groups.[22] *In vivo* experiments have suggested that **2** acts as an effective antioxidant at pharmacologically relevant concentrations,[21] although direct evidence for **2** radical scavenging activity and protection against lipid peroxidation is lacking.[15]

Moreover, colchiceine has been demonstrated to be less toxic than colchicine in the evaluation of the effect on cytochrome P450 (CYP) expression. It has been concluded that colchiceine is not cytotoxic in primary human hepatocytes.[23] Interestingly, colchiceine has better antifibrotic properties than colchicine, it can be used as a **1** substitute with anticipated fewer side-effects.[20] Colchiceine, a closely related structural analogue of colchicine with a C-ring tropolone, has been shown to be a potent inhibitor of microtubule assembly *in vitro.* The mechanism of inhibition is mediated through binding to tubulin although potentially not through the colchicine receptor site.[24]

The formation of complexes between colchicines and cations has not been the subject of great interest. Only Mackay et al. in 1998 obtained hydrated crystals of copper(II) colchiceine, belonging to the tetragonal space group.[16] In this structure the tropolonic oxygens, from two colchiceine molecules, are coordinated to the copper atom in this bis-chelated complex to form a square planar arrangement. The positions of three water molecules are clearly defined. An intricate hydrogen-bonding system links the complex and water molecules into a three-dimensional network in the crystal.[16]

In the previous work we have reported the coordination process of colchicine with iodides and perchlorates monovalent metal ions (lithium, sodium and potassium salts).[25] Although colchicine is a very important commercially available alkaloid, its complexes (except in Ref. 25) and complexes of colchicine derivatives like colchiceine have not been thoroughly characterized yet.

The complexation process of colchiceine with monovalent metal ions has not been studied yet. This fact has prompted us to obtain and examine colchiceine complexes **3**–**8** with monovalent cations: Li^+, Na^+ and K^+. Molecular structures for all obtained colchiceine complexes were established by of 1D and 2D NMR, IR, UV and mass spectral analysis. From the practical point of view this process of complexation can be useful for isolation colchicines from plant extracts.

EXPERIMENTAL

Material

Colchiceine was synthesized from colchicine (Aldrich) by acidic hydrolysis in the mild conditions.[26] Obtained colchiceine is a monohydrate compound. The colchiceine complexes **3-8** with salts ($LiClO_4$, LiI, $NaClO_4$, NaI, KI, KSCN from Aldrich) were obtained by dissolving of the respective salts and colchiceine in the 1:1 ratio in methanol. Complexes were prepared from colchiceine (96 mg, 0,25 mmol) and lithium iodide (33 mg, 0,25 mmol), lithium perchlorate (27 mg, 0.25 mmol), sodium iodide (38 mg, 0.25 mmol), sodium perchlorate (31 mg, 0.25 mmol), potassium iodide (42 mg, 0.25 mmol), potassium thiocyanate 24 mg, 0.25 mmol), respectively. Each mixture was stirred for 24h in room temperature. The crystalline products were obtained by slow evaporation of the solvent.

Carbon atom numbering of colchicine **1** and colchiceine **2** is shown in Figure 1.

(3) Complex Colchiceine - Lithium Iodide

103 mg; yield 84 %; m.p. 110–112 °C, Anal. Calcd. for $C_{21}H_{23}NO_6 \cdot LiI \cdot 3.5H_2O$: C, 43.22; H, 5.11; N, 2.41. Found: C, 43.49; H, 5.31; N, 2.55. IR(KBr) $\tilde{\nu}/cm^{-1}$: 3411 m, 3246 m, 2998 w, 2937 w, 2856 w, 1656 s, 1602 s, 1540 m, 1489 m, 1454 m, 1276 m, 1195 s, 1139 m, 483 w. IR (nujol/fluorolub) $\tilde{\nu}/cm^{-1}$: 3397 m, 3243 m, 3045 w, 2952 w, 2922 w, 2853 w, 2727 w, 1655 s, 1602 s, 1537 m, 1488 m, 1452 m, 1404 w,

1349 w, 1274 w, 484 w (vs, very strong; s, strong; m, medium; w, weak; and also in further text regarding IR spectra ($\tilde{\nu}$/cm^{-1})). ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 6.79 (s, HC-4), 2.15 (m, H_2C-5), 2.55 (m, H_2C-5), 1.94 (m, H_2C-6), 2.13 (m, H_2 C-6), 4.35 (m, HC-7), 7.30 (s, HC-8), 7.31 (d, J = 11.81 Hz, HC-11), 7.13 (d, J = 11.81 Hz, H C-12), 3.77 (s, CH_3O-1), 3.51 (s, CH_3O-2), 3.83 (s, CH_3O-3), 1.88 (s, H_3C-14), 8.63 (d, J = 7.14 Hz, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 149.68 (C-1), 125.60 (C-1a), 140.48 (C-2), 152.82 (C-3), 107.70 (C-4), 133.69 (C-4a), 29.24 (C-5), 36.50 (C-6), 51.61 (C-7), 149.94 (C-7a), 123.91 (C-8), 172.00 (C-9), 168.54 (C-10), 118.07 (C-11), 139.91 (C-12), 134.54 (C-12a), 60.71 (CH_3O-1), 60.69 (CH_3O-2), 55.87 (CH_3O-3), 168.90 (C-13), 22.50 (C-14). ^{1}H NMR (300 MHz, CD_3CN) δ/ppm: 6.74 (s, HC-4), 2.15 (m, H_2C-5), 2.55 (m, H_2C-5), 2.09 (m, H_2C-6), 2.25 (m, H_2C-6), 4.41 (m, HC-7), 7.51 (s, HC-8), 7.25 (d, J = 7.48 Hz, HC-11), 7.48 (d, J = 7.25 Hz, HC-12), 3.59 (s, CH_3O-1), 3.82 (s, CH_3O-2), 3.87 (s, CH_3O-3), 1.94 (s, H_3C-14), 7.65 (d, J = 4.41 Hz, NH). ^{13}C NMR (75 MHz, CD_3CN) δ/ppm: 151.04 (C-1), 126.37 (C-1a), 141.74 (C-2), 154.39 (C-3), 108.44 (C-4), 135.75 (C-4a), 30.03 (C-5), 37.87 (C-6), 53.53 (C-7), 151.31 (C-7a), 124.85 (C-8), 172.94 (C-9), 168.43 (C-10), 118.20 (C-11), 142.30 (C-12), 136.24 (C-12a), 61.62 (CH_3O-1), 61.30 (CH_3O-2), 56.53 (CH_3O-3), 171.23 (C-13), 22.77 (C-14). UV-visible in CH_3OH λ_{max}/nm (ε / mol^{-1} dm^3 cm^{-1}): 349.5 (17444), 224 (35853).

(4) Complex Colchiceine - Sodium Iodide

131 mg; yield 93 %; m.p. 98–100 °C, Anal. Calcd. for $C_{21}H_{23}NO_6$·NaI·3H_2O: C, 41.38; H, 4.76; N, 2.29. Found: C, 41.70; H, 4.71; N, 2.27. IR (KBr) $\tilde{\nu}$/cm^{-1}: 3436 m, 3252 m, 2998 w, 2937 w, 2856 w, 2836 w, 1659 s, 1608 s, 1541 m, 1489 m, 1453 m, 1275 m, 1196 m, 1137 m, 483 w. IR (nujol/fluorolub) $\tilde{\nu}$/cm^{-1}: 3407 m, 3245 m, 3046 m, 2953 m, 2937 w, 2855 w, 2728 w, 1659 s, 1606 s, 1534 m, 1489 m, 1455 m, 1404 m, 1349 m, 1276 m, 484 w. ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 6.81 (s, HC-4), 2.13 (m, H_2C-5), 2.53 (m, H_2C-5), 1.97 (m, H_2C-6), 2.11 (m, H_2C-6), 4.34 (m, HC-7), 7.32 (s, HC-8), 7.34 (d, J = 11.81 Hz, HC-11), 7.16 (d, J = 11.81 Hz, HC-12), 3.79 (s, CH_3O-1), 3.53 (s, CH_3O-2), 3.85 (s, CH_3O-3), 1.88 (s, H_3C-14), 8.65 (d, J = 7.32 Hz, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 49.75 (C-1), 125.56 (C-1a), 140.50 (C-2), 152.88 (C-3), 107.74 (C-4), 133.98 (C-4a), 29.24 (C-5), 36.91 (C-6), 51.65 (C-7), 149.95 (C-7a), 124.07 (C-8), 171.74 (C-9), 168.53 (C-10), 118.12 (C-11), 140.00 (C-12), 134.56 (C-12a), 60.74 (CH_3O-1), 60.72 (CH_3O-2), 55.91 (CH_3O-3), 168.61 (C-13), 22.51 (C-14). ^{1}H NMR (300 MHz, CD_3CN) δ/ppm: 6.74 (s, HC-4), 2.21 (m, H_2C-5), 2.54 (m, H_2C-5), 2.08 (m, H_2C-6), 2.25 (m, H_2C-6), 4.42 (m, HC-7), 7.53 (s, HC-8), 7.24 (d, J = 7.48 Hz, HC-11), 7.48 (d, J = 7.24 Hz, HC-12), 3.60 (s, CH_3O-1), 3.84 (s, CH_3O-2), 3.87 (s, CH_3O-3), 1.95 (s, H_3C-14), 7.54 (d, J = 4.42 Hz, NH). ^{13}C NMR (75 MHz, CD_3CN) δ/ppm: 150.99 (C-1), 126.46 (C-1a), 141.67 (C-2), 154.26 (C-3), 108.40 (C-4), 135.78 (C-4a), 30.05 (C-5), 37.86 (C-6), 53.37 (C-7), 151.30 (C-7a), 124.81 (C-8), 173.06 (C-9), 168.41 (C-10), 117.88 (C-11), 142.13 (C-12), 135.95 (C-12a), 61.53 (CH_3O-1), 61.30 (CH_3O-2), 56.50 (CH_3O-3), 170.42 (C-13), 22.77 (C-14). UV-visible in CH_3OH λ_{max}/nm (ε/ mol^{-1} dm^3 cm^{-1}): 350 (16762), 223 (36109).

(5) Complex Colchiceine - Potassium Iodide

135 mg; yield 98 %; m.p. 94–96 °C, Anal. Calcd. for $C_{21}H_{23}NO_6$·KI·2H_2O: C, 42.82; H, 4.56; N, 2.38. Found: C, 42.72; H, 4.42; N, 2.31. IR (KBr) $\tilde{\nu}$/cm^{-1}: 3438 m, 3359 m, 3128 m, 2997 w, 2938 w, 2859 w, 2833 w, 1653 s, 1611 s, 1553 m, 1490 m, 1454 m, 1276 m, 1195 m, 1136 m, 482 w. IR (nujol/fluorolub) $\tilde{\nu}$/cm^{-1}: 3418 m, 3244 m, 3051 w, 2953 w, 2926 w, 2855 w, 2725 w, 1657 s, 1609 s, 1543 m, 1489 m, 1453 m, 1403 m, 1349 m, 1275 m, 484 w. ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 6.81 (s, HC-4), 2.13 (m, H_2C-5), 2.54 (m, H_2C-5), 1.98 (m, H_2C-6), 2.12 (m, H2C-6), 4.37 (m, HC-7), 7.34 (s, HC-8), 7.36 (d, J = 11.81 Hz, HC-11), 7.18 (d, J = 11.81 Hz, HC-12), 3.80 (s, CH_3O-1), 3.54 (s, CH_3O-2), 3.85 (s, CH_3O-3), 1.89 (s, H_3C-14), 8.65 (d, J = 7.31Hz, 1H – NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 149.75 (C-1), 125.49 (C-1a), 140.46 (C-2), 152.85 (C-3), 107.71 (C-4), 134.07 (C-4a), 29.19 (C-5), 36.88 (C-6), 51.62 (C-7), 149.91 (C-7a), 124.07 (C-8), 171.57 (C-9), 168.32 (C-10), 118.08 (C-11), 139.99 (C-12), 134.52 (C-12a), 60.70 (CH_3O-1), 60.67 (CH_3O-2), 55.88 (CH_3O-3), 168.53 (C-13), 22.48 (C-14). ^{1}H NMR (300 MHz, CD_3CN) δ/ppm: 6.74 (s, HC-4), 2.21 (m, H_2C-5), 2.55 (m, H_2C-5), 2.03 (m, H_2C-6), 2.19 (m, H_2C-6), 4.42 (m, HC-7), 7.52 (s, HC-8), 7.25 (d, J = 7.48 Hz, HC-11), 7.48 (d, J = 7.25 Hz, HC-12), 3.61 (s, CH_3O-1), 3.84 (s, CH_3O-2), 3.87 (s, CH_3O-3), 1.94 (s, H_3C-14), 7.44 (d, J = 4.42 Hz, NH). ^{13}C NMR (75 MHz, CD_3CN) δ/ppm: 151.00 (C-1), 126.48 (C-1a), 141.70 (C-2), 154.30 (C-3), 108.41 (C-4), 135.78 (C-4a), 30.06 (C-5), 37.86 (C-6), 53.35 (C-7), 151.29 (C-7a), 124.75 (C-8), 173.09 (C-9), 168.24 (C-10), 117.66 (C-11), 142.12 (C-12), 135.94 (C-12a), 61.49 (CH_3O-1), 61.28 (CH_3O-2), 56.49 (CH_3O-3), 170.17 (C-13), 22.74 (C-14). UV-visible in CH_3OH λ_{max}/nm (ε/ mol^{-1} dm^3 cm^{-1}): 350.5 (17292), 222.5 (39813).

(6) Complex Colchiceine - Lithium Perchlorate

114 mg; yield 94 %; m.p. 100–102 °C, Anal. Calcd. for $C_{21}H_{23}NO_6$·$LiClO_4$·2.5H_2O: C, 46.93; H, 5.21; N, 2.61. Found: C, 46.54; H, 5.11; N, 2.55. IR (KBr) $\tilde{\nu}$/cm^{-1}: 3394 m, 3245 m, 3059 w, 3000 w, 2939 w, 2859 w, 2838 w, 1654 s, 1606 s, 1543 m, 1489 m, 1456 m, 1277 m, 1196 m, 1143 m, 1087 vs, 1044 m, 918 w, 625 w, 484 w. IR (nujol/fluorolub) $\tilde{\nu}$/cm^{-1}: 3573 m, 3526 m, 3366 m, 2951 w, 2925 w, 2855 w, 2726 w, 1629 s, 1611 s, 1552 m, 1491 m, 1456 m, 1404 m, 1349 m, 1278 m, 1094 vs, 1011 m, 920 w, 625 w, 483 w. ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 6.79 (s, HC-4), 2.13 (m, H_2C-5), 2.55 (m, H_2C-5), 1.94 (m, H_2C-6), 2.11 (m, H_2C-6), 4.34 (m, HC-7), 7.31 (s, HC-8), 7.33 (d, J = 11.81 Hz, HC-11), 7.15 (d, J = 11.81 Hz, HC-12), 3.77 (s, CH_3O-1), 3.51 (s, CH_3O-2), 3.83 (s, CH_3O-3), 1.88 (s, H_3C-14), 8.64 (d, J = 7.42 Hz, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 149.82 (C-1),

125.59 (C-1a), 140.55 (C-2), 152.95 (C-3), 107.76 (C-4), 134.14 (C-4a), 29.29 (C-5), 36.97 (C-6), 51.69 (C-7), 150.02 (C-7a), 124.18 (C-8), 171.71 (C-9), 168.47 (C-10), 118.16 (C-11), 140.09 (C-12), 134.63 (C-12a), 60.80 (CH_3O-1), 60.77 (CH_3O-2), 55.91 (CH_3O-3), 168.69 (C-13), 22.55 (C-14). ^{1}H NMR (300 MHz, CD_3CN) δ/ppm: 6.74 (s, HC-4), 2.22 (m, H_2C-5), 2.56 (m, H_2C-5), 1.96 (m, H_2C-6), 2.26 (m, H_2C-6), 4.37 (m, HC-7), 7.46 (s, HC-8), 7.26 (d, J = 7.48 Hz, HC-11), 7.48 (d, J = 7.25 Hz, HC-12), 3.60 (s, CH_3O-1), 3.83 (s, CH_3O-2), 3.87 (s, CH_3O-3), 1.92 (s, H_3C-14), 7.48 (d, J = 4.37 Hz, NH). ^{13}C NMR (75 MHz, CD_3CN) δ/ppm: 151.08 (C-1), 126.41 (C-1a), 141.78 (C-2), 154.45 (C-3), 108.48 (C-4), 135.81 (C-4a), 30.06 (C-5), 37.91 (C-6), 53.49 (C-7), 151.19 (C-7a), 125.07 (C-8), 172.98 (C-9), 168.48 (C-10), 117.95 (C-11), 142.36 (C-12), 136.29 (C-12a), 61.65 (CH_3O-1), 61.35 (CH_3O-2), 56.54 (CH_3O-3), 170.08 (C-13), 22.69 (C-14). UV-visible in CH_3OH λ_{max}/nm (ε/ mol^{-1} dm^3 cm^{-1}): 348.5 (16192), 231 (27017).

(7) Complex Colchiceine - Sodium Perchlorate

125 mg; yield 93 %; m.p. 102–104 °C, Anal. Calcd. for $C_{21}H_{23}NO_6 \cdot NaClO_4 \cdot 2H_2O$: C, 47.27; H, 4.95; N, 2.57. Found: C, 47.24; H, 4.87; N, 2.52. IR (KBr) $\tilde{\nu}$/cm^{-1}: 3364 m, 3261 m, 3063 m, 2998 w, 2940 w, 2858 w, 2839 w, 1662 s, 1611 s, 1544 m, 1490 m, 1455 m, 1276 m, 1196 m, 1140 m, 1108 m, 1090 vs, 1043 m, 918 w, 626 w, 484 w. IR (nujol/fluorolub) $\tilde{\nu}$/cm^{-1}: 3538 m, 3363 m, 3260 m, 2996 w, 2939 w, 2858 w, 2725 w, 1659 s, 1609 s, 1540 m, 1489 m, 1454 m, 1405 m, 1350 m, 1277 m, 1092 vs, 1044 m, 919 w, 624 w, 484 w. ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 6.80 (s, HC-4), 2.13 (m, H_2C-5), 2.53 (m, H_2C-5), 1.96 (m, H_2C-6), 2.11 (m, H_2C-5), 4.35 (m, HC-7), 7.33 (s, HC-8), 7.35 (d, J = 11.81 Hz, HC-11), 7.17 (d, J = 11.81 Hz, HC-12), 3.79 (s, CH_3O-1), 3.53 (s, CH_3O-2), 3.85 (s, CH_3O-3), 1.88 (s, H_3C-14), 8.65 (d, J =7.36 Hz, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 150.03 (C-1), 125.74 (C-1a), 140.74 (C-2), 153.16 (C-3), 107.89 (C-4), 134.33 (C-4a), 29.27 (C-5), 36.96 (C-6), 51.73 (C-7), 150.21 (C-7a), 124.36 (C-8), 171.95 (C-9), 168.67 (C-10), 118.32 (C-11), 140.28 (C-12), 134.81 (C-12a), 60.84 (CH_3O-1), 60.81 (CH_3O-2), 55.73 (CH_3O-3), 168.94 (C-13), 22.52 (C-14). ^{1}H NMR (300 MHz, CD_3CN) δ/ppm: 6.73 (s, HC-4). 2.21 (m, H_2C-5), 2.56 (m, H_2C-5), 1.97 (m, H_2C-6), 2.25 (m, H_2C-6), 4.40 (m, HC-7), 7.47 (s, HC-8), 7.25 (d, J = 7.49 Hz, HC-11), 7.49 (d, J =7.24 Hz, HC-12), 3.61 (s, CH_3O-1), 3.84 (s, CH_3O-2), 3.87 (s, CH_3O-3), 1.90 (s, H_3C-14), 7.54 (d, J =4.39 Hz, NH). ^{13}C NMR (75 MHz, CD_3CN) δ/ppm: 151.09 (C-1), 126.50 (C-1a), 141.78 (C-2), 154.39 (C-3), 108.49 (C-4), 135.86 (C-4a), 30.11 (C-5), 37.93 (C-6), 53.41 (C-7), 150.32 (C-7a), 124.91 (C-8), 173.02 (C-9), 168.38 (C-10), 117.82 (C-11), 142.28 (C-12), 136.18 (C-12a), 61.59 (CH_3O-1), 61.38 (CH_3O-2), 56.56 (CH_3O-3), 170.46 (C-13), 22.71 (C-14). UV-visible in CH_3OH λ_{max}/nm (ε/ mol^{-1} dm^3 cm^{-1}): 349 (16917), 231 (28146).

(8) Complex Colchiceine - Potassium Thiocyanate

109 mg; yield 91 %; m.p. = 98–100 °C, Anal. Calcd. for $C_{21}H_{23}NO_6 \cdot KSCN \cdot 2.5H_2O$: C, 50.09; H, 5.31; N, 5.31. Found: C, 49.98; H, 5.36; N, 5.29. IR (KBr) $\tilde{\nu}$/cm^{-1}: 3360 m, 3256 m, 3052 w, 2999 w, 2938 w, 2856 w, 2837 w, 1661 s, 1609 s, 1544 m, 1489 m, 1453 m, 1274 m, 1196 m, 1136 m, 2051 m. IR (nujol/fluorolub) $\tilde{\nu}$/cm^{-1}: 3370 m, 3249 m, 3052 w, 2952 w, 2936 w, 2856 w, 2727 w, 1658 s, 1608 s, 1539 m, 1488 m, 1456 m, 1404 m, 1348 m, 1274 m, 2054 s. ^{1}H NMR (300 MHz, DMSO-d_6) δ/ppm: 6.82 (s, HC-4), 2.14 (m, H_2C-5), 2.53 (m, H_2C-5), 1.96 (m, H_2C-6), 2.11 (m, H_2C-6), 4.36 (m, HC-7), 7.33 (s, HC-8), 7.36 (d, J = 11.81 Hz, HC-11), 7.18 (d, J = 11.81 Hz, HC-12), 3.79 (s, CH_3O-1), 3.53 (s, CH_3O-2), 3.85 (s, CH_3O-3), 1.88 (s, H3C-14), 8.67 (d, J = 7.42 Hz, NH). ^{13}C NMR (75 MHz, DMSO-d_6) δ/ppm: 150.04 (C-1), 125.73 (C-1a), 140.72 (C-2), 153.16 (C-3), 107.89 (C-4), 134.36 (C-4a), 29.26 (C-5), 36.95 (C-6), 51.73 (C-7), 150.24 (C-7a), 124.38 (C-8), 171.92 (C-9), 168.63 (C-10), 118.32 (C-11), 140.29 (C-12), 134.80 (C-12a), 60.84 (CH_3O-1), 60.81 (CH_3O-2), 55.95 (CH_3O-3), 168.93 (C-13), 22.51 (C-14), 129.78 (SCN). ^{1}H NMR (300 MHz, CD_3CN) δ/ppm: 6.73 (s, HC-4), 2.22 (m, H_2C-5), 2.54 (m, H_2C-5), 1.99 (m, H_2C-6), 2.24 (m, H_2C-6), 4.39 (m, HC-7), 7.48 (s, HC-8), 7.23 (d, J = 7.48 Hz, HC-11), 7.48 (d, J =7.23 Hz, HC-12), 3.61 (s, CH_3O-1), 3.82 (s, CH_3O-2), 3.88 (s, CH_3O-3), 1.92 (s, H_3C-14), 7.44 (d, J = 4.39 Hz, NH). ^{13}C NMR (75 MHz, CD_3CN) δ/ppm: 151.15 (C-1), 126.59 (C-1a), 141.81 (C-2), 154.37 (C-3), 108.48 (C-4), 135.85 (C-4a), 30.14 (C-5), 37.92 (C-6), 53.36 (C-7), 151.36 (C-7a), 124.83 (C-8), 173.03 (C-9), 168.49 (C-10), 117.71 (C-11), 142.16 (C-12), 136.10 (C-12a), 61.55 (CH_3O-1), 61.34 (CH_3O-2), 56.54 (CH_3O-3), 170.69 (C-13), 22.70 (C-14), 131.49 (SCN). UV-visible in CH_3OH λ_{max}/nm (ε/ mol^{-1} dm^3 cm^{-1}): 350 (16200), 230 (28341).

(2) Colchiceine $C_{21}H_{23}NO_6 \cdot H_2O$, m.p. 148–150 °C.

Physical Measurements

The NMR spectra of **2** and its 1:1 complexes **3**–**8** (0.07 mol L^{-1}) with monovalent metal cations salts were recorded in CD_3CN solutions using a Varian Gemini 300 MHz spectrometer. All spectra were locked to deuterium resonance of CD_3CN. The ^{1}H NMR measurements in CD_3CN were carried out at the operating frequency 300.075 MHz; flip angle 45°; spectral width 4500 Hz; acquisition time 2.0 s; relaxation delay 1.0 s and T = 293.0 K, using TMS as the internal standard. No window function or zero filling was used. Digital resolution was 0.2 Hz per point. The error of chemical shift value was 0.01 ppm. ^{13}C NMR spectra were recorded at the operating frequency 75.454 MHz; pw = 60°; sw = 19000 Hz; at = 1.8 s; d_1 = 1.0 s; T = 293.0 K and TMS as the internal standard. Line broadening parameters were 0.5 or 1 Hz. The error of chemical shift value was 0.01 ppm. The ^{1}H and ^{13}C NMR signals were assigned for each species using one or two-dimensional (COSY, HETCOR, HMBC in inverse mode) spectra.

Table 1. Value of λ_{max} and ε in UV-Vis spectra of **2** and its complexes **3–8** in (CH_3OH)

Compound	λ_{max1} / nm	ε_1 / (mol^{-1} dm^3 cm^{-1})	λ_{max2} / nm	ε_2 / (mol^{-1} dm^3 cm^{-1})
2	350.5	34887	243.5	62699
3	349.5	17444	224	35853
4	350	16762	223	36109
5	350.5	17292	222.5	39813
6	348.5	16192	231	27017
7	349	16917	231	28146
8	350	16200	230	28341

The FT-IR spectra of **2** and its 1:1 complex **3–8** (0.07 mol dm^{-3}) were recorded in the mid infrared region in KBr pellets, fluorolub/ nujol mixture for obtained complexes **3–8** and in CD_3CN for colchiceine and its complex **6** with $LiClO_4$. The spectra were taken with an IFS 113v FT-IR spectrophotometer (Bruker, Karlsruhe) equipped with a DTGS detector; resolution 2 cm^{-1}, NSS=125. A cell with Si windows and wedge-shaped layers was used to avoid interferences (mean layer thickness 170 μm). Each FT-IR spectrum was measured by acquisition of 64 scans. All manipulations with the substances were performed in a carefully dried and CO_2-free glove box.

The UV-Vis spectra were recorded in methanol by JASCO V-550 spectrophotometer at 200–600 nm range.

Liquid secondary ion mass spectra (FAB MS) were obtained with AMD 604 two sector mass spectrometer of reverse B/E geometry, made by AMD Intectra (Germany). A CsI gun supplied the primary ion beam (12 keV, Cs^+). The secondary ion beam was accelerated to 8 kV. Samples of the complexes studied were dissolved in 3-nitrobenzyl alcohol (Aldrich).

Elemental analysis of colchiceine complexes was carried out by means of a Elementarz Analyser Vario EL III. Melting point was determined on BUCHI SMP-20. Melt-Temp II apparatus (Laboratory Devices Inc.)

RESULTS AND DISCUSSION

Colchiceine and respective inorganic salts ($LiClO_4$, LiI, $NaClO_4$, NaI, KSCN, KI) at the ratio 1:1 M were dissolved in methanol and were stirred for 24h at room temperature. Compounds **3–8** were obtained as pale to dark yellow solids with very good yields. The complexes were studied by spectral analysis: UV-Vis, ^{1}H and ^{13}C NMR, FT-IR and MS FAB.

UV-Vis Measurements

The UV-Vis spectrum of colchiceine **2** in methanol (Table 1.) shows two bands at λ_{max1} 350 and λ_{max2} 243.5 nm assigned to the tropolonic and benzenoic moieties in the colchiceine molecule, respectively. The values of ε_1 and ε_2 change much in comparison to colchiceine but those of ε_2 are higher for the colchiceine complexes with iodides **3–5** and lower for the complexes with perchlorate salts **6–7** and thiocyanate **8**. The UV-Vis data for the 1:1 complexes of colchicine with monovalent cations **3**–**8** in methanol show the influence of salts only on the λ_{max2} and ε_2 values, which means that the effect of coordination process is stronger on benzenoic moiety than on tropolonic moiety. In the UV-Vis spectra of the benzenoic moiety, hypsochromic shifts were observed for all obtained colchiceine complexes **3–8** of both λ_{max1} and λ_{max2} for iodide and perchlorate salts. The hypsochromic shifts of λ_{max1} for tropolonic moiety is very weak. The hypsochromic shift of λ_{max2} observed for colchiceine complexes with iodide salts **3–5** was stronger than that for perchlorates **6–7** and thiocyanate **8**, and it was the strongest for the complex of colchiceine with potassium iodide **5**, which means that the type of anion also influences the coordination process.

NMR Measurements

The NMR spectra of colchiceine complexes **3–8** were recordered in two different solvents to compare a possible interaction between the ligand (colchiceine) and the solvent as well as to establish the effect of the nature of solvents on the values of chemical shifts. The ^{1}H and ^{13}C NMR data of **2** and its complexes with iodides **3–5**, perchlorates **6–7** and thiocyanate **8** in DMSO-d_6 and in CD_3CN solution are given in Experimental Section.

^{1}H NMR Spectra

The chemical shift of proton at OH (hydroxyl group at C-10) in **2** cannot be obtained experimentally on account of the very low activation energy. Colchiceine exists in the form of two tautomers, which follows from intramolecular proton transfer on the tropolone moiety (between two oxygens at C-9 and C-10).[27]

The ^{1}H NMR spectra of colchiceine complexes **3–8** do not show significant changes in the values of chemical shifts of respective hydrogen atoms, as has been also observed earlier for other biologically active compounds like monensin, oligomycin and complexes of colchicine.[25,28–32]

The slightest changes of proton chemical shifts have been observed for the amide group. Apart from these values, ^{1}H NMR spectra give no additional information on the structure of the complexes studied.

Table 2. Selected chemical shifts of protons in the ^{1}H NMR in CD_3CN

Hydrogen atom	Chemical shifts (ppm), δ_H multiplicity, J / Hz						
	2	**3**	**4**	**5**	**6**	**7**	**8**
H_2C-5	2.18 m	2.15 m	2.21 m	2.21 m	2.22 m	2.21 m	2.22 m
	2.54 m	2.55 m	2.54 m	2.55 m	2.56 m	2.56 m	2.54 m
H_2C-6	2.09 m	2.09 m	2.08 m	2.03 m	1.96 m	1.97 m	1.99 m
	2.23 m	2.25 m	2.25 m	2.19 m	2.26 m	2.25 m	2.24 m
HC-8	7.48 s	7.51 s	7.53 s	7.52 s	7.46 s	7.47 s	7.48 s
HC-11	7.24 d *7.48*	7.25 d *7.48*	7.24 d *7.48*	7.25 d *7.48*	7.26 d *7.48*	7.25 d *7.49*	7.23 d *7.48*
HN	7.16 d *6.32*	7.65d *4.41*	7.54 d *4.42*	7.44 d *4.42*	7.48 d *4.37*	7.54 d *4.39*	7.44 d *4.39*

The ^{1}H NMR spectra of colchiceine complexes **3–8** in acetonitrile, similarly as those of colchiceine complexes **3–8** in DMSO-d_6, show the signals of protons assigned to three *o*-methoxyl groups whose positions do not change much after the complexation process. Some selected data on proton chemical shifts in acetonitrile are given in the Table 2. Slight changes in chemical shifts are observed for methylene protons at carbon atoms C5 and C6 (ring B). The proton at the carbon atom C-8 observed as a singlet showed considerable change in chemical shift for colchiceine complex with sodium perchlorate **4**.

The differences in chemical shifts of protons of the acetamide substituent at C-7 position gave the greatest changes, as a result of the complexation process, changing from 7.16 ppm in **2** to 7.44–7.65 ppm in **3–8**. The proton signal whose position was the most changed in this region of the spectra of complexes **3–8** was observed in colchiceine complex with lithium perchlorate **6**. The changes in the values of chemical shift decrease in the following order $Li^+ > Na^+ > K^+$, which suggests that the larger the cation, the weaker the complex interaction with the acetamide group. Figure 2. shows the changes in the position of the chemical shift of proton at NH group of acetamide substituent in colchiceine and its complexes with lithium iodide **3** and perchlorate **6**.

If only lithium cation had impact on the complexation process in both complexes **3** and **6**, the position of the chemical shift of proton from the NH group would be almost the same, Figure 2. It can be easily observed that the type of anion also influences the position of proton in the acetamide group.

^{13}C NMR spectra

Some selected data on chemical shifts in ^{13}C NMR spectra of colchiceine complexes **3–8** in DMSO-d_6 are given in the Table 3. ^{13}C NMR spectra of these complexes are much more informative than ^{1}H NMR spectra. The chemical shifts of *o*-methoxy carbons do not change much after the complexation process and the most shifted ones are those in complex **7** and **8**. The differences in chemical shifts of

Table 3. Selected carbon-13 chemical shifts for colchiceine **2** and its complexes **3**–**8** (in DMSO-d_6)

Carbon atom	Chemical shifts (δ/ppm)						
	2	**3**	**4**	**5**	**6**	**7**	**8**
C-1	149.73	149.68	149.75	149.75	149.82	150.03	150.04
C-1a	125.53	125.60	125.56	125.49	125.59	125.74	125.73
C-2	140.49	140.48	140.50	140.46	140.55	140.74	140.72
C-3	152.87	152.82	152.88	152.85	152.95	153.16	153.16
C-4a	134.05	133.69	133.98	134.07	134.14	134.33	134.36
C-7	51.58	51.61	51.65	51.62	51.69	51.73	51.73
C-7a	149.95	149.94	149.95	149.91	150.02	150.21	150.24
C-8	124.06	123.91	124.07	124.07	124.18	124.36	124.38
C-9	171.57	172.00	171.74	171.57	171.71	171.95	171.92
C-10	168.35	168.54	168.53	168.32	16.47	168.67	168.63
C-11	118.07	118.07	118.12	118.08	118.16	118.32	118.32
C-12	139.96	139.91	140.00	139.99	140.09	140.28	140.29
C-12a	134.52	134.54	134.56	134.52	134.63	134.81	134.80
CH_3O-1	60.70	60.71	60.74	60.70	60.80	60.84	60.84
CH_3O-2	60.66	60.69	60.72	60.67	60.77	60.81	60.81
CH_3O-3	55.82	55.87	55.91	55.88	55.91	55.73	55.95
C-13	168.49	168.90	168.61	168.53	168.69	168.94	168.93

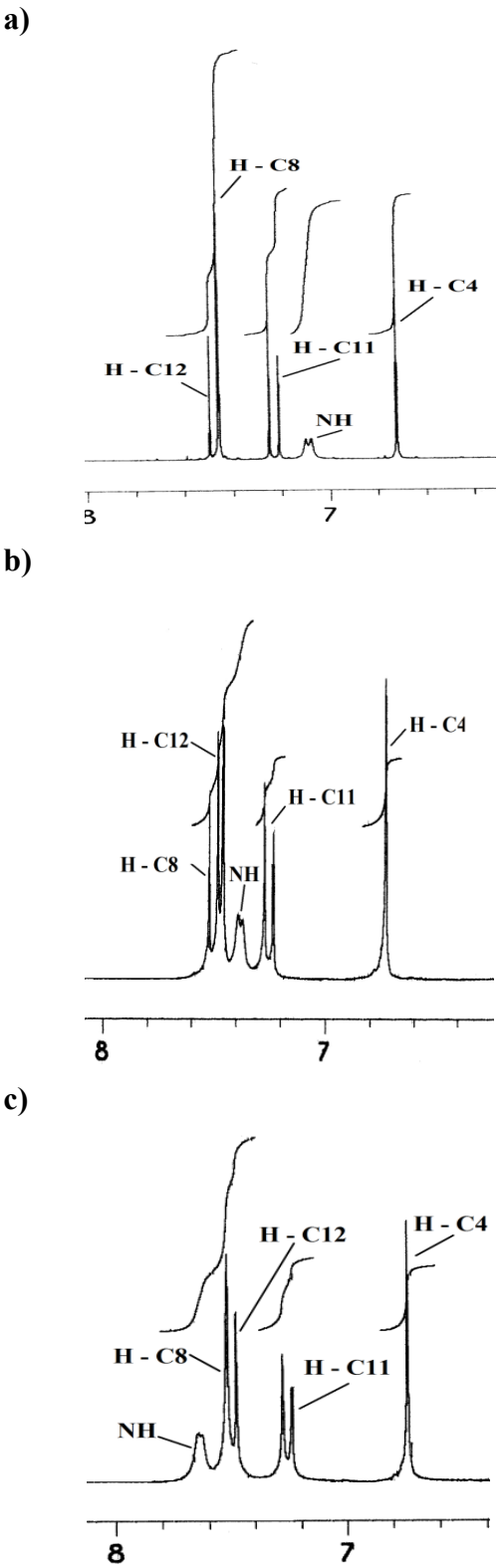

Figure 2. ^{1}H NMR spectra (CD_3CN) in the range of 6.4–8.2 ppm of: a) colchiceine **2**, b) complex with lithium perchlorate **6** and c) complex with lithium iodide **3**.

carbon atom C-13 of carbonyl group do not depend on the type of monovalent cation. Signal assigned to carbonyl carbon atom C-13 is most shifted in **3**, **6** and **7** ranging from 168.90 to 168.95 ppm (168.49 ppm in **2)**. Signal of carbonyl carbon atom C-9 for colchiceine appears at 171.57 ppm, but for colchiceine complexes with lithium iodide **3** shifts to 172 ppm. The chemical shifts of carbonyl carbon atom C-9 decrease in the following order $Li^+ \rightarrow Na^+ \rightarrow K^+$ for iodides and $Na^+ \rightarrow K^+ \rightarrow Li^+$ for perchlorates **6** and **7** and thiocyanate **8**. This observation suggests that in complexation process in colchiceine complex with lithium iodide **3** both carbonyl groups are involved. Some changes after complexation process are also visible in chemical shifts for carbon atoms on ring B. For carbon atoms C-7 and C-7a the greatest change in chemical shifts are observed in colchiceine complex **7** and **8**. The carbon atoms C-11, C-12 and C-12a on the C ring shift much in colchiceine complexes **7** and **8**. For carbon atoms on ring A C-1, C-1a, C-2, C-3, C-4a the greatest change in chemical shifts are observed in colchiceine complex **7** and **8**.

Some selected ^{13}C NMR chemical shifts of colchiceine complexes **3–8** are given in the Table 4. The ^{13}C NMR spectra of colchiceine complexes **3–8** in CD_3CN similarly to those of complexes **3–8** in DMSO-d_6, show the signals of carbons assigned to three *o*-methoxyl groups do not change much after the complexation process. The chemical shifts of carbon atoms from the three *o*-methoxyl groups decrease in the following order $Li^+ \rightarrow Na^+ \rightarrow K^+$, both for iodides and perchlorates.

The chemical shifts of carbon atoms show that both carbonyl group at C-7 position (acetamide group) and C-9 in the tropolone ring C are involved in the coordination. The complexation process of Li^+, Na^+ and K^+ cations results in changes in the chemical shifts of the carbon atoms of the tropolone ring C of colchiceine and at the acetamide substituent (carbon from carbonyl group) at C-7 position. The chemical shifts of carbon atom C-13 of acetamide group depend on the type of monovalent cation and increase in the following order $Li^+ \rightarrow Na^+ \rightarrow K^+$ for iodides **3–5** and $K^+ \rightarrow Li^+ \rightarrow Na^+$ for perchlorates **6–7** and thiocyanate **8**. The most upfield shift of C-13 carbonyl carbon atom was observed in **6** (complex with lithium perchlorate), while the most downfield shift was found in **8** with respect to **2**. On the other hand signal of carbonyl carbon atom C-9 in colchiceine appears at 173.30 ppm, but for colchiceine complexes with lithium iodide **3** shifts slightly downfield to 172.94 ppm. The chemical shifts of carbonyl carbon atom C-9 decrease in the following order $Li^+ \rightarrow Na^+ \rightarrow K^+$ for iodides and for perchlorates **6** and **7** and thiocyanate **8**.

FT-IR Measurements

To be able to make comparison to the results of the previous work on colchicine complexes,[25] the IR spectra of

Table 4. Selected chemical shifts of carbon atoms in the ^{13}C NMR in CD_3CN

Carbon atom	Chemical shifts (ppm) CD_3CN						
	2	**3**	**4**	**5**	**6**	**7**	**8**
C-1	151.44	151.04	150.99	151.00	151.08	151.09	151.15
C-1a	126.83	126.37	126.46	126.48	126.41	126.50	126.59
C-2	142.15	141.74	141.67	141.70	141.78	141.78	141.81
C-3	154.71	154.39	154.26	154.30	154.45	154.39	154.37
C-4a	136.29	135.75	135.78	135.78	135.81	135.86	135.85
C-5	30.21	30.03	30.05	30.06	30.06	30.11	30.14
C-7	53.47	53.53	53.37	53.35	53.49	53. 41	53.36
C-7a	151.56	151.31	151.30	151.29	151.19	150.32	151.36
C-9	173.30	172.94	173.06	173.09	172.98	173.02	173.03
C-10	168.74	168.43	168.41	168.24	168.48	168.38	168.49
C-11	117.81	118.20	117.88	117.66	117.95	117.82	117.71
C-12	142.43	142.30	142.13	142.12	142.36	142.28	142.16
C-12a	136.07	136.24	135.95	135.94	136.29	136.18	136.10
CH_3O-1	61.64	61.62	61.53	61.49	61.65	61.59	61.55
CH_3O-2	61.46	61.30	61.30	61.28	61.35	61.38	61.34
CH_3O-3	56.66	56.53	56.50	56.49	56.54	56.56	56.54
C-13	170.43	171.23	170.42	170.17	170.08	170.46	170.69

colchiceine and its complexes **3–8** were collected both in solid state – KBr pellets and in a nujol/fluorolub while those of **2** and **6** in CD_3CN solutions. Initial examination of these spectra showed that some differences in position, shape and intensity of respective bands can be observed in spectra obtained in KBr pellets, nujol/fluorolub and in CD_3CN solutions.

The FT-IR spectra of colchiceine complexes **3–5** with monovalent cations and, for comparison, the spectrum of colchiceine **2** all in KBr pellets are shown in Figure 3 and Figure 1S (Supporting Information).

From crystallographic studies it is well known that colchiceine in the solid state exist as a monohydrate. In the FT-IR spectrum, the stretching vibration of amine group observed as a broad band with a maximum at 3357 cm^{-1}. The same band is also observed in the spectra of colchiceine complexes with monovalent cations **3–8,** but the maximum of the broad band is shifted toward higher wavenumbers depending on the type of cation in the following order K→ Na→ Li for iodides **3–5** and K ≈ Na → Li in perchlorates **6–7** and thiocyanate **8**. This observation indicates that in the solid, not only the cation but also the type of anion has impact on the complexation process. The strongest shift of this band is observed in **3**. Cation and anion together with water molecules are involved in the complexation process. In the region of stretching vibrations of two carbonyl groups of tropolone and the acetamide group, respectively, these two bands are slightly shifted from 1611 cm^{-1} and 1653 cm^{-1} in **2** to the region of 1602–1610 cm^{-1} and 1662–1653 cm^{-1} in colchiceine complexes **3–8**. The strongest shift of the band assigned to the carbonyl group of tropolone ring C is observed in colchiceine complex with lithium iodide **3.** The strongest shift of the band assigned to the acetamide group is observed in

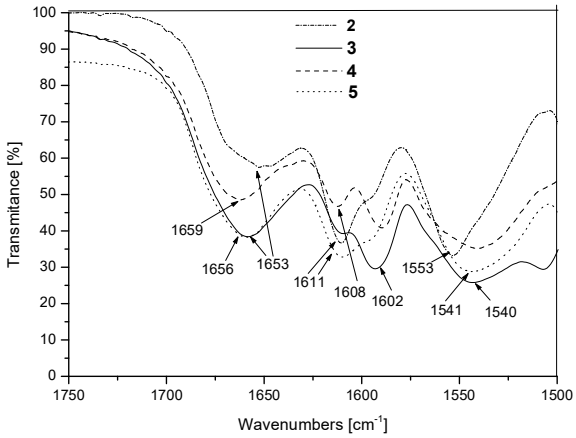

Figure 3. FT-IR spectra (KBr) of colchiceine **2** and its complexes with lithium iodide **3**, sodium iodide **4** and

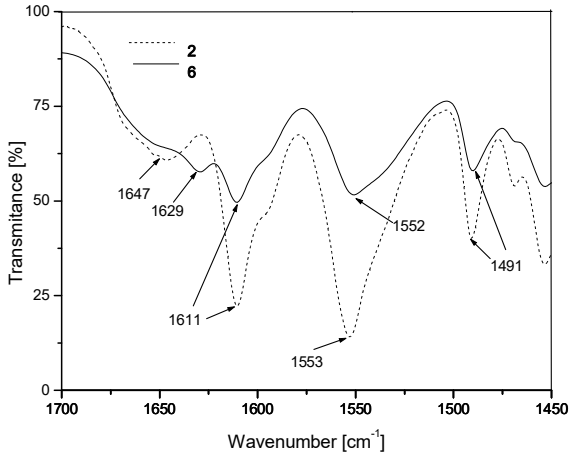

Figure 4. FT-IR spectra (nujol/fluorolub) of Colchiceine Complexes with lithium perchlorate **6** in the region of carbonyl group 1700–1450cm^{-1}.

colchiceine complex with sodium perchlorate **7**.

The FT-IR spectra of **1** show very characteristic absorption bands of tropolone ring in the colchicine molecule, appearing in the region of 1624–1605 cm^{-1}, 1570–1538 cm^{-1} and 1280–1250 cm^{-1},[26] the first two are assigned to the stretching vibrations of $\tilde{\nu}_{(C=C)}$, but in the IR spectra of colchiceine these bands appear in the region of 1553 cm^{-1} and 1276 cm^{-1}. It was supposed that in the tropolone ring C, the active hydrogen was statically located between the two oxygen atoms (on hydroxyl and carbonyl group) in such a way that the resonance was possible without zwitterion formation by shifts of the double bond and OH bonds as indicated.[33] The FT-IR spectra of colchiceine complexes **3–8** show the bands assigned to the stretching vibrations of amine group and hydroxyl group in the region of 3650–2500 cm^{-1}. These bands are shifted towards higher wavenumbers in comparison to their position in the spectrum of **2**, which is a result of the presence of water molecules in the crystalline structure of colchiceine complexes **3–8**.

The FT-IR spectra in the nujol/fluorolub of respective complex **6** are shown in Figures 2S–3S (Supporting Information) and Figure 4, colchiceine is present as a monohydrate and in its FT-IR spectra the band assigned to water molecule is present.[34]

The stretching vibrations of this water molecule is observed in the FT-IR spectra as a band with a maximum at about 3447 cm^{-1}. The same bands are also observed in the spectra of the colchiceine complexes with Li^+, Na^+ and K^+ cations, but their maximum is slightly shifted towards lower wavenumbers, in a way dependent on the type of cation in the following sequence: $K^+ \rightarrow Na^+ \rightarrow Li^+$ in iodides, which was also observed for the complexes of colchicine,[25] and $Li^+ \rightarrow Na^+ \rightarrow K^+$ in perchlorates. In the FT-IR spectrum, the NH stretching vibrations of colchiceine produces the band with a maximum at 3359 cm^{-1}. In the FT-IR spectra of colchiceine complexes the same band is observed but shifted towards lower wavenumbers, to 3245 cm^{-1} in complexes with iodide salts. The bands assigned to NH stretching vibrations of colchiceine complexes with iodides are much more shifted than those of the colchiceine complexes with perchlorates. The absence of any band in the usual carbonyl range (1720–1670 cm^{-1}) can be explained by the conjunction of carbonyl group with unsaturated group or aromatic ring that lowers the carbonyl frequencies. In the region of stretching vibrations of C=O group two bands are present, one assigned to the tropolone moiety and the other to acetamide groups. The shape and intensity of bands differ from those in the spectrum of colchiceine. The band of carbonyl group of tropolone moiety does not shifts much in comparison to the band assigned to the carbonyl group from acetamide, which shifts towards higher wavenumbers from 1647 cm^{-1} in **2** to 1655–1657 cm^{-1} in complexes **3–8** as a result of complexation process. Only in complex **6** this carbonyl band shifts toward lower wavenumbers from 1647 cm^{-1} to 1629 cm^{-1}. The strongest shift of the bands assigned to $\tilde{\nu}_{(CO)}$ vibrations of acetamide group, from 1647 cm^{-1} in colchiceine to 1657 cm^{-1}, was observed in its complex with K^+ **5,** while for the tropolone carbonyl group the greatest shift was from 1611 cm^{-1} in **2** to 1602 cm^{-1} in complex **3** with lithium iodide.

The bands assigned to the tropolone moiety (unsaturated resonant system of ring C) appear at 1611cm^{-1}, 1553 cm^{-1}, 1349 cm^{-1} and 1278cm^{-1} in the FT-IR spectra of colchicine **1** and colchiceine **2**. As expected, the same bands appear in the FT-IR spectra of colchiceine complexes **3–8** at somewhat different frequencies and with different relative intensities.

The FT-IR spectra of **2** and its complex with lithium perchlorate **6** in CD_3CN solutions were also obtained and the data are given in Table 5 and shown in Figure 5 and Figure 4S (Supporting Information). For comparison, the data for colchicine **1** and its complex with lithium perchlorate **1a**, are also given.[25] Changes in the shape and intensity of the bands are easily observed. The bands assigned to the stretching vibrations of NH group are shifted to the lower wavenumbers from 3371 cm^{-1} for **2** to 3358 cm^{-1} for its complex **6** with Li^+ cation. In the region of stretching vibrations of C=O bonds of the carbonyl group of tropolone and acetamide groups, two bands appear and shift towards lower wavenumbers in comparison to the FT-IR spectrum of colchiceine. After the complexation process, the band assigned to the stretching vibrations of carbonyl group of acetamide group is shifted from 1681 cm^{-1} for **2** to 1662 cm^{-1} for **6,** which implies that the carbonyl group of

Table 5. Wavenumbers (cm^{-1}) in FT-IR spectra in acetonitrile for **2** and its complex **6** and colchicine **1** and its complex with lithium perchlorate **1a**.[25]

Assignments	Wavenumbers / cm^{-1}			
	2	**6**	**1**	**1a**
*v*OH	3538	3536	3544	3539
*v*NH	3371	3358	3369	3367
*v*CH	3206	3229	3095	3163
	3167	3160	3090	3002
	2998	2998	2943	2944
*v*C=O	1681	1660	1680	1664
*v*C=O	1617	1615	1618	1614
*v*C=C	1596	1596	1591	1592
*v*C=C	1556	1551	1575	1573

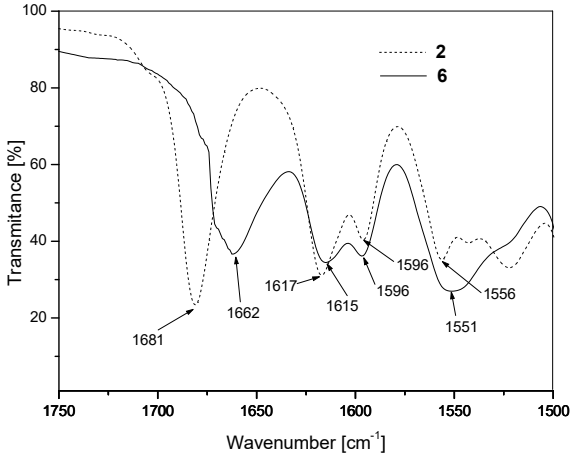

Figure 5. FT-IR spectra of Colchiceine **2** and its Complex with lithium perchlorate **6** (CD_3CN): 1700–1575cm^{-1}.

acetamide substituent at C-7 rather is involved in the complexation process rather than the carbonyl group of tropolone ring C. As can be concluded the complexation process involves especially the carbonyl group of acetamide substituent at C-7 rather than the carbonyl group of tropolone ring C.

The presence of free perchlorate anions ClO_4^- is manifested by four fundamental vibration bands: symmetric stretching band at ≈ 932 cm^{-1}, symmetric bending band at ≈ 460 cm^{-1}, asymmetric stretching band at ≈ 1115 cm^{-1} and asymmetric bending band at ≈ 630 cm^{-1}.[35,36] The IR spectra of colchiceine complexes: with lithium perchlorate **6** and with sodium perchlorate **7** show the bands assigned to perchlorate anions and their wavenumbers are given in the Table 6 and Figure 6. The broad band at ≈ 1115 cm^{-1} assigned to asymmetric stretching is shifted towards lower wavenumbers as a result of complexation process and also in case of presence of water molecules in complexes **6** and **7**. These observations were made for the spectra of the samples in KBr pellet, nujol/fluorolub suspension and for complex **6** for CD_3CN solution, which means that the medium in which the IR spectra were recorded (solid state, suspension or solution) did not affect behavior of the band assigned to ClO_4^- group. The symmetric bending band usually present at ≈ 460 cm^{-1} after the complexation process is shifted towards higher wavenumbers for **6** and **7** to 484 cm^{-1}, but these shifts are irrespective of the type of cation and the medium in which IR spectra were recorded (solid state, suspension or solution). The symmetric stretching band is shifted towards lower wavenumbers 918–920 cm^{-1} for the IR spectra in KBr and nujol/fluorolub and this change does not depend on type of cation, but in CD_3CN solution for complex **6** only a slight change by 2cm^{-1} is observed.

FAB MS Measurements

In Fast Atom Bombardment (FAB) MS mass spectra, molecular ions of colchiceine with respective monovalent metal ions as Li^+, Na^+ and K^+ in stoichiometry 1:1 can be observed, respectively. The relevant data are given in the Table 7.

For all complexes, except **5**, formation of complexes in stoichiometry in 2:1 is also observed. For complex **6**

Table 6. Wavenumbers of free perchlorate anion and in complexed form of ClO_4^-: as colchiceine complex with lithium perchlorate **6** and colchiceine complex with sodium perchlorate **7**

Assignment	Wavenumbers / cm^{-1}					
	Free ClO_4^-	**6**	**6**	**6**	**7**	**7**
		KBr	nujol/fluorolub	CD_3CN	KBr	nujol/fluorolub
1. ν_{as}	≈ 1115	1087 1044	1094 1011	1104 1100 1096	1108 1090 1043	1092 1044
2. ν_{sym}	≈ 932	918	920	930	918	919
3. δ_{as}	≈ 630	625	625	625	626	624
4. δ_{sym}	≈ 460	484	483	483	484	484

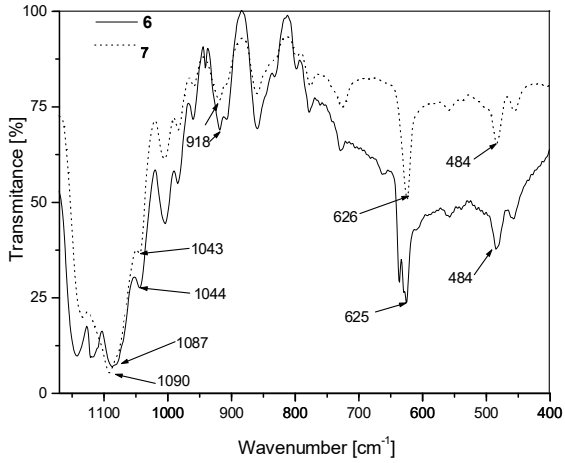

Figure 6. FT-IR spectra of colchiceine complex with lithium perchlorate **6** and colchiceine complex with sodium perchlorate **7** in the range of 1200–400cm^{-1} ClO_4^- anion.

signals at *m*/*z* = 392 and also at *m*/*z* = 778 appear, which indicates not only 1:1 but also 2:1 stoichiometry, of two colchiceine molecules and one lithium cation. Moreover, in **6** two other signals with low intensity (%) were observed, which can be assigned to much more complicated stoichiometry with perchlorate anion at *m*/*z* = 498, which indicates a connection colchiceine molecule to two lithium cations and one perchlorate anion and also *m*/*z* = 884 two colchiceine molecules to two lithium cations and one perchlorate anion. It can be easily observed that formation of complicated stoichiometry depends on the kind of cation and decreases in the order lithium, sodium to potassium cation.

CONCLUSIONS

Series of new colchiceine complexes were obtained and described by spectral analysis. In the present work it was found that exchanging just one substituent in colchicine molecule at C-10 carbon atom from methoxyl to hydroxyl group on tropolone ring C causes huge changes in complexes formation. The strongest changes in the ^{1}H and ^{13}C NMR and in the FT-IR spectra were observed for colchiceine complexes with lithium iodide and lithium perchlorate.

In our previous work it has been found that colchicine forms complexes with lithium, sodium and potassium iodides, perchlorates and thiocyanate. In comparison to our previous work of colchicine complexes, colchiceine forms complexes in which in complexation process perchlorate anions are also involved, what was observed especially in colchiceine complex with lithium perchlorate.

Supplementary Information. Supporting information to the paper is enclosed to the electronic version of the article at: http://dx.doi.org/10.5562/cca2871.

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Table 7. Main signals in FAB MS mass spectra of colchiceine complexes **3**–**8**

Complex	Col-M$^+$(%)	Col-2M$^+$X$^-$ (%)	2Col-M$^+$ (%)	2Col-2M$^+$X$^-$ (%)
3	392 (100)	-	778 (5)	-
4	408 (90)	-	794 (4)	-
5	424 (88)	-	-	-
6	392 (98)	498 (10)	778 (5)	884 (5)
7	408 (85)	-	794 (3)	-
8	424 (80)	-	810 (2)	-

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Supporting Information

Colchiceine Complexes with Lithium, Sodium and Potassium Salts - Spectroscopic Studies

Joanna Kurek, Piotr Barczyński

Figures of FT-IR spectra

Figure 1S. FT IR spectra (KBr) of colchiceine **2** and its complexes with lithium iodide **3**, sodium iodide **4** and potassium iodide **5** at the range 3750-2500cm^{-1}

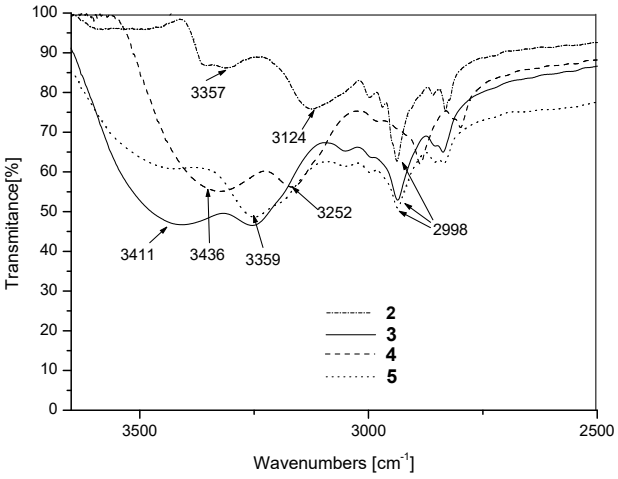


Figure 2S. FT IR spectra (nujol/fluorolub) of colchiceine complex with lithium perchlorate **6** at the range 4000-400cm^{-1}

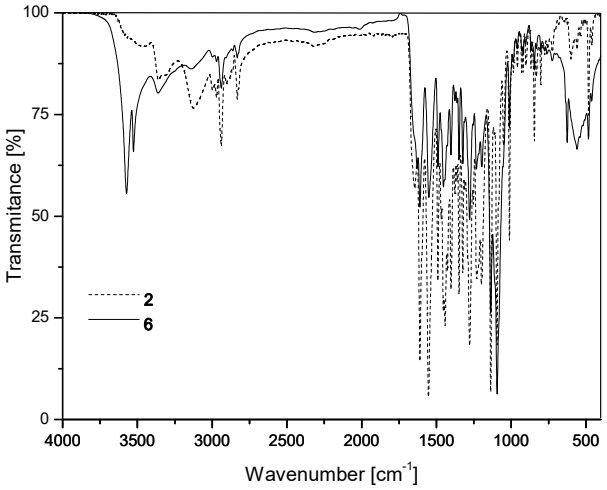


Figure 3S. FT IR spectra (nujol/fluorolub) of Colchiceine Complexes with lithium perchlorate **6** in the region of 3750-2750cm^{-1}

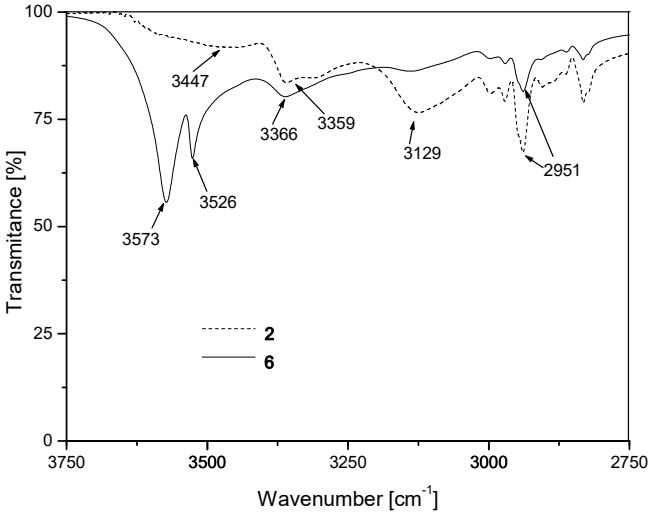
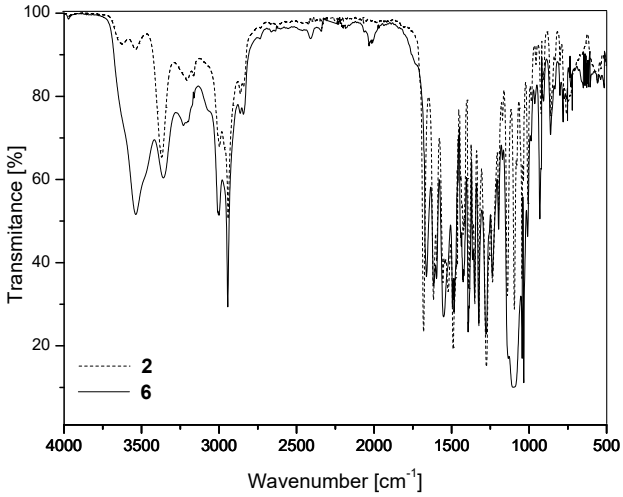


Figure 4S. FT IR spectra of Colchiceine **2** and its Complex with lithium perchlorate **6** (CD_3CN): a) 4000-500cm^{-1}, b) 3750-2750cm^{-1}

a)



b)